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J. Biol. Chem. (223)

Ezoe, S., I. Matsumura, et al. (2005). "GATA Transcription Factors Inhibit Cytokine-dependent Growth and Survival of a Hematopoietic Cell Line through the Inhibition of STAT3 Activity." J. Biol. Chem. **280**(13): 13163-13170.

http://www.jbc.org/cgi/content/abstract/280/13/13163

Although GATA-1 and GATA-2 were shown to be essential for the development of hematopoietic cells by gene targeting experiments, they were also reported to inhibit the growth of hematopoietic cells. Therefore, in this study, we examined the effects of GATA-1 and GATA-2 on cytokine signals. A tamoxifen-inducible form of GATA-1 (GATA-1/ERT) showed a minor inhibitory effect on interleukin-3 (IL-3)-dependent growth of an IL-3-dependent cell line Ba/F3. On the other hand, it drastically inhibited TPO-dependent growth and gp130-mediated growth/survival of Ba/F3. Similarly, an estradiol-inducible form of GATA-2 (GATA-2/ER) disrupted thrombopoietin (TPO)-dependent growth and gp130-mediated growth/survival of Ba/F3. As for this mechanism, we found that both GATA-1 and GATA-2 directly bound to STAT3 both in vitro and in vivo and inhibited its DNA-binding activity in gel shift assays and chromatin immunoprecipitation assays, whereas they hardly affected STAT5 activity. In addition, endogenous GATA-1 was found to interact with STAT3 in normal megakaryocytes, suggesting that GATA-1 may inhibit STAT3 activity in normal hematopoietic cells. Furthermore, we found that GATA-1 suppressed STAT3 activity through its N-zinc finger domain. Together, these results suggest that, besides the roles as transcription factors, GATA family proteins modulate cytokine signals through protein-protein interactions, thereby regulating the growth and survival of hematopoietic cells.

Maxwell, M. A., M. E. Cleasby, et al. (2005). "Nur77 Regulates Lipolysis in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN THE {beta}-ADRENERGIC AND AN ORPHAN NUCLEAR HORMONE RECEPTOR PATHWAY." J. Biol. Chem. **280**(13): 12573-12584.

http://www.jbc.org/cgi/content/abstract/280/13/12573

Skeletal muscle is a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure and is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. Excessive caloric intake is sensed by the brain and induces {beta}-adrenergic receptor ({beta}-AR)-mediated adaptive thermogenesis. {beta}-AR null mice develop severe obesity on a high fat diet. However, the target gene(s), target tissues(s), and molecular mechanism involved remain obscure. We observed that 30-60 min of {beta}-AR agonist (isoprenaline) treatment of C2C12 skeletal muscle cells strikingly activated (>100-fold) the expression of the mRNA encoding the nuclear hormone receptor, Nur77. In contrast, the expression of other nuclear receptors that regulate lipid and carbohydrate metabolism was not induced. Stable transfection of Nur77-specific small interfering RNAs (siNur77) into skeletal muscle cells repressed endogenous

Nur77 mRNA expression. Moreover, we observed attenuation of gene and protein expression associated with the regulation of energy expenditure and lipid homeostasis, for example AMP-activated protein kinase {gamma}3, UCP3, CD36, adiponectin receptor 2, GLUT4, and caveolin-3. Attenuation of Nur77 expression resulted in decreased lipolysis. Finally, in concordance with the cell culture model, injection and electrotransfer of siNur77 into mouse tibialis cranialis muscle resulted in the repression of UCP3 mRNA expression. This study demonstrates regulatory cross-talk between the nuclear hormone receptor and {beta}-AR signaling pathways. Moreover, it suggests Nur77 modulates the expression of genes that are key regulators of skeletal muscle lipid and energy homeostasis. In conclusion, we speculate that Nur77 agonists would stimulate lipolysis and increase energy expenditure in skeletal muscle and suggest selective activators of Nur77 may have therapeutic utility in the treatment of obesity.

Agorio, A., C. Chalar, et al. (2003). "Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of Echinococcus granulosus Thioredoxin Glutathione Reductase." J. Biol. Chem. **278**(15): 12920-12928.

http://www.jbc.org/cgi/content/abstract/278/15/12920

Thioredoxin and glutathione systems are the major thiol-dependent redox systems in animal cells. They transfer via the reversible oxidoreduction of thiols the reducing equivalents of NADPH to numerous substrates and substrate reductases and constitute major defenses against oxidative stress. In this study, we cloned from the helminth parasite Echinococcus granulosus two trans-spliced mRNA variants that encode thioredoxin glutathione reductases (TGR). These variants code for mitochondrial and cytosolic selenocysteine-containing isoforms that possess identical glutaredoxin (Grx) and thioredoxin reductase (TR) domains and differ exclusively in their N termini. Western blot analysis of subcellular fractions with specific anti-TGR antibodies showed that TGR is present in both compartments. The biochemical characterization of the native purified TGR suggests that the Grx and TR domains of the enzyme can function either coupled or independently of each other, because the Grx domain can accept electrons from either TR domains or the glutathione system and the TR domains can transfer electrons to either the fused Grx domain or to E. granulosus thioredoxin.

Kaneto, H., A. Sharma, et al. (2002). "Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation." J. Biol. Chem. **277**(15): 12998-13006.

http://www.jbc.org/cgi/content/abstract/277/15/12998

Insulin biosynthesis and secretion are critical for pancreatic [beta]-cell function, but both are impaired under diabetic conditions. We have found that hyperglycemia induces the expression of the basic helix-loop-helix transcription factor c-Myc in islets in several different diabetic models. To examine the possible implication of c-Myc in [beta]-cell dysfunction, c-Myc was overexpressed in isolated rat islets using adenovirus. Adenovirus-mediated c-Myc overexpression suppressed both insulin gene transcription and glucose-stimulated insulin secretion. Insulin protein content, determined by immunostaining, was markedly decreased in c-Myc-overexpressing cells. In gel-shift assays c-Myc bound to the E-box in the insulin gene promoter region. Furthermore, in [beta]TC1, MIN6, and HIT-T15 cells and primary rat islets, wild type insulin gene promoter activity was dramatically decreased by c-Myc overexpression, whereas the activity of an E-box mutated insulin promoter was not affected. In HeLa and HepG2 cells c-Myc exerted a suppressive effect on the insulin promoter activity only in the presence of NeuroD/BETA2 but not PDX-1. Both c-Myc and NeuroD can bind the E-box element in the insulin promoter, but unlike NeuroD, the c-Myc transactivation domain lacked the ability to activate insulin gene expression. Additionally p300, a

co-activator of NeuroD, did not function as a co-activator of c-Myc. In conclusion, increased expression of c-Myc in [beta]-cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. This mechanism may explain some of the [beta]-cell dysfunction found in diabetes.

Chiang, M.-C., Y.-C. Lee, et al. (2005). "cAMP-response Element-binding Protein Contributes to Suppression of the A2A Adenosine Receptor Promoter by Mutant Huntingtin with Expanded Polyglutamine Residues." J. Biol. Chem. **280**(14): 14331-14340.

http://www.jbc.org/cgi/content/abstract/280/14/14331

Huntington's disease is a neurodegenerative disease resulting from a CAG (glutamine) trinucleotide expansion in exon 1 of the Huntingtin (Htt) gene. The role of the striatum-enriched A2A adenosine receptor (A2A-R) in Huntington's disease has attracted much attention lately. In the present study, we found that expression of mutant Htt with expanded poly(Q) significantly reduced the transcript levels of the endogenous A2A-R in PC12 cells and primary striatal neurons. Cotransfection of various promoter constructs of the A2A-R gene and an expression construct of poly(Q)-expanded Htt revealed that the Htt mutant suppressed the core promoter activity of the A2A-R gene. Stimulation of the A2A-R using CGS21680 forskolin, and a constitutively active cAMP-response element-binding protein (CREB) mutant elevated the reduced promoter activity of the A2A-R gene by mutant Htt. Moreover, the effect of CGS was blocked by an A2A-R-selective antagonist (CSC), two inhibitors of protein kinase A, and two dominant negative mutants of (CREB). The protein kinase A/CREB pathway therefore is involved in regulating A2A-R promoter activity. Consistently, an atypical CRE site (TCCAGG) is located in the core promoter region of the A2A-R gene. Electrophoretic gel mobility shift assay and mutational inactivation further demonstrated the functional binding of CREB to the core promoter region and showed that expression of poly(Q)-expanded Htt abolished the binding of CREB to this site. Stimulation of the A2A-R restored the reduced CREB binding caused by the mutant and concurrently reduced mutant Htt aggregation. Collectively, the poly(Q)-expanded mutant Htt suppressed expression of the A2A-R by inhibiting its core promoter at least partially by preventing CREB binding.

Chang, W.-T. and A. M. Huang (2004). "{alpha}-Pal/NRF-1 Regulates the Promoter of the Human Integrin-associated Protein/CD47 Gene." J. Biol. Chem. **279**(15): 14542-14550.

http://www.jbc.org/cgi/content/abstract/279/15/14542

Integrin-associated protein (IAP or CD47) is expressed in a variety of tissues, including the nervous system and immune system. To understand how cells control the expression of the IAP gene, we cloned the 5'-proximal region of the human IAP gene and investigated IAP promoter activity by transient transfection. RT-PCR confirmed the expression of IAP transcripts in human neuroblastoma IMR-32 and hepatoma HepG2 cells. Deletion analysis identified a core promoter of the human IAP gene located between nucleotide positions -232 and -12 relative to the translation initiation codon in these two cell lines. Site-directed mutagenesis and gel electrophoretic mobility shift assay identified a {alpha}-Pal/NRF-1 binding element within the IAP core promoter. Supershift assays using the {alpha}-Pal/NRF-1 antiserum confirmed the binding of this transcription factor on the {alpha}-Pal/NRF-1 site. Overexpression of the DNA binding domain of {alpha}-Pal/NRF-1 in cells enhanced DNA-{alpha}-Pal/NRF-1 binding in vitro. Furthermore, overexpression of full-length {alpha}-Pal/NRF-1 significantly enhanced IAP promoter activity while overexpression of dominant-negative mutant reduced promoter activity both in the cultured human cell lines and primary mouse cortical cells. These results revealed that {alpha}-Pal/NRF-1 is an essential transcription factor in the regulation of human IAP gene expression.

Cheng, D., T. C. Nelson, et al. (2003). "Identification of Acyl Coenzyme A:Monoacylglycerol Acyltransferase 3, an Intestinal Specific Enzyme Implicated in Dietary Fat Absorption." J. Biol. Chem. **278**(16): 13611-13614.

http://www.jbc.org/cgi/content/abstract/278/16/13611

Acyl coenzyme A:monoacylglycerol acyltransferase (MGAT) catalyzes the synthesis of diacylglycerol using 2-monoacylglycerol and fatty acyl coenzyme A. This enzymatic reaction is believed to be an essential and rate-limiting step for the absorption of fat in the small intestine. Although the first MGAT-encoding cDNA, designated MGAT1, has been recently isolated, it is not expressed in the small intestine and hence cannot account for the high intestinal MGAT enzyme activity that is important for the physiology of fat absorption. In the current study, we report the identification of a novel MGAT, designated MGAT3, and present evidence that it fulfills the criteria to be the elusive intestinal MGAT. MGAT3 encodes a ~36-kDa transmembrane protein that is highly homologous to MGAT1 and -2. In humans, expression of MGAT3 is restricted to gastrointestinal tract with the highest level found in the ileum. At the cellular level, recombinant MGAT3 is localized to the endoplasmic reticulum. Recombinant MGAT3 enzyme activity produced in insect Sf9 cells selectively acylates 2-monoacylglycerol with higher efficiency than other stereoisomers. The molecular identification of MGAT3 will facilitate the evaluation of using intestinal MGAT as a potential point of intervention for antiobesity therapies.

Ortega, A. L., J. Carretero, et al. (2003). "Tumor Cytotoxicity by Endothelial Cells. IMPAIRMENT OF THE MITOCHONDRIAL SYSTEM FOR GLUTATHIONE UPTAKE IN MOUSE B16 MELANOMA CELLS THAT SURVIVE AFTER IN VITRO INTERACTION WITH THE HEPATIC SINUSOIDAL ENDOTHELIUM." J. Biol. Chem. **278**(16): 13888-13897.

http://www.jbc.org/cgi/content/abstract/278/16/13888

High GSH content associates with high metastatic activity in B16-F10 melanoma cells cultured to low density (LD B16M). GSH homeostasis was investigated in LD B16M cells that survive after adhesion to the hepatic sinusoidal endothelium (HSE). Invasive B16M (iB16M) cells were isolated using anti-Met-72 monoclonal antibodies and flow cytometry-coupled cell sorting. HSE-derived NO and H2O2 caused GSH depletion and a decrease in [gamma]-glutamylcysteine synthetase activity in iB16M cells. Overexpression of [gamma]-glutamylcysteine synthetase heavy and light subunits led to a rapid recovery of cytosolic GSH, whereas mitochondrial GSH (mtGSH) further decreased during the first 18 h of culture. NO and H2O2 damaged the mitochondrial system for GSH uptake (rates in iB16M were approximately 75% lower than in LD B16M cells). iB16M cells also showed a decreased activity of mitochondrial complexes II, III, and IV, less O2 consumption, lower ATP levels, higher O[IMG]img001.gif" ALT="<UP>₂</UP>"> and H2O2 production, and lower mitochondrial membrane potential. In vitro growing iB16M cells maintained high viability (>98%) and repaired HSE-induced mitochondrial damages within 48 h. However, iB16M cells with low mtGSH levels were highly susceptible to TNF-[alpha]-induced oxidative stress and death. Therefore depletion of mtGSH levels may represent a critical target to challenge survival of invasive cancer cells.

Dorsam, G. and E. J. Goetzl (2002). "Vasoactive Intestinal Peptide Receptor-1 (VPAC-1) Is a Novel Gene Target of the Hemolymphopoietic Transcription Factor Ikaros." J. Biol. Chem. **277**(16): 13488-13493.

http://www.jbc.org/cgi/content/abstract/277/16/13488

Vasoactive intestinal peptide and its G-protein-coupled receptors, VPAC-1 and VPAC-2, are highly expressed in the immune system and modulate diverse T cell functions. The human VPAC-1 5'-flanking region (1.4 kb) contains four high affinity Ikaros (IK) consensus sequences. Ikaros native protein from T cell nuclear extracts and IK-1 and IK-2 recombinant proteins recognized an IK high affinity binding motif in the VPAC-1 promoter in electrophoretic mobility shift assays by a sequence-specific mechanism, and anti-IK antibodies supershifted this complex. Stable NIH-3T3 clones overexpressing IK-1 or IK-2 isoforms were generated to investigate Ikaros regulation of endogenous VPAC-1 expression as assessed by quantifying VPAC-1 mRNA and protein. By traditional and fluorometric-based kinetic reverse transcription-PCR and 125I-labeled vasoactive intestinal peptide binding, both IK-1 and IK-2 suppressed endogenous VPAC-1 expression in NIH-3T3 clones by a range of 50-93%. When a series of nested deletions of the VPAC-1 luciferase reporter construct were transiently transfected into IK-2 clones there was up to a 41% decrease in transcriptional activity compared with vector control. Two major IK-2 binding domains also were identified at [-]1076 to [-]623 bp and at [-]222 to [-]35 bp, respectively. As both Ikaros and its novel target VPAC-1 are highly expressed in T cells, this system may be a dominant determinant of the VPAC-1 expression in immune responses.

Graham, D. E., H. Xu, et al. (2002). "Identification of Coenzyme M Biosynthetic Phosphosulfolactate Synthase. A NEW FAMILY OF SULFONATE-BIOSYNTHESIZING ENZYMES." J. Biol. Chem. **277**(16): 13421-13429.

http://www.jbc.org/cgi/content/abstract/277/16/13421

The hyperthermophilic euryarchaeon Methanococcus jannaschii uses coenzyme M (2-mercaptoethanesulfonic acid) as the terminal methyl carrier in methanogenesis. We describe an enzyme from that organism, (2R)-phospho-3-sulfolactate synthase (ComA), that catalyzes the first step in coenzyme M biosynthesis. ComA catalyzed the stereospecific Michael addition of sulfite to phosphoenolpyruvate over a broad range of temperature and pH conditions. Substrate and product analogs moderately inhibited activity. This enzyme has no significant sequence similarity to previously characterized enzymes; however, its Mg2+-dependent enzyme reaction mechanism may be analogous to one proposed for enolase. A diverse group of microbes and plants have homologs of ComA that could have been recruited for sulfolactate or sulfolipid biosyntheses.

Pruitt, K., A. S. Ulku, et al. (2005). "RAS mediated loss of the pro-apoptotic response protein par-4 is mediated by DNA hypermethylation through RAF-independent and RAF-dependent signaling cascades in epithelial cells." J. Biol. Chem.: M503083200.

http://www.jbc.org/cgi/content/abstract/M503083200v1

The apoptosis promoting protein, Par-4, has been shown to be downregulated in Ras-transformed NIH 3T3 fibroblasts through the Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway. Since mutations of the ras gene are most often found in tumors of epithelial origin, we explored the signaling pathways utilized by oncogenic Ras to downregulate Par-4 in RIE-1 and ROSE epithelial cells. We determined that constitutive activation of the Raf, phosphatidylinositol 3-kinase (PI3K), or Ral guanine nucleotide exchange factor effector pathway alone was not sufficient to downregulate Par-4 in RIE-1 or ROSE cells. However, treatment of Ras-transformed RIE-1 or ROSE cells with the MEK inhibitors U0126 or PD98059 increased Par-4 protein expression. Thus, while oncogenic Ras utilizes the Raf/MEK/ERK pathway to down

modulate Par-4 in both fibroblasts and epithelial cells, Ras activation of an additional signaling pathway(s) is required to achieve the same outcome in epithelial cells. Methylation-specific PCR showed the Par-4 promoter is methylated in Ras transformed cells through a MEK-dependent pathway and treatment with the DNA methyltransferase inhibitor, azadeoxycytidine, restored Par-4 mRNA transcript and protein levels suggesting that the mechanism for Ras-mediated downregulation of Par-4 is by promoter methylation. Support for this possibility is provided by our observation that Ras transformation was associated with upregulated expression of the Dnmt1 and Dnmt3 DNA methyltransferases. Finally, ectopic Par-4 expression significantly reduced Ras-mediated growth in soft agar but not morphological transformation highlighting the importance of Par-4 downregulation in specific aspects of Ras-mediated transformation of epithelial cells.

Kaneto, H., T.-a. Matsuoka, et al. (2005). "A Crucial Role of MafA as a Novel Therapeutic Target for Diabetes." J. Biol. Chem. **280**(15): 15047-15052.

http://www.jbc.org/cgi/content/abstract/280/15/15047

MafA, a recently isolated pancreatic {beta}-cell-specific transcription factor, is a potent activator of insulin gene transcription. In this study, we show that MafA overexpression, together with PDX-1 (pancreatic and duodenal homeobox factor-1) and NeuroD, markedly increases insulin gene expression in the liver. Consequently, substantial amounts of insulin protein were induced by such combination. Furthermore, in streptozotocin-induced diabetic mice, MafA overexpression in the liver, together with PDX-1 and NeuroD, dramatically ameliorated glucose tolerance, while combination of PDX-1 and NeuroD was much less effective. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes.

Valineva, T., J. Yang, et al. (2005). "The Transcriptional Co-activator Protein p100 Recruits Histone Acetyltransferase Activity to STAT6 and Mediates Interaction between the CREB-binding Protein and STAT6." J. Biol. Chem. **280**(15): 14989-14996.

http://www.jbc.org/cgi/content/abstract/280/15/14989

STAT6 is a critical regulator of transcription for interleukin-4 (IL-4)-induced genes. Activation of gene expression involves recruitment of coactivator proteins that function as bridging factors connecting sequence-specific transcription factors to the basal transcription machinery, and as chromatin-modifying enzymes. Coactivator proteins CBP/p300 have been implicated in regulation of transcription in all STATs. CBP is also required for STAT6-mediated gene activation, but the underlying molecular mechanisms are still elusive. In this study we investigated the mechanisms by which STAT6 recruits CBP and chromatin-modifying activities to the promoter. Our results indicate that while STAT1-interacted directly with CBP, the interaction between STAT6 and CBP was found to be mediated through p100 protein, a coactivator protein that has previously been shown to stimulate the transcription of IL-4-induced genes. The staphylococcal nuclease-like (SN)-domains of p100 directly interacted with amino acids 1099-1758 of CBP, while p100 did not associate with SRC-1, another coactivator of STAT6. p100 was found to recruit histone acetyltransferase (HAT) activity to STAT6 in vivo. Chromatin immunoprecipitation studies demonstrated that p100 increases the STAT6-p100-CBP ternary complex formation in the human Ig{epsilon} promoter. p100 also increased the amount of acetylated histone H4 at the Ig{epsilon} promoter, and siRNAs directed against p100 effectively inhibited Ig{epsilon} reporter gene expression. Our results suggest that p100 has an important role in the assembly of STAT6 transcriptosome, and that p100 stimulates IL-4-dependent transcription by mediating interaction between STAT6 and CBP and recruiting chromatin modifying activities to STAT6-responsive promoters.

Di Carlo, A., R. De Mori, et al. (2004). "Hypoxia Inhibits Myogenic Differentiation through Accelerated MyoD Degradation." J. Biol. Chem. **279**(16): 16332-16338.

http://www.jbc.org/cgi/content/abstract/279/16/16332

Cells undergo a variety of biological responses when placed in hypoxic conditions, including alterations in metabolic state and growth rate. Here we investigated the effect of hypoxia on the ability of myogenic cells to differentiate in culture. Exposure of myoblasts to hypoxia strongly inhibited multinucleated myotube formation and the expression of differentiation markers. We showed that hypoxia reversibly inhibited MyoD, Myf5, and myogenin expression. One key step in skeletal muscle differentiation involves the up-regulation of the cell cycle-dependent kinase inhibitors p21 and p27 as well as the product of the retinoblastoma gene (pRb). Myoblasts cultured under hypoxic conditions in differentiation medium failed to up-regulate both p21 and pRb despite the G1 cell cycle arrest, as evidenced by p27 accumulation and pRb hypophosphorylation. Hypoxia-dependent inhibition of differentiation was associated with MyoD degradation by the ubiquitin-proteasome pathway. MyoD overexpression in C2C12 myoblasts overrode the differentiation block imposed by hypoxic conditions. Thus, hypoxia by inducing MyoD degradation blocked accumulation of early myogenic differentiation markers such as myogenin and p21 and pRb, preventing both permanent cell cycle withdraw and terminal differentiation. Our study revealed a novel anti-differentiation effect exerted by hypoxia in myogenic cells and identified MyoD degradation as a relevant target of hypoxia.

Hoffmann, C., M. Pop, et al. (2004). "The Yersinia pseudotuberculosis Cytotoxic Necrotizing Factor (CNFY) Selectively Activates RhoA." J. Biol. Chem. **279**(16): 16026-16032.

http://www.jbc.org/cgi/content/abstract/279/16/16026

The cytotoxic necrotizing factors (CNF)1 and CNF2 from pathogenic Escherichia coli strains activate RhoA, Rac1, and Cdc42 by deamidation of Gln63 (RhoA) or Gln61 (Rac and Cdc42). Recently, a novel cytotoxic necrotizing factor termed CNFY was identified in Yersinia pseudotuberculosis strains (Lockman, H. A., Gillespie, R. A., Baker, B. D., and Shakhnovich, E. (2002) Infect. Immun. 70, 2708-2714). We amplified the cnfy gene from genomic DNA of Y. pseudotuberculosis, cloned and expressed the recombinant protein, and studied its activity. Recombinant GST-CNFY induced morphological changes in HeLa cells and caused an upward shift of RhoA in SDS-PAGE, as is known for GST-CNF1 and GST-CNF2. Mass spectrometric analysis of GST-CNFY-treated RhoA confirmed deamidation at Glu63. Treatment of RhoA, Rac1, and Cdc42 with GST-CNFY decreased their GTPase activities, indicating that all of these Rho proteins could serve as substrates for GST-CNFY in vitro. In contrast, RhoA, but not Rac or Cdc42, was the substrate of GST-CNFY in culture cells. GST-CNFY caused marked stress fiber formation in HeLa cells after 2 h. In contrast to GST-CNF1, formation of filopodia or lamellipodia was not induced with GST-CNFY. Accordingly, effector pull-down experiments with lysates of toxin-treated cells revealed strong activation of RhoA but no activation of Rac1 or Cdc42 after 6 h of GST-CNFY-treatment. Moreover, in rat hippocampal neurons, GST-CNFY results in the retraction of neurites, indicating RhoA activation. In contrast, no activation of Rac or Cdc42 was found. Altogether, our data suggest that CNFY from Y. pseudotuberculosis is a strong, selective activator of RhoA, which can be used as a powerful tool for constitutive RhoA activation without concomitant activation of Rac1 or Cdc42.

Priest, J. W. and S. L. Hajduk (2003). "Trypanosoma brucei Cytochrome c1 Is Imported into Mitochondria

Along an Unusual Pathway." J. Biol. Chem. **278**(17): 15084-15094.

http://www.jbc.org/cgi/content/abstract/278/17/15084

In most eukaryotic organisms, cytochrome c1 is encoded in the nucleus, translated on cytosolic ribosomes, and directed to its final destination in the mitochondrial inner membrane by a bipartite, cleaved, amino-terminal presequence. However, in the kinetoplastids and euglenoids, the cytochrome c1 protein has been shown to lack a cleaved presequence; a single methionine is removed from the amino terminus upon maturation, and the sequence upstream of the heme-binding site is generally shorter than that of the other eukaryotic homologs. We have used a newly developed mitochondrial protein import assay system from Trypanosoma brucei to demonstrate that the T. brucei cytochrome c1 protein is imported along a non-conservative pathway similar to that described for the inner membrane carrier proteins of other organisms. This pathway requires external ATP and an external protein receptor but is not absolutely dependent on a membrane potential or on ATP hydrolysis in the mitochondrial matrix. We propose the cytochrome c1 import in T. brucei is a two-step process first involving a membrane potential independent translocation across the outer mitochondrial membrane followed by heme attachment and a membrane potential-dependent insertion into the inner membrane.

Vanden Abeele, F., M. Roudbaraki, et al. (2003). "Store-operated Ca2+ Current in Prostate Cancer Epithelial Cells. ROLE OF ENDOGENOUS Ca2+ TRANSPORTER TYPE 1." J. Biol. Chem. **278**(17): 15381-15389.

http://www.jbc.org/cgi/content/abstract/278/17/15381

Ca2+ influx via store-operated channels (SOCs) following stimulation of the plasma membrane receptors is the key event controlling numerous processes in nonexcitable cells. The human transient receptor potential vanilloid type 6 channel, originally termed Ca2+ transporter type 1 (CaT1) protein, is one of the promising candidates for the role of endogenous SOC, although investigations of its functions have generated considerable controversy. In order to assess the role of CaT1 in generating endogenous store-operated Ca2+ current (ISOC) in the lymph node carcinoma of the prostate (LNCaP) human prostate cancer epithelial cell line, we manipulated its endogenous levels by means of antisense hybrid depletion or pharmacological up-regulation (antiandrogen treatment) combined with functional evaluation of ISOC. Antisense hybrid depletion of CaT1 decreased ISOC in LNCaP cells by ~50%, whereas enhancement of CaT1 levels by 60% in response to Casodex treatment potentiated ISOC by 30%. The functional characteristics of ISOC in LNCaP cells were similar in many respects to those reported for heterologously expressed CaT1, although 2-aminoethoxydiphenyl borate sensitivity and lack of constitutive current highlighted notable departures. Our results suggest that CaT1 is definitely involved in ISOC, but it may constitute only a part of the endogenous SOC, which in general may be a heteromultimeric channel composed of homologous CaT1 and other transient receptor potential subunits.

Kruger, R. P., H. C. Winter, et al. (2002). "Cloning, Expression, and Characterization of the Galalpha 1,3Gal High Affinity Lectin from the Mushroom Marasmius oreades." J. Biol. Chem. **277**(17): 15002-15005.

http://www.jbc.org/cgi/content/abstract/277/17/15002

The purification and unique carbohydrate binding properties, including blood group B-specific agglutination and preferential binding to Gal[alpha]1,3Gal-containing sugar epitopes, of the

Marasmius oreades agglutinin (MOA) are reported in an accompanying paper (Winter, H. C., Mostafapour, K., and Goldstein, I. J. (2002) J. Biol. Chem. 277, 14996-15001). Here we describe the cloning, characterization, and expression of MOA. MOA was digested with trypsin and endoproteinase Asp-N, and the peptide fragments were purified by high performance liquid chromatography. Amino acid sequence data were obtained for eight peptides. Using oligonucleotides deduced from the peptide sequences for a reverse transcriptase-PCR, a 41-base pair cDNA was obtained. The 41-base pair fragment allowed the generation a full-length cDNA using 5' and 3' rapid amplification of cDNA ends. MOA cDNA encodes a protein of 293 amino acids that contains a ricin domain. These carbohydrate binding domains were first described in subunits of bacterial toxins and are also commonly found in polysaccharide-degrading enzymes. Whereas these proteins are known to display a variety of sugar binding specificities, none to date are known to share MOA's high affinity for Gal[alpha]1,3Gal and Gal[alpha]1,3Gal[beta]1,4GlcNAc. Recombinantly expressed and purified MOA retains the specificity and affinity observed with the native protein. This study provides the basis for analyzing the underlying cause for the unusual binding specificity of MOA.

Morohashi, Y., N. Hatano, et al. (2002). "Molecular Cloning and Characterization of CALP/KChIP4, a Novel EF-hand Protein Interacting with Presenilin 2 and Voltage-gated Potassium Channel Subunit Kv4." J. Biol. Chem. **277**(17): 14965-14975.

http://www.jbc.org/cgi/content/abstract/277/17/14965

Presenilin (PS) genes linked to early-onset familial Alzheimer's disease encode polytopic membrane proteins that are presumed to constitute the catalytic subunit of [gamma]-secretase, forming a high molecular weight complex with other proteins. During our attempts to identify binding partners of PS2, we cloned CALP (calsenilin-like protein)/KChIP4, a novel member of calsenilin/KChIP protein family that interacts with the C-terminal region of PS. Upon co-expression in cultured cells, CALP was directly bound to and co-localized with PS2 in endoplasmic reticulum. Overexpression of CALP did not affect the metabolism or stability of PS complex, and [gamma]-cleavage of [beta]APP or Notch site 3 cleavage was not altered. However, co-expression of CALP and a voltage-gated potassium channel subunit Kv4.2 reconstituted the features of A-type K+ currents and CALP directly bound Kv4.2, indicating that CALP functions as KChIPs that are known as components of native Kv4 channel complex. Taken together, CALP/KChIP4 is a novel EF-hand protein interacting with PS as well as with Kv4 that may modulate functions of a subset of membrane proteins in brain.

Tang, S. and Z.-M. Zheng (2002). "Kaposi's Sarcoma-associated Herpesvirus K8 Exon 3 Contains Three 5'-Splice Sites and Harbors a K8.1 Transcription Start Site." J. Biol. Chem. **277**(17): 14547-14556.

http://www.jbc.org/cgi/content/abstract/277/17/14547

Kaposi's sarcoma-associated herpesvirus (KSHV) K8 and K8.1 open reading frames are juxtaposed and span from nucleotide (nt) 74850 to 76695 of the virus genome. A K8 pre-mRNA overlaps the entire K8.1 coding region, and alternative splicing of KSHV K8 and K8.1 pre-mRNAs each produces three isoforms ([alpha], [beta], and [gamma]) of the mRNAs. We have mapped the 5' end of the K8.1 RNA in butyrate-induced KSHV-positive JSC-1 cells to nt 75901 in the KSHV genome and have shown that exon 3 of the K8 pre-mRNA in JSC-1 cells covers most part of the intron 3 defined previously and has three 5'-splice sites (ss), respectively, at nt 75838, 76155, and 76338. Selection of the nt 75838 5'-ss dictates the K8 mRNA production and overwhelms the RNA processing. Alternative selection of other two 5'-ss is feasible and leads to production of two additional bicistronic mRNAs, K8/K8.1[alpha] and -[beta]. However, the novel bicistronic K8/K8.1

mRNAs translated a little K8 and no detectable K8.1 proteins in 293 cells. Data suggest that production of the K8/K8.1 mRNAs may be an essential way to control K8 mRNAs, especially K8[alpha], to a threshold at RNA processing level.

Tynan, F. E., N. A. Borg, et al. (2005). "The high resolution structures of highly bulged viral epitopes bound to the major histocompatability class I: Implications for T-cell receptor engagement and T-cell immunodominance." J. Biol. Chem.: M503060200.

http://www.jbc.org/cgi/content/abstract/M503060200v1

Although HLA class I alleles can bind epitopes up to 14 amino acids in length, little is known about the immunogenicity or the responding T-cell repertoire against such determinants. Here we describe a HLA-B*3508-restricted CTL response to a 13-mer viral epitope (LPEPLPQGQLTAY). The rigid, centrally-bulged epitope generates a biased T-cell response. Only the N-terminal face of the peptide bulge is critical for recognition by the dominant clonotype, SB27. The SB27 public TcR associates slowly onto the bulged pMHC complex, suggesting significant remodelling upon engagement. The broad antigen binding cleft of HLA-B*3508 represents a critical feature for engagement of the public TcR, as the narrower binding cleft of HLA-B*3501LPEPLPQGQLTAY, which differs from HLA-B*3508 by a single amino acid polymorphism (Arg 156'Leu), interacts poorly with the dominant TcR. Biased TcR usage in this CTL response appears to reflect a dominant role of the prominent pMHC-I surface.

Mitsutake, S., T.-J. Kim, et al. (2004). "Ceramide Kinase Is a Mediator of Calcium-dependent Degranulation in Mast Cells." J. Biol. Chem. **279**(17): 17570-17577.

http://www.jbc.org/cgi/content/abstract/279/17/17570

Ceramide kinase (CERK) catalyzes the conversion of ceramide to ceramide 1-phosphate (C1P) and is known to be activated by calcium. Although several groups have examined the functions of CERK and its product C1P, the functions of C1P and CERK are not understood. We studied the RBL-2H3 cell line, a widely used model for mast cells, and found that CERK and C1P are required for activation of the degranulation process in mast cells. We found that C1P formation was enhanced during activation induced by IgE/antigen or by Ca2+ ionophore A23187. The formation of C1P required the intracellular elevation of Ca2+. We generated RBL-2H3 cells that stably express CERK, and when these cells were treated with A23187, a concomitant C1P formation was observed and degranulation increased 4-fold, compared with mock transfectants. The cell-permeable N-acetylsphingosine (C2-ceramide), a poor substrate of CERK, inhibited both the formation of C1P and degranulation, indicating that C1P formation was necessary for degranulation. Exogenous introduction of CERK into permeabilized RBL-2H3 cells caused degranulation. We identified a cytosolic localization of CERK that provides exposure to cytosolic Ca2+. Taken together, these results indicate that C1P formation is a necessary step in the degranulation pathway in RBL-2H3 cells.

Tardif, K. D., K. Mori, et al. (2004). "Hepatitis C Virus Suppresses the IRE1-XBP1 Pathway of the Unfolded Protein Response." J. Biol. Chem. **279**(17): 17158-17164.

http://www.jbc.org/cgi/content/abstract/279/17/17158

Hepatitis C virus (HCV) gene expression disrupts normal endoplasmic reticulum (ER) functions

and induces ER stress. ER stress results from the accumulation of unfolded or misfolded proteins in the ER; cells can alleviate this stress by degrading or refolding these proteins. The IRE1-XBP1 pathway directs both protein refolding and degradation in response to ER stress. Like IRE1-XBP1, other branches of the ER stress response mediate protein refolding. However, IRE1-XBP1 can also specifically activate protein degradation. We show here that XBP1 expression is elevated in cells carrying HCV subgenomic replicons, but XBP1 trans-activating activity is repressed. This prevents the IRE1-XBP1 transcriptional induction of EDEM (ER degradation-enhancing {alpha}-mannosidase-like protein). The mRNA expression of EDEM is required for the degradation of misfolded proteins. Consequently, misfolded proteins are stable in cells expressing HCV replicons. HCV may suppress the IRE1-XBP1 pathway to stimulate the synthesis of its viral proteins. IRE1{alpha}-null MEFs, a cell line with a defective IRE1-XBP1 pathway, show elevated levels of HCV IRES-mediated translation. Therefore, HCV may suppress the IRE1-XBP1 pathway to not only promote HCV expression but also to contribute to the persistence of the virus in infected hepatocytes.

Tian, B., D. E. Nowak, et al. (2005). "Identification of Direct Genomic Targets Downstream of the Nuclear Factor-{kappa}B Transcription Factor Mediating Tumor Necrosis Factor Signaling." J. Biol. Chem. **280**(17): 17435-17448.

http://www.jbc.org/cgi/content/abstract/280/17/17435

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine that controls expression of inflammatory genetic networks. Although the nuclear factor-{kappa}B (NF-{kappa}B) pathway is crucial for mediating cellular TNF responses, the complete spectrum of NF-{kappa}B-dependent genes is unknown. In this study, we used a tetracycline-regulated cell line expressing an NF-{kappa}B inhibitor to systematically identify NF-{kappa}B-dependent genes. A microarray data set generated from a time course of TNF stimulation in the presence or absence of NF-{kappa}B signaling was analyzed. We identified 50 unique genes that were regulated by TNF (Pr(F) <0.001) and demonstrated a change in signal intensity of {+/-} 3-fold relative to control. Of these, 28 were NF-{kappa}B-dependent, encoding proteins involved in diverse cellular activities. Quantitative real-time PCR assays of eight characterized NF-{kappa}B-dependent genes and five genes not previously known to be NF-{kappa}B-dependent (Gro-{beta} and-{gamma}, I{kappa}B{epsilon}, interleukin (IL)-7R, and Naf-1) were used to determine whether they were directly or indirectly NF-{kappa}B regulated. Expression of constitutively active enhanced green fluorescent{middle dot}NF-{kappa}B/Rel A fusion protein transactivated all but IL-6 and IL-7R in the absence of TNF stimulation. Moreover, TNF strongly induced all 12 genes in the absence of new protein synthesis. High probability NF-{kappa}B sites in novel genes were predicted by binding site analysis and confirmed by electrophoretic mobility shift assay. Chromatin immunoprecipitation assays show the endogenous I{kappa}B{alpha}/{epsilon}, Gro-{beta}/{gamma}, and Naf-1 promoters directly bound NF-{kappa}B/Rel A in TNF-stimulated cells. Together, these studies systematically identify the direct NF-{kappa}B-dependent gene network downstream of TNF signaling, extending our knowledge of biological processes regulated by this pathway.

Nishiya, T. and A. L. DeFranco (2004). "Ligand-regulated Chimeric Receptor Approach Reveals Distinctive Subcellular Localization and Signaling Properties of the Toll-like Receptors." J. Biol. Chem. **279**(18): 19008-19017.

http://www.jbc.org/cgi/content/abstract/279/18/19008

Toll-like receptors (TLRs) are sensors for the detection of invading infectious agents and can initiate innate immune responses. Because the innate immune system induces an appropriate

defense against different pathogens, different TLR signaling domains may have unique properties that are responsible for eliciting distinctive responses to different types of pathogens. To test this hypothesis, we created ligand-regulated TLR chimeric receptors composed of the extracellular region of TLR4 and the transmembrane and cytoplasmic regions of other TLRs and expressed these chimeras in macrophages lacking endogenous TLR4. Interestingly, the chimeras between TLR4 and either TLR3, TLR7, or TLR9 were localized completely intracellularly whereas other chimeras were expressed on the cell surface. Lipopolysaccharide (LPS), a ligand for these chimeras, induced the activation of nuclear factor {kappa}B and mitogen-activated protein kinases and the subsequent production of pro-inflammatory cytokines in macrophages expressing TLR4, TLR4/TLR5, or TLR4/TLR8 chimeras but not in macrophages expressing TLR4/TLR1, TLR4/TLR2, or TLR4/TLR6 chimeras. Co-expression of unresponsive chimeras in some combinations (chimeras with TLR1+TLR2 or TLR2+TLR6 but not TLR1+TLR6) resulted in LPS responsiveness, indicating functional complementarity. Furthermore, the pair of TLR2+TLR6 chimera required approximately 10-fold less LPS to induce the same responses compared with the TLR1+TLR2 pair. Finally, LPS induced effective interferon-{beta} production and subsequent Stat1 phosphorylation in macrophages expressing full-length TLR4 but not other cell surface TLR chimeras. These results suggest that the functions of TLRs are diversified not only in their extracellular regions for ligand recognition but also in their transmembrane and cytoplasmic regions for subcellular localization and signaling properties.

Nagaoka, K., H. Nojima, et al. (2003). "Regulation of Blastocyst Migration, Apposition, and Initial Adhesion by a Chemokine, Interferon {gamma}-inducible Protein 10 kDa (IP-10), during Early Gestation." J. Biol. Chem. **278**(31): 29048-29056.

http://www.jbc.org/cgi/content/abstract/278/31/29048

For a pregnancy to be established, initial apposition and adhesion of the blastocyst to maternal endometrium must occur in a coordinated manner; however, a key factor(s) that mediates the trophoblast cell migration and attachment to the apical surface of the endometrium has not been identified. In this study, we examined the effect of an endometrial chemokine, interferon-{gamma}-inducible protein 10 kDa (IP-10), on conceptus migration to the endometrial epithelium. We first studied endometrial IP-10 mRNA expression, which was localized in the subepithelial stromal region, and detected the protein in the uterine flushing media during early pregnancy. Expression of IP-10 mRNA by the endometrium of cyclic animals was stimulated by the addition of a conceptus factor interferon-tau (IFN-{tau}). Immunofluorescent analysis revealed that IP-10 receptor, CXCR3, was localized in the trophoblast cells, to which biotinylated-recombinant caprine IP-10 (rcIP-10) bound. Chemotaxis assay indicated that rcIP-10 stimulated the migration of trophoblast cells, and the effects of rcIP-10 were neutralized by the pretreatment with an anti-IP-10 antibody. Adhesive activity of trophoblast cells to fibronectin was promoted by rcIP-10, and the effect was inhibited by the use of anti-IP-10 antibody. Further adhesion experiments demonstrated that binding of trophoblast cells to fibronectin was completely inhibited by a peptide of the Arg-Gly-Asp (RGD) sequence, which binds to integrins {alpha}5{beta}1, {alpha}V{beta}1, {alpha}V{beta}3, and {alpha}V{beta}5, whereas non-binding peptide containing Arg-Gly-Glu (RGE) had minimal effects. More importantly, rcIP-10 promoted the adhesion of trophoblast cells to primary cells isolated from endometrial epithelium. Furthermore, rcIP-10 stimulated the expression of integrin {alpha}5, {alpha}V, and {beta}3 subunit mRNA in trophoblast cells. These findings suggest that endometrial IP-10 regulates the establishment of apical interactions between trophoblast and epithelial cells during early gestation.

Rumberger, J. M., T. Wu, et al. (2003). "Role of Hexosamine Biosynthesis in Glucose-mediated Up-regulation of Lipogenic Enzyme mRNA Levels: EFFECTS OF GLUCOSE, GLUTAMINE, AND GLUCOSAMINE ON GLYCEROPHOSPHATE DEHYDROGENASE, FATTY ACID SYNTHASE,

AND ACETYL-CoA CARBOXYLASE mRNA LEVELS." J. Biol. Chem. **278**(31): 28547-28552.

http://www.jbc.org/cgi/content/abstract/278/31/28547

Glucose uptake into adipose and liver cells is known to up-regulate mRNA levels for various lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). To determine whether the hexosamine biosynthesis pathway (HBP) mediates glucose regulation of mRNA expression, we treated primary cultured adipocytes for 18 h with insulin (25 ng/ml) and either glucose (20 mM) or glucosamine (2 mM). A ribonuclease protection assay was used to quantitate mRNA levels for FAS, ACC, and glycerol-3-P dehydrogenase (GPDH). Treatment with insulin and various concentrations of D-glucose increased mRNA levels for FAS (280%), ACC (93%), and GPDH (633%) in a dose-dependent manner (ED50 8-16 mM). Mannose similarly elevated mRNA levels, but galactose and fructose were only partially effective. L-glucose had no effect. Omission of glutamine from the culture medium markedly diminished the stimulatory effect of glucose on mRNA expression. Since glutamine is a crucial amide donor in hexosamine biosynthesis, we interpret these data to mean that glucose flux through the HBP is linked to regulation of lipogenesis through control of gene expression. Further evidence for hexosamine regulation was obtained using glucosamine, which is readily transported into adipocytes where it directly enters the HBP. Glucosamine was 15-30 times more potent than glucose in elevating FAS, ACC, and GPDH mRNA levels (ED50 [~]0.5 mM). In summary: 1) GPDH, FAS, and ACC mRNA levels are upregulated by glucose; 2) glucose-induced up-regulation requires glutamine; and 3) mRNA levels for lipogenic enzymes are up-regulated by glucosamine. Hyperglycemia is the hallmark of diabetes mellitus and leads to insulin resistance, impaired glucose metabolism, and dyslipidemia. We postulate that disease pathophysiology may have a common underlying factor, excessive glucose flux through the HBP.

Basu, S. S., M. J. Karbarz, et al. (2002). "Expression Cloning and Characterization of the C28 Acyltransferase of Lipid A Biosynthesis in Rhizobium leguminosarum." J. Biol. Chem. **277**(32): 28959-28971.

http://www.jbc.org/cgi/content/abstract/277/32/28959

An unusual feature of lipid A from plant endosymbionts of the Rhizobiaceae family is the presence of a 27-hydroxyoctacosanoic acid (C28) moiety. An enzyme that incorporates this acyl chain is present in extracts of Rhizobium leguminosarum, Rhizobium etli, and Sinorhizobium meliloti but not Escherichia coli. The enzyme transfers 27-hydroxyoctacosanate from a specialized acyl carrier protein (AcpXL) to the precursor Kdo2 ((3-deoxy-D-manno-octulosonic acid)2)-lipid IVA. We now report the identification of five hybrid cosmids that direct the overexpression of this activity by screening ~4000 lysates of individual colonies of an R. leguminosarum 3841 genomic DNA library in the host strain S. meliloti 1021. In these heterologous constructs, both the C28 acyltransferase and C28-AcpXL are overproduced. Sequencing of a 9-kb insert from cosmid pSSB-1, which is also present in the other cosmids, shows that acpXL and the lipid A acyltransferase gene (lpxXL) are close to each other but not contiguous. Nine other open reading frames around lpxXL were also sequenced. Four of them encode orthologues of fatty acid and/or polyketide biosynthetic enzymes. AcpXL purified from S. meliloti expressing pSSB-1 is fully acylated, mainly with 27-hydroxyoctacosanoate. Expression of lpxXL in E. coli behind a T7 promoter results in overproduction in vitro of the expected R. leguminosarum acyltransferase, which is C28-AcpXL-dependent and utilizes (3-deoxy-D-manno-octulosonic acid)2-lipid IVA as the acceptor. These findings confirm that lpxXL is the structural gene for the C28 acyltransferase. LpxXL is distantly related to the lauroyltransferase (LpxL) of E. coli lipid A biosynthesis, but highly significant LpxXL orthologues are present in Agrobacterium tumefaciens, Brucella melitensis, and all sequenced strains of Rhizobium, consistent with the occurrence of long secondary acyl chains in the lipid A molecules of these organisms.

Beekman, J. M., J. E. Bakema, et al. (2004). "Modulation of Fc{gamma}RI (CD64) Ligand Binding by Blocking Peptides of Periplakin." J. Biol. Chem. **279**(32): 33875-33881.

http://www.jbc.org/cgi/content/abstract/279/32/33875

Fc{gamma}RI requires both the intracellular domain of the {alpha}-chain and associated leukocyte Fc receptor (FcR) {gamma}-chains for its biological function. We recently found the C terminus of periplakin to selectively interact with the cytoplasmic domain of the Fc{gamma}RI {alpha}-chain. It thereby enhances the capacity of Fc{gamma}RI to bind, internalize, and present antigens on MHC class II. Here, we characterized the domains involved in Fc{gamma}RI-periplakin interaction using truncated and alanine-substituted Fc{gamma}RI mutants and randomly mutagenized periplakin. This allowed us to design TAT peptides that selectively interfered with endogenous Fc{gamma}RI-periplakin interactions. The addition of these peptides to Fc{gamma}RI-expressing cells modulated Fc{gamma}RI ligand binding, as assessed by erythrocyte-antibody-rosetting. These data support a dominant-negative role of C-terminal periplakin for Fc{gamma}RI biological activity and implicate periplakin as a novel regulator of Fc{gamma}RI in immune cells.

Horibata, Y., K. Sakaguchi, et al. (2004). "Unique Catabolic Pathway of Glycosphingolipids in a Hydrozoan, Hydra magnipapillata, Involving Endoglycoceramidase." J. Biol. Chem. **279**(32): 33379-33389.

http://www.jbc.org/cgi/content/abstract/279/32/33379

Endoglycoceramidase (EGCase; EC 3.2.1.123) is an enzyme capable of cleaving the glycosidic linkage between oligosaccharides and ceramides of various glycosphingolipids. We detected strong EGCase activity in animals belonging to Cnidaria, Mollusca, and Annelida and cloned the enzyme from a hydra, Hydra magnipapillata. The hydra EGCase, consisting of 517 amino acid residues, showed 19.2% and 50.2% identity to the Rhodococcus and jellyfish EGCases, respectively. The recombinant hydra enzyme, expressed in CHOP (Chinese hamster ovary cells expressing polyoma LT antigen) cells, hydrolyzed [14C]GM1a to produce [14C]ceramide with a pH optimum at 3.0-3.5. Whole mount in situ hybridization and immunocytochemical analysis revealed that EGCase was widely expressed in the endodermal layer, especially in digestive cells. GM1a injected into the gastric cavity was incorporated and then directly catabolized by EGCase to produce GM1a-oligosaccharide and ceramide, which were further degraded by exoglycosidases and ceramidase, respectively. However, hydra exoglycosidases did not hydrolyze GM1a directly. These results indicate that the EGCase is indispensable for the catabolic processing of dietary glycosphingolipids in hydra, demonstrating the unique catabolic pathway for glycosphingolipids in the animal.

Schmidt, C., B. Li, et al. (2003). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF POINT MUTATIONS THAT "SILENCE" A CONSTITUTIVELY ACTIVE MUTANT M3 RECEPTOR AND GREATLY IMPAIR RECEPTOR/G PROTEIN COUPLING." J. Biol. Chem. **278**(32): 30248-30260.

http://www.jbc.org/cgi/content/abstract/278/32/30248

The M3 muscarinic receptor is a prototypical member of the class I family of G protein-coupled receptors (GPCRs). To facilitate studies on the structural mechanisms governing M3 receptor

activation, we generated an M3 receptor-expressing yeast strain (Saccharomyces cerevisiae) that requires agonist-dependent M3 receptor activation for cell growth. By using receptor random mutagenesis followed by a genetic screen in yeast, we initially identified a point mutation at the cytoplasmic end of transmembrane domain (TM) VI (Q490L) that led to robust agonist-independent M3 receptor signaling in both yeast and mammalian cells. To explore further the molecular mechanisms by which point mutations can render GPCRs constitutively active, we subjected a region of the Q490L mutant M3 receptor that included TM V-VII to random mutagenesis. We then applied a yeast genetic screen to identify second-site mutations that could suppress the activating effects of the Q490L mutation and restore wild-type receptor-like function to the Q490L mutant receptor. This analysis led to the identification of 12 point mutations that allowed the Q490L mutant receptor to function in a fashion similar to the wild-type receptor. These amino acid substitutions mapped to two distinct regions of the M3 receptor, the exofacial segments of TM V and VI and the cytoplasmic ends of TM V-VII. Strikingly, in the absence of the activating Q490L mutation, all recovered point mutations severely reduced the efficiency of receptor/G protein coupling, indicating that the targeted residues play important roles in receptor activation and/or receptor/G protein coupling. This strategy should be generally applicable to identify sites in GPCRs that are critically involved in receptor function.

Hellberg, A., J. Poole, et al. (2002). "Molecular Basis of the Globoside-deficient Pk Blood Group Phenotype. IDENTIFICATION OF FOUR INACTIVATING MUTATIONS IN THE UDP-N-ACETYLGALACTOSAMINE: GLOBOTRIAOSYLCERAMIDE 3-beta -N-ACETYLGALACTOSAMINYLTRANSFERASE GENE." J. Biol. Chem. **277**(33): 29455-29459.

http://www.jbc.org/cgi/content/abstract/277/33/29455

The biochemistry and molecular genetics underlying the related carbohydrate blood group antigens P, Pk, and LKE in the GLOB collection and P1 in the P blood group system are complex and not fully understood. Individuals with the rare but clinically important erythrocyte phenotypes P1k and P2k lack the capability to synthesize P antigen identified as globoside, the cellular receptor for Parvo-B19 virus and some P-fimbriated Escherichia coli. As in the ABO system, naturally occurring antibodies, anti-P of the IgM and IgG class with hemolytic and cytotoxic capacity, are formed. To define the molecular basis of the Pk phenotype we analyzed the full coding region of a candidate gene reported in 1998 as a member of the 3-[beta]-galactosyltransferase family but later shown to possess UDP-N-acetylgalactosamine:globotriaosylceramide 3-[beta]-N-acetylgalactosaminyltransferase or globoside synthase activity. Homozygosity for different nonsense mutations (C202 [right-arrow] T and 538insA) resulting in premature stop codons was found in blood samples from two individuals of the P2k phenotype. Two individuals with P1k and P2k phenotypes were homozygous for missense mutations causing amino acid substitutions (E266A or G271R) in a highly conserved region of the enzymatically active carboxyl-terminal domain in the transferase. We conclude that crucial mutations in the globoside synthase gene cause the Pk phenotype.

Kaneto, H., G. Xu, et al. (2002). "Involvement of c-Jun N-terminal Kinase in Oxidative Stress-mediated Suppression of Insulin Gene Expression." J. Biol. Chem. **277**(33): 30010-30018.

http://www.jbc.org/cgi/content/abstract/277/33/30010

Oxidative stress, which is found in pancreatic [beta]-cells in the diabetic state, suppresses insulin gene transcription and secretion, but the signaling pathways involved in the [beta]-cell dysfunction induced by oxidative stress remain unknown. In this study, subjecting rat islets to oxidative stress activates JNK, p38 MAPK, and protein kinase C, preceding the decrease of insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type (DN) JNK, but not

the p38 MAPK inhibitor SB203580 nor the protein kinase C inhibitor GF109203X, protected insulin gene expression and secretion from oxidative stress. Moreover, wild type JNK overexpression suppressed both insulin gene expression and secretion. These results were correlated with changes in the binding of the important transcription factor PDX-1 to the insulin promoter; adenoviral overexpression of DN-JNK preserved PDX-1 DNA binding activity in the face of oxidative stress, whereas wild type JNK overexpression decreased PDX-1 DNA binding activity. Furthermore, to examine whether suppression of the JNK pathway can protect [beta]-cells from the toxic effects of hyperglycemia, rat islets were infected with DN-JNK expressing adenovirus or control adenovirus and transplanted under renal capsules of streptozotocin-induced diabetic nude mice. In mice receiving DN-JNK overexpressing islets, insulin gene expression in islet grafts was preserved, and hyperglycemia was ameliorated compared with control mice. In conclusion, activation of JNK is involved in the reduction of insulin gene expression by oxidative stress, and suppression of the JNK pathway protects [beta]-cells from oxidative stress.

Zhang, D., R. C. M. Simmen, et al. (2002). "Secretory Leukocyte Protease Inhibitor Mediates Proliferation of Human Endometrial Epithelial Cells by Positive and Negative Regulation of Growth-associated Genes." J. Biol. Chem. **277**(33): 29999-30009.

http://www.jbc.org/cgi/content/abstract/277/33/29999

Secretory leukocyte protease inhibitor (SLPI) inhibits chymotrypsin, trypsin, elastase, and cathepsin G. This protein also exhibits proliferative effects, although little is known about the molecular mechanisms underlying this activity. We have generated SLPI-ablated epithelial sublines by stably transfecting the Ishikawa human endometrial cell line with an antisense human SLPI RNA expression vector. We demonstrate a positive correlation between cellular SLPI production and proliferation. We further show that Ishikawa sublines expressing low to undetectable SLPI have correspondingly increased and decreased expression, respectively, of transforming growth factor-[beta]1 and cyclin D1 genes, relative to parental cells. SLPI selectively increased cyclin D1 gene expression, with the effect occurring in part at the level of promoter activity. Cellular SLPI levels negatively influenced the anti-proliferative and pro-apoptotic insulin-like growth factor-binding protein-3 expression. We also identified lysyl oxidase, a phenotypic inhibitor of the ras oncogenic pathway and a tumor suppressor, as SLPI-repressed gene, whose expression is up-regulated by transforming growth factor-[beta]1. Our results suggest that SLPI acts at the node(s) of at least three major interacting growth inhibitory pathways. Because expression of SLPI is generally high in epithelial cells exhibiting abnormal proliferation such as in carcinomas, SLPI may define a novel pathway by which cellular growth is modulated.

Dave, V., T. Childs, et al. (2004). "Nuclear Factor of Activated T Cells Regulates Transcription of the Surfactant Protein D Gene (Sftpd) via Direct Interaction with Thyroid Transcription Factor-1 in Lung Epithelial Cells." J. Biol. Chem. **279**(33): 34578-34588.

http://www.jbc.org/cgi/content/abstract/279/33/34578

Surfactant protein D (SP-D) plays critical roles in host defense, surfactant homeostasis, and pulmonary immunomodulation. Here, we identify a role of nuclear factor of activated T cells (NFATs) in regulation of murine SP-D gene (Sftpd) transcription. An NFAT-dependent enhancer modulated by NFATs or calcineurin and sensitive to cyclosporin was identified in the Sftpd promoter. Ionomycin and phorbol 12-myristate 13-acetate further increased the activity of this enhancer, whereas VIVIT, a potent NFAT inhibitor peptide, selectively interfered with the calcineurin-NFAT interaction and abolished enhancer function. Gel supershift and DNase I protection assays identified DNA elements that bind NFAT in the Sftpd promoter. Calcineurin and

NFATc3 proteins were detected in the embryonic and adult mouse lung epithelium, and the mRNA expression profiles of the NFATs were similar in immortalized mouse lung epithelial cells and alveolar epithelial type II cells. NFATc3 and TTF-1 activated the Sftpd promoter, synergized transcription, co-immunoprecipitated from mouse lung epithelial cells, and physically interacted in vitro. Components of the calcineurin/NFAT pathway were identified in respiratory epithelial cells of the lung that potentially augment rapid assembly of a multiprotein transcription complex on Sftpd promoter inducing SP-D expression.

Dettwiler, S., C. Aringhieri, et al. (2004). "Distinct Sequence Motifs within the 68-kDa Subunit of Cleavage Factor Im Mediate RNA Binding, Protein-Protein Interactions, and Subcellular Localization." J. Biol. Chem. **279**(34): 35788-35797.

http://www.jbc.org/cgi/content/abstract/279/34/35788

Cleavage factor Im (CF Im) is required for the first step in pre-mRNA 3'-end processing and can be reconstituted in vitro from its heterologously expressed 25- and 68-kDa subunits. The binding of CF Im to the pre-mRNA is one of the earliest steps in the assembly of the cleavage and polyadenylation machinery and facilitates the recruitment of other processing factors. We identified regions in the subunits of CF Im involved in RNA binding, protein-protein interactions, and subcellular localization. CF Im68 has a modular domain organization consisting of an N-terminal RNA recognition motif and a C-terminal alternating charge domain. However, the RNA recognition motif of CF Im68 on its own is not sufficient to bind RNA but is necessary for association with the 25-kDa subunit. RNA binding appears to require a CF Im68/25 heterodimer. Whereas multiple protein interactions with other 3'-end-processing factors are detected with CF Im25, CF Im68 interacts with SRp20, 9G8, and hTra2{beta}, members of the SR family of splicing factors, via its C-terminal alternating charge domain. This domain is also required for targeting CF Im68 to the nucleus. However, CF Im68 does not concentrate in splicing speckles but in foci that partially colocalize with paraspeckles, a subnuclear component in which other proteins involved in transcriptional control and RNA processing have been found.

Lu, S., Y. Yao, et al. (2002). "Overexpression of Apolipoprotein A-IV Enhances Lipid Transport in Newborn Swine Intestinal Epithelial Cells." J. Biol. Chem. **277**(35): 31929-31937.

http://www.jbc.org/cgi/content/abstract/277/35/31929

Apolipoprotein A-IV (apoA-IV) has myriad functions, including roles as a post-prandial satiety factor and lipid antioxidant. ApoA-IV is expressed in mammalian small intestine and is up-regulated in response to lipid absorption. In newborn swine jejunum, a high fat diet acutely induces a 7-fold increase in apoA-IV expression. To determine whether apoA-IV plays a role in the transport of absorbed lipid, swine apoA-IV was overexpressed in a newborn swine enterocyte cell line, IPEC-1, followed by analysis of the expression of genes related to lipoprotein assembly and lipid transport, as well as quantitation of lipid synthesis and secretion. A full-length swine apoA-IV cDNA was cloned, sequenced, and inserted into a Vp and Rep gene-deficient adeno-associated viral vector, containing the cytomegalovirus immediate early promoter/enhancer and neomycin resistance gene, and was used to transfect IPEC-1 cells. Control cells were transfected with the same vector minus the apoA-IV insert. Using neomycin selection, apoA-IV-overexpressing (+AIV) and control ([-]AIV) clones were isolated for further study. Both undifferentiated ([-]D) and differentiated (+D) +AIV cells expressed 40- to 50-fold higher levels of apoA-IV mRNA and both intracellular and secreted apoA-IV protein compared with [-]AIV cells. Expression of other genes was not affected by apoA-IV overexpression in a manner that would contribute to enhanced lipid secretion. +D +AIV cells secreted 4.9-fold more labeled triacylglycerol (TG), 4.6-fold more labeled cholesteryl ester (CE), and 2-fold more labeled

phospholipid (PL) as lipoproteins, mostly in the chylomicron/very low density lipoprotein (VLDL) density range. ApoA-IV overexpression in IPEC-1 cells enhances basolateral TG, CE, and PL secretion in chylomicron/VLDL particles. This enhancement is not associated with up-regulation of other genes involved in lipid transport. ApoA-IV may play a role in facilitating enterocyte lipid transport, particularly in the neonate receiving a diet of high fat breast milk.

Wang, Y. L., K. A. Frauwirth, et al. (2002). "Thiazolidinedione Activation of Peroxisome Proliferator-activated Receptor gamma Can Enhance Mitochondrial Potential and Promote Cell Survival." J. Biol. Chem. **277**(35): 31781-31788.

http://www.jbc.org/cgi/content/abstract/277/35/31781

Thiazolidinediones (TZDs) are widely used for treatment of type 2 diabetes mellitus. Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]) is the molecular target of TZDs and is believed to mediate the apoptotic effects of this class of drugs in a variety of cell types, including B and T lymphocytes. The finding that TZDs induce lymphocyte death has raised concerns regarding whether TZDs might further impair immune functions in diabetics. To address this issue, we investigated the roles of PPAR[gamma] and TZDs in lymphocyte survival. PPAR[gamma] was up-regulated upon T cell activation. As previously reported, PPAR[gamma] agonists induced T cell death in a dose-dependent manner. However, the concentrations of TZD needed to cause T cell death were above those needed to induce PPAR[gamma]-dependent transcription. Surprisingly, at concentrations that induce optimal transcriptional activation, TZD activation of PPAR[gamma] protected cells from apoptosis following growth factor withdrawal. The survival-enhancing effects depended on both the presence and activation of PPAR[gamma]. Measurements of mitochondrial potential revealed that PPAR[gamma] activation enhanced the ability of cells to maintain their mitochondrial potential. These data indicate that activation of PPAR[gamma] with TZDs can promote cell survival and suggest that PPAR[gamma] activation may potentially augment the immune responses of diabetic patients.

Lau, P., S. J. Nixon, et al. (2004). "ROR{alpha} Regulates the Expression of Genes Involved in Lipid Homeostasis in Skeletal Muscle Cells: CAVEOLIN-3 AND CPT-1 ARE DIRECT TARGETS OF ROR." J. Biol. Chem. **279**(35): 36828-36840.

http://www.jbc.org/cgi/content/abstract/279/35/36828

The staggerer mice carry a deletion in the ROR{alpha} gene and have a prolonged humoral response, overproduce inflammatory cytokines, and are immunodeficient. Furthermore, the staggerer mice display lowered plasma apoA-I/-II, decreased plasma high density lipoprotein cholesterol and triglycerides, and develop hypo-{alpha}-lipoproteinemia and atherosclerosis. However, relatively little is known about ROR{alpha} in the context of target tissues, target genes, and lipid homeostasis. For example, ROR{alpha} is abundantly expressed in skeletal muscle, a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure. This lean tissue is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. In particular, the role of ROR{alpha} in skeletal muscle metabolism has not been investigated, and the contribution of skeletal muscle to the ROR-/- phenotype has not been resolved. We utilize ectopic dominant negative ROR{alpha} expression in skeletal muscle cells to understand the regulatory role of RORs in this major mass peripheral tissue. Exogenous dominant negative ROR{alpha} expression in skeletal muscle cells represses the endogenous levels of ROR{alpha} and -{gamma} mRNAs and ROR-dependent gene expression. Moreover, we observed attenuated expression of many genes involved in lipid homeostasis. Furthermore, we show that the muscle carnitine palmitoyltransferase-1 and caveolin-3 promoters are directly

regulated by ROR and coactivated by p300 and PGC-1. This study implicates RORs in the control of lipid homeostasis in skeletal muscle. In conclusion, we speculate that ROR agonists would increase fatty acid catabolism in muscle and suggest selective activators of ROR may have therapeutic utility in the treatment of obesity and atherosclerosis.

Schwarzer, C., T. E. Machen, et al. (2004). "NADPH Oxidase-dependent Acid Production in Airway Epithelial Cells." J. Biol. Chem. **279**(35): 36454-36461.

http://www.jbc.org/cgi/content/abstract/279/35/36454

The purpose of this study was to determine the role of NADPH oxidase in H+ secretion by airway epithelia. In whole cell patch clamp recordings primary human tracheal epithelial cells (hTE) and the human serous gland cell line Calu-3 expressed a functionally similar zincblockable plasma membrane H+ conductance. However, the rate of H+ secretion of confluent epithelial monolayers measured in Ussing chambers was 9-fold larger in hTE compared with Calu-3. In hTE H+ secretion was blocked by mucosal ZnCl2 and the NADPH oxidase blockers acetovanillone and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), whereas these same blockers had no effect in Calu-3. We determined levels of transcripts for the NADPH oxidase transmembrane isoforms (Nox1 through -5, Duox1 and -2, and p22phox) and found Duox1, -2, and p22phox to be highly expressed in hTE, as well as the intracellular subunits p40phox, p47phox, and p67phox. In contrast, Calu-3 lacked transcripts for Duox1, p40phox, and p47phox. Anti-Duox antibody staining resulted in prominent apical staining in hTE but no significant staining in Calu-3. When treated with amiloride to block the Na+/H+ exchanger, intracellular pH in hTE acidified at significantly higher rates than in Calu-3, and treatment with AEBSF blocked acidification. These data suggest a role for an apically located Duox-based NADPH oxidase during intracellular H+ production and H+ secretion, but not in H+ conduction.

Hidaka, K., J. J. Caffrey, et al. (2002). "An Adjacent Pair of Human NUDT Genes on Chromosome X Are Preferentially Expressed in Testis and Encode Two New Isoforms of Diphosphoinositol Polyphosphate Phosphohydrolase." J. Biol. Chem. **277**(36): 32730-32738.

http://www.jbc.org/cgi/content/abstract/277/36/32730

Combinatorial expression of the various isoforms of diphosphoinositol synthases and phosphohydrolases determines the rates of phosphorylation/dephosphorylation cycles that have been functionally linked to vesicle trafficking, stress responses, DNA repair, and apoptosis. We now describe two new 19-kDa diphosphoinositol polyphosphate phosphohydrolases (DIPPs), named types 3[alpha] and 3[beta], which possess the canonical Nudix-type catalytic motif flanked on either side by short Gly-rich sequences. The two enzymes differ only in that Pro-89 in the [alpha] form is replaced by Arg-89 in the [beta] form, making the latter ~2-fold more active in vitro. Another Nudix substrate, diadenosine hexaphosphate, was hydrolyzed less efficiently (kcat/Km = 0.2 x 105 M[-]1 s[-]1) compared with diphosphoinositol polyphosphates (kcat/Km = 2-40 x 105 M[-]1 s[-]1). Catalytic activity in vivo was established by individual overexpression of the human (h) DIPP3 isoforms in HEK293 cells, which reduced cellular levels of diphosphoinositol polyphosphates by 40-50%. The hDIPP3 mRNA is preferentially expressed in testis, accompanied by relatively weak expression in the brain, contrasting with hDIPP1 and hDIPP2 which are widely expressed. The hDIPP3 genes (NUDT10 encodes hDIPP3[alpha]; NUDT11 encodes hDIPP3[beta]) are only 152 kbp apart at p11.22 on chromosome X and probably arose by duplication. Transcription of both genes is inactivated on one of the X chromosomes of human females to maintain appropriate gene dosage. The hDIPP3 pair add tissue-specific diversity to the molecular mechanisms regulating diphosphoinositol polyphosphate turnover.

Lee, H.-W., D.-H. Ahn, et al. (2002). "Phorbol 12-Myristate 13-Acetate Up-regulates the Transcription of MUC2 Intestinal Mucin via Ras, ERK, and NF-kappa B." J. Biol. Chem. **277**(36): 32624-32631.

http://www.jbc.org/cgi/content/abstract/277/36/32624

MUC2 is a secretory mucin normally expressed by goblet cells of the intestinal epithelium. It is overexpressed in mucinous type colorectal cancers but down-regulated in colorectal adenocarcinoma. Phorbol 12-myristate 13-acetate (PMA) treatment of colon cancer cell lines increases MUC2 expression, so we have undertaken a detailed analysis of the effects of PMA on the promoter activity of the 5'-flanking region of the MUC2 gene using stably and transiently transfected promoter reporter vectors. Protein kinase C inhibitors (bisindolylmaleimide, calphostin C) and inhibitors of mitogen-activated protein/extracellular signal regulated kinase kinase (MEK) (PD98059 and U0126) suppressed up-regulation of MUC2. Src tyrosine kinase inhibitor PP2, a protein kinase A inhibitor (KT5720), and a p38 inhibitor (SB 203580) did not affect transcription. Western blotting and reverse transcription-PCR analysis confirmed these results. In addition, co-transfections with mutants of Ras, Raf, and MEK showed that the induction of MUC2 promoter activity by PMA required these three signaling proteins. Our results demonstrate that PMA activates protein kinase C, stimulating MAP kinase through a Ras- and Raf-dependent mechanism. An important role for nuclear factor [kappa]B (NF-[kappa]B) was also demonstrated using the inhibitor caffeic acid phenethyl ester and electrophoretic mobility shift assays. Such identification of pathways involved in MUC2 up-regulation by PMA in the HM3 colon cancer cell line may serve as a model for the effects of cytokines and growth factors, which regulate MUC2 expression during the progression of colorectal cancer.

Arai, H., T. Furuya, et al. (2004). "Neurotoxic Effects of Lipopolysaccharide on Nigral Dopaminergic Neurons Are Mediated by Microglial Activation, Interleukin-1{beta}, and Expression of Caspase-11 in Mice." J. Biol. Chem. **279**(49): 51647-51653.

http://www.jbc.org/cgi/content/abstract/279/49/51647

The endotoxin lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, selectively induces degeneration of substantia nigral (SN) dopaminergic neurons via activation of microglial cells in rats and mice. Caspase-11 plays a crucial role in LPS-induced septic shock in mice. We examined the mechanism of LPS neurotoxicity on SN dopaminergic neurons in C57BL/6 mice and caspase-11 knockout mice. Mice were stereotaxically injected with LPS into the SN on one side and vehicle into the SN of the other side. Immunohistochemistry, Western blotting analysis, enzyme-linked immunosorbent assay, and reverse transcriptase-PCR were performed to evaluate damage of SN dopaminergic neurons and activation of microglial cells. Intranigral injection of LPS at 1 or 3 {micro}g/{micro}l/site decreased tyrosine hydroxylase-positive neurons and increased microglial cells in the SN compared with the contralateral side injected with vehicle at days 7 and 14 post-injection in C57BL/6 mice. Intranigral injection of LPS at 3 {micro}g/{micro}l/site induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection, and the expression of caspase-11-positive cells in the SN at 8 and 12 h post-injection. Moreover, LPS at 3 {micro}g/{micro}l/site increased interleukin-1{beta} content in the ventral midbrain at 12 and 24 h post-injection. LPS failed to elicit these responses in caspase-11 knockout mice. Our results indicate that the neurotoxic effects of LPS on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1{beta}, and caspase-11 expression in mice.

Deroo, T., T. Denayer, et al. (2004). "Global Inhibition of Lef1/Tcf-dependent Wnt Signaling at Its Nuclear End Point Abrogates Development in Transgenic Xenopus Embryos." J. Biol. Chem. **279**(49): 50670-50675.

http://www.jbc.org/cgi/content/abstract/279/49/50670

Analysis of canonical Wnt signaling during vertebrate development by means of knock-out or transgenic approaches is often hampered by functional redundancy as well as pathway bifurcations downstream of the manipulated components. We report the design of an optimized chimera capable of blocking transcriptional activation of Lef1/Tcf-{beta}-catenin target genes, thus enabling intervention with the canonical Wnt pathway at its nuclear end point. This construct was made hormone-inducible, both functionally and transcriptionally, and was transgenically integrated in Xenopus embryos. Down-regulation of target genes was clearly observed upon treatment of these embryos with dexamethasone. In addition, exposure of variously aged transgenic embryos to dexamethasone caused complex phenotypes with many new but also several recognizable features stemming from inhibition of canonical Wnt signaling. At least in some tissues, a significant reduction in cell proliferation and an increase in programmed cell death appeared to underlie these phenotypes. Our inducible transgenic system can serve a broad range of experimental settings designed to unveil new functional aspects of Lef1/Tcf-{beta}-catenin signaling during vertebrate embryogenesis.

Allaman-Pillet, N., J. Storling, et al. (2003). "Calcium- and Proteasome-dependent Degradation of the JNK Scaffold Protein Islet-brain 1." J. Biol. Chem. **278**(49): 48720-48726.

http://www.jbc.org/cgi/content/abstract/278/49/48720

In models of type 1 diabetes, cytokines induce pancreatic {beta}-cell death by apoptosis. This process seems to be facilitated by a reduction in the amount of the islet-brain 1/JNK interacting protein 1 (IB1/JIP1), a JNK-scaffold with an anti-apoptotic effect. A point mutation S59N at the N terminus of the scaffold, which segregates in diabetic patients, has the functional consequence of sensitizing cells to apoptotic stimuli. Neither the mechanisms leading to IB1/JIP1 down-regulation by cytokines nor the mechanisms leading to the decreased capacity of the S59N mutation to protect cells from apoptosis are understood. Here, we show that IB1/JIP1 stability is modulated by intracellular calcium. The effect of calcium depends upon JNK activation, which primes the scaffold for ubiquitination-mediated degradation via the proteasome machinery. Furthermore, we observe that the S59N mutation decreases IB1/JIP1 stability by sensitizing IB1/JIP1 to calcium- and proteasome-dependent degradation. These data indicate that calcium influx initiated by cytokines mediates ubiquitination and degradation of IB1/JIP1 and may, therefore, provide a link between calcium influx and JNK-mediated apoptosis in pancreatic {beta}-cells.

Reglier-Poupet, H., C. Frehel, et al. (2003). "Maturation of Lipoproteins by Type II Signal Peptidase Is Required for Phagosomal Escape of Listeria monocytogenes." J. Biol. Chem. **278**(49): 49469-49477.

http://www.jbc.org/cgi/content/abstract/278/49/49469

Lipoproteins of Gram-positive bacteria are involed in a broad range of functions such as substrate binding and transport, antibiotic resistance, cell signaling, or protein export and folding. Lipoproteins are also known to initiate both innate and adaptative immune responses. However, their role in the pathogenicity of intracellular microorganisms is yet poorly understood. In Listeria monocytogenes, a Gram-positive facultative intracellular human pathogen, surface proteins have

important roles in the interactions of the microorganism with the host cells. Among the putative surface proteins of L. monocytogenes, lipoproteins constitute the largest family. Here, we addressed the role of the signal peptidase (SPase II), responsible for the maturation of lipoproteins in listerial pathogenesis. We identified a gene, lsp, encoding a SPase II in the genome of L. monocytogenes and constructed a {Delta}lsp chromosomal deletion mutant. The mutant strain fails to process several lipoproteins demonstrating that lsp encodes a genuine SPase II. This defect is accompanied by a reduced efficiency of phagosomal escape during infection of eucaryotic cells, and leads to an attenuated virulence. We show that lsp gene expression is strongly induced when bacteria are still entrapped inside phagosomes of infected macrophages. The data presented establish, thus, that maturation of lipoproteins is critical for efficient phagosomal escape of L. monocytogenes, a process temporally controlled by the regulation of Lsp production in infected cells.

Kang, Z., A. Pirskanen, et al. (2002). "Involvement of Proteasome in the Dynamic Assembly of the Androgen Receptor Transcription Complex." J. Biol. Chem. **277**(50): 48366-48371.

http://www.jbc.org/cgi/content/abstract/277/50/48366

We have used the chromatin immunoprecipitation technique to analyze the formation of the androgen receptor (AR) transcription complex onto prostate-specific antigen (PSA) and kallikrein 2 promoters in LNCaP cells. Our results show that loading of holo-AR and recruitment of RNA polymerase II to the promoters occur transiently. The cyclic nature of AR transcription complex assembly is also illustrated by transient association of coactivators GRIP1 and CREB-binding protein and acetylated histone H3 with the PSA promoter. Treatment of cells with the pure antiandrogen bicalutamide also elicits occupancy of the promoter by AR. In contrast to the agonist-liganded AR, bicalutamide-bound receptor is not capable of recruiting polymerase II, GRIP1, or CREB-binding protein, indicating that the conformation of AR bound to anti-androgen is not competent to assemble transcription complexes. Proteasome is involved in the regulation of AR-dependent transcription, as a proteasome inhibitor, MG-132, prevents the release of the receptor from the PSA promoter, and it also blocks the androgen-induced PSA mRNA accumulation. Furthermore, occupancy of the PSA promoter by the 19 S proteasome subcomplex parallels that by AR. Collectively, formation of the AR transcription complex, encompassing AR, polymerase II, and coactivators, on a regulated promoter is a cyclic process involving proteasome function.

Paulussen, A., A. Raes, et al. (2002). "A Novel Mutation (T65P) in the PAS Domain of the Human Potassium Channel HERG Results in the Long QT Syndrome by Trafficking Deficiency." J. Biol. Chem. **277**(50): 48610-48616.

http://www.jbc.org/cgi/content/abstract/277/50/48610

The congenital long QT syndrome is a cardiac disease characterized by an increased susceptibility to ventricular arrhythmias. The clinical hallmark is a prolongation of the QT interval, which reflects a delay in repolarization caused by mutations in cardiac ion channel genes. Mutations in the HERG (human ether-a-go-go-related gene KCNH2 can cause a reduction in IKr, one of the currents responsible for cardiac repolarization. We describe the identification and characterization of a novel missense mutation T65P in the PAS (Per-Arnt-Sim) domain of HERG, resulting in defective trafficking of the protein to the cell membrane. Defective folding of the mutant protein could be restored by decreased cell incubation temperature and pharmacologically by cisapride and E-4031. When trafficking was restored by growing cells at 27 {degrees}C, the kinetics of the mutated channel resembled that of wild-type channels although the rate of activation, deactivation, and recovery from inactivation were accelerated. No positive evidence for

the formation of heterotetramers was obtained by co-expression of wild-type with mutant subunits at 37 {degrees}C. As a consequence the clinical symptoms may be explained rather by haploinsufficiency than by dominant negative effects. This study is the first to relate a PAS domain mutation in HERG to a trafficking deficiency at body temperature, apart from effects on channel deactivation.

Jackers, P., G. Szalai, et al. (2004). "Ets-dependent Regulation of Target Gene Expression during Megakaryopoiesis." J. Biol. Chem. **279**(50): 52183-52190.

http://www.jbc.org/cgi/content/abstract/279/50/52183

Megakaryopoiesis is the process by which hematopoietic stem cells in the bone marrow differentiate into mature megakaryocytes. The expression of megakaryocytic genes during megakaryopoiesis is controlled by specific transcription factors. Fli-1 and GATA-1 transcription factors are required for development of megakaryocytes and promoter analysis has defined in vitro functional binding sites for these factors in several megakaryocytic genes, including GPIIb, GPIX, and C-MPL. Herein, we utilize chromatin immunoprecipitation to examine the presence of Ets-1, Fli-1, and GATA-1 on these promoters in vivo. Fli-1 and Ets-1 occupy the promoters of GPIIb, GPIX, and C-MPL genes in both Meg-01 and CMK11-5 cells. Whereas GPIIb is expressed in both Meg-01 and CMK11-5 cells, GPIX and C-MPL are only expressed in the more differentiated CMK11-5 cells. Thus, in vivo occupancy by an Ets factor is not sufficient to promote transcription of some megakaryocytic genes. GATA-1 and Fli-1 are both expressed in CMK11-5 cells and co-occupy the GPIX and C-MPL promoters. Transcription of all three megakaryocytic genes is correlated with the presence of acetylated histone H3 and phosphorylated RNA polymerase II on their promoters. We also show that exogenous expression of GATA-1 in Meg-01 cells leads to the expression of endogenous c-mpl and gpIX mRNA. Whereas GPIIb, GPIX, and C-MPL are direct target genes for Fli-1, both Fli-1 and GATA-1 are required for formation of an active transcriptional complex on the C-MPL and GPIX promoters in vivo. In contrast, GPIIb expression appears to be independent of GATA-1 in Meg-01 cells.

Li, Q., A. K.-K. Ching, et al. (2004). "A Death Receptor-associated Anti-apoptotic Protein, BRE, Inhibits Mitochondrial Apoptotic Pathway." J. Biol. Chem. **279**(50): 52106-52116.

http://www.jbc.org/cgi/content/abstract/279/50/52106

BRE, brain and reproductive organ-expressed protein, was found previously to bind the intracellular juxtamembrane domain of a ubiquitous death receptor, tumor necrosis factor receptor 1 (TNF-R1), and to down-regulate TNF-{alpha}-induced activation of NF-{kappa}B. Here we show that BRE also binds to another death receptor, Fas, and upon overexpression conferred resistance to apoptosis induced by TNF-{alpha}, anti-Fas agonist antibody, cycloheximide, and a variety of stress-related stimuli. However, down-regulation of the endogenous BRE by small interfering RNA increased apoptosis to TNF-{alpha}, but nottoetoposide, indicating that the physiological antiapoptotic role of this protein is specific to death receptor-mediated apoptosis. We further demonstrate that BRE mediates antiapoptosis by inhibiting the mitochondrial apoptotic machinery but without translocation to the mitochondria or nucleus or down-regulation of the cellular level of truncated Bid. Dissociation of BRE rapidly from TNF-R1, but not from Fas, upon receptor ligation suggests that this protein interacts with the death inducing signaling complex during apoptotic induction. Increased association of BREwith phosphorylated, sumoylated, and ubiquitinated proteins after death receptor stimulation was also detected. We conclude that in contrast to the truncated Bid that integrates mitochondrial apoptosis to death receptor-triggered apoptotic cascade, BRE inhibits the integration. We propose that BRE inhibits, by ubiquitination-like activity, components in or proximal to the death-inducing signaling complexes that are

necessary for activation of the mitochondria.

Miyakawa-Naito, A., P. Uhlen, et al. (2003). "Cell Signaling Microdomain with Na,K-ATPase and Inositol 1,4,5-Trisphosphate Receptor Generates Calcium Oscillations." J. Biol. Chem. **278**(50): 50355-50361.

http://www.jbc.org/cgi/content/abstract/278/50/50355

Recent studies indicate novel roles for the ubiquitous ion pump, Na,K-ATPase, in addition to its function as a key regulator of intracellular sodium and potassium concentration. We have previously demonstrated that ouabain, the endogenous ligand of Na,K-ATPase, can trigger intracellular Ca2+ oscillations, a versatile intracellular signal controlling a diverse range of cellular processes. Here we report that Na,K-ATPase and inositol 1,4,5-trisphosphate (InsP3) receptor (InsP3R) form a cell signaling microdomain that, in the presence of ouabain, generates slow Ca2+ oscillations in renal cells. Using fluorescent resonance energy transfer (FRET) measurements, we detected a close spatial proximity between Na,K-ATPase and InsP3R. Ouabain significantly enhanced FRET between Na,K-ATPase and InsP3R. The FRET effect and ouabain-induced Ca2+ oscillations were not observed following disruption of the actin cytoskeleton. Partial truncation of the NH2 terminus of Na,K-ATPase catalytic {alpha}1-subunit abolished Ca2+ oscillations and downstream activation of NF-{kappa}B. Ouabain-induced Ca2+ oscillations occurred in cells expressing an InsP3 sponge and were hence independent of InsP3 generation. Thus, we present a novel principle for a cell signaling microdomain where an ion pump serves as a receptor.

Xiao, J., P. Jethanandani, et al. (2003). "Regulation of {alpha}7 Integrin Expression during Muscle Differentiation." J. Biol. Chem. **278**(50): 49780-49788.

http://www.jbc.org/cgi/content/abstract/278/50/49780

Expression of the laminin-binding {alpha}7 integrin is tightly regulated during myogenic differentiation, reflecting required functions that range from cell motility to formation of stable myotendinous junctions. However, the exact mechanism controlling {alpha}7 expression in a tissue- and differentiation-specific manner is poorly understood. This report provides evidence that {alpha}7 gene expression during muscle differentiation is regulated by the c-Myc transcription factor. In myoblasts, {alpha}7 is expressed at basal levels, but following conversion to myotubes the expression of the integrin is strongly elevated. The increased {alpha}7 mRNA and protein levels following myogenic differentiation are inversely correlated with c-Myc expression. Transfection of myoblasts with the c-Myc transcription factor down-regulated {alpha}7 expression, whereas overexpression of Madmyc, a dominant-negative c-Myc chimera, induced elevated {alpha}7 expression. Functional analysis with site-specific deletions identified a specific double E-box sequence in the upstream promoter region (-2.0 to -2.6 kb) that is responsible for c-Myc-induced suppression of {alpha}7 expression. DNA-protein binding assays and supershift analysis revealed that c-Myc forms a complex with this double E-box sequence. Our results suggest that the interaction of c-Myc with this promoter region is an important regulatory element controlling {alpha}7 integrin expression during muscle development and myotendinous junction formation.

Kumar, S., L. Gupta, et al. (2004). "Inducible Peroxidases Mediate Nitration of Anopheles Midgut Cells Undergoing Apoptosis in Response to Plasmodium Invasion." J. Biol. Chem. **279**(51): 53475-53482.

http://www.jbc.org/cgi/content/abstract/279/51/53475

Plasmodium berghei invasion of Anopheles stephensi midgut cells causes severe damage, induces expression of nitric-oxide synthase, and leads to apoptosis. The present study indicates that invasion results in tyrosine nitration, catalyzed as a two-step reaction in which nitric-oxide synthase induction is followed by increased peroxidase activity. Ookinete invasion induced localized expression of peroxidase enzymes, which catalyzed protein nitration in vitro in the presence of nitrite and H2O2. Histochemical stainings revealed that when a parasite migrates laterally and invades more than one cell, the pattern of induced peroxidase activity is similar to that observed for tyrosine nitration. In Anopheles gambiae, ookinete invasion elicited similar responses; it induced expression of 5 of the 16 peroxidase genes predicted by the genome sequence and decreased mRNA levels of one of them. One of these inducible peroxidases has a C-terminal oxidase domain homologous to the catalytic moiety of phagocyte NADPH oxidase and could provide high local levels of superoxide anion ([IMG]f1.gif" BORDER="0">), that when dismutated would generate the local increase in H2O2 required for nitration. Chemically induced apoptosis of midgut cells also activated expression of four ookinete-induced peroxidase genes, suggesting their involvement in general apoptotic responses. The two-step nitration reaction provides a mechanism to precisely localize and circumscribe the toxic products generated by defense reactions involving nitration. The present study furthers our understanding of the biochemistry of midgut defense reactions to parasite invasion and how these may influence the efficiency of malaria transmission by anopheline mosquitoes.

Inagaki, T., S. Suzuki, et al. (2003). "The Retinoic Acid-responsive Proline-rich Protein Is Identified in Promyeloleukemic HL-60 Cells." J. Biol. Chem. **278**(51): 51685-51692.

http://www.jbc.org/cgi/content/abstract/278/51/51685

To identify new genes that retinoic acid activates, we employed an mRNA differential display technique and screened for genes that are differentially expressed in promyeloleukemic HL-60 cells incubated in the presence of all-trans-retinoic acid (ATRA) compared with the absence of ATRA. We cloned the coding region of a retinoic acid-induced gene from a human thymus library, which was the mRNA encoding the 666-amino acid human homologue of mouse proline-rich protein 76. We have designated it RARP1 (retinoic acid response proline-rich protein 1). Transcription of an [~]2.4-kbp mRNA occurred mainly in organs with immune functions, such as thymus, spleen, and peripheral leukocytes. Cycloheximide blocked the ATRA-induced expression. In megakaryocyte-like human erythroleukemia HEL cells, the amount of RARP1 mRNA was high, but it was low in human T-lymphoblastoid Jurkat cells. A specific antibody against RARP1 recognized a 110-kDa protein, which accumulates after incubation of HL-60 cells with ATRA. In immunohistochemical experiments, strong RARP1 staining was observed in the megakaryocytes of bone marrow and spleen, and heterogeneous stain was seen in thymus. Transcriptional studies showed that RARP1 expression impaired the transactivation through activator protein1 and serum responseelement in all cell lines we checked, whereas it did not affect the transactivation through cAMP-response element in the same cell lines. Further analysis demonstrated that proline-rich regions of RARP1 are the functional regions regulated for suppression of activator protein1 transactivation. These data suggest that ATRA-inducible RARP1 selectively affects signal transduction and may contribute to myeloid and megakaryocytic differentiation.

Mesquita, P., N. Jonckheere, et al. (2003). "Human MUC2 Mucin Gene Is Transcriptionally Regulated by Cdx Homeodomain Proteins in Gastrointestinal Carcinoma Cell Lines." J. Biol. Chem. **278**(51): 51549-51556.

http://www.jbc.org/cgi/content/abstract/278/51/51549

In intestinal metaplasia and 30% of gastric carcinomas, MUC2 intestinal mucin and the intestine-specific transcription factors Cdx-1 and Cdx-2 are aberrantly expressed. The involvement of Cdx-1 and Cdx-2 in the intestinal development and their role in transcription of several intestinal genes support the hypothesis that Cdx-1 and/or Cdx-2 play important roles in the aberrant intestinal differentiation program of intestinal metaplasia and gastric carcinoma. To clarify the mechanisms of transcriptional regulation of the MUC2 mucin gene in gastric cells, pGL3 deletion constructs covering 2.6 kb of the human MUC2 promoter were used in transient transfection assays, enabling us to identify a relevant region for MUC2 transcription in all gastric cell lines. To evaluate the role of Cdx-1 and Cdx-2 in MUC2 transcription we performed co-transfection experiments with expression vectors encoding Cdx-1 and Cdx-2. In two of the four gastric carcinoma cell lines and in all colon carcinoma cell lines we observed transactivation of the MUC2 promoter by Cdx-2. Using gel shift assays we identified two Cdx-2 binding sites at -177/-171 and -191/-187. Only simultaneous mutation of the two sites resulted in inhibition of Cdx-2-mediated transactivation of MUC2 promoter, implying that both Cdx-2 sites are active. Finally, stable expression of Cdx-2 in a gastric cell line initially not expressing Cdx-2, led to induction of MUC2 expression. In conclusion, this work demonstrates that Cdx-2 activates the expression of MUC2 mucin gene in gastric cells, inducing an intestinal transdifferentiation phenotype that parallels what is observed both in intestinal metaplasia and some gastric carcinomas.

Ohshima, T. and K. Shimotohno (2003). "Transforming Growth Factor-{beta}-mediated Signaling via the p38 MAP Kinase Pathway Activates Smad-dependent Transcription through SUMO-1 Modification of Smad4." J. Biol. Chem. **278**(51): 50833-50842.

http://www.jbc.org/cgi/content/abstract/278/51/50833

Post-translational modifications such as ubiquitination, phosphorylation, and acetylation play important roles in the regulation of Smad-mediated functions. Here, we demonstrate that Smad4 is covalently modified by SUMO-1, which was characterized recently as a key modulator of many transcription factors. Sumoylation of Smad4 mainly occurs at lysine 159, located in the linker region, and facilitates Smad-dependent transcriptional activation. Furthermore, we show that the PIAS family proteins, PIAS1 and PIASx{beta}, function as E3 ligase factors for Smad4. Intriguingly, sumoylation of Smad4 was strongly enhanced by TGF-{beta}-induced activation of the p38 MAP kinase pathway but not the Smad pathway. Activation of p38 not only stabilized PIASx{beta} protein but also enhanced PIASx{beta} gene expression, suggesting that PIAS-mediated sumoylation of Smad4 is regulated by the p38 MAP kinase pathway. These findings illustrate a novel regulatory mechanism by which Smad-dependent transcriptional activation cooperatively modulates Smad proteins through receptor-mediated phosphorylation and sumoylation.

Piechotta, K., J. Lu, et al. (2002). "Cation Chloride Cotransporters Interact with the Stress-related Kinases Ste20-related Proline-Alanine-rich Kinase (SPAK) and Oxidative Stress Response 1 (OSR1)." J. Biol. Chem. **277**(52): 50812-50819.

http://www.jbc.org/cgi/content/abstract/277/52/50812

Cells respond to stress stimuli by mounting specific responses. During osmotic and oxidative stress, cation chloride cotransporters, e.g. Na-K-2Cl and K-Cl cotransporters, are activated to maintain fluid/ion homeostasis. Here we report the interaction of the stress-related serine-threonine kinases Ste20-related proline-alanine-rich kinase (SPAK) and oxidative stress response

1 (OSR1) with the cotransporters KCC3, NKCC1, and NKCC2 but not KCC1 and KCC4. The interaction was identified using yeast two-hybrid assays and confirmed via glutathione S-transferase pull-down experiments. Evidence for in vivo interaction was established by co-immunoprecipitation of SPAK from mouse brain with anti-NKCC1 antibody. The interacting region of both kinases comprises the last 100 amino acids of the protein. The SPAK/OSR1 binding motif on the cotransporters consists of nine residues, starting with an (R/K)FX(V/I) sequence followed by five additional residues that are essential for binding but for which no consensus was found. Immunohistochemical analysis of choroid plexus epithelium revealed co-expression of NKCC1 and SPAK on the apical membrane. In contrast, in choroid plexus epithelium from NKCC1 null mice, SPAK immunostaining was found in the cytoplasm. We conclude that several cation chloride co-transporters interact with SPAK and/or OSR1, and we hypothesize that this interaction might play a role during the initiation of the cellular stress response.

Xu, G., L.-x. Pan, et al. (2002). "Regulation of the Farnesoid X Receptor (FXR) by Bile Acid Flux in Rabbits." J. Biol. Chem. **277**(52): 50491-50496.

http://www.jbc.org/cgi/content/abstract/277/52/50491

We investigated the roles of hydrophobic deoxycholic acid (DCA) and hydrophilic ursocholic acid (UCA) in the regulation of the orphan nuclear farnesoid X receptor (FXR) in vivo. Rabbits with bile fistula drainage (removal of the endogenous bile acid pool), rabbits with bile fistula drainage and replacement with either DCA or UCA, and intact rabbits fed 0.5% cholic acid (CA) (enlarged endogenous bile acid pool) were studied. After bile fistula drainage, cholesterol 7[alpha]-hydroxylase (CYP7A1) mRNA and activity levels increased, FXR-mediated transcription was decreased, and FXR mRNA and nuclear protein levels declined. Replacing the enterohepatic bile acid pool with DCA restored FXR mRNA and nuclear protein levels and activated FXR-mediated transcription as evidenced by the increased expression of its target genes, SHP and BSEP, and decreased CYP7A1 mRNA level and activity. Replacing the bile acid pool with UCA also restored FXR mRNA and nuclear protein levels but did not activate FXR-mediated transcription, because the SHP mRNA level and CYP7A1 mRNA level and activity were unchanged. Feeding CA to intact rabbits expanded the bile acid pool enriched with the FXR high affinity ligand, DCA. FXR-mediated transcription became activated as shown by increased SHP and BSEP mRNA levels and decreased CYP7A1 mRNA level and activity but did not change FXR mRNA or nuclear protein levels. Thus, both hydrophobic and hydrophilic bile acids are effective in maintaining FXR mRNA and nuclear protein levels. However, the activating ligand (DCA) in the enterohepatic flux is necessary for FXR-mediated transcriptional regulation, which leads to down-regulation of CYP7A1.

Cabral, W. A., A. Fertala, et al. (2002). "Procollagen with Skipping of alpha 1(I) Exon 41 Has Lower Binding Affinity for alpha 1(I) C-telopeptide, Impaired in Vitro Fibrillogenesis, and Altered Fibril Morphology." J. Biol. Chem. **277**(6): 4215-4222.

http://www.jbc.org/cgi/content/abstract/277/6/4215

Previous in vitro data on type I collagen self-assembly into fibrils suggested that the amino acid 776-796 region of the [alpha]1(I) chain is crucial for fibril formation because it serves as the recognition site for the telopeptide of a docking collagen monomer. We used a natural collagen mutation with a deletion of amino acids 766-801 to confirm the importance of this region for collagen fibril formation. The proband has type III osteogenesis imperfecta and is heterozygous for a COL1A1 IVS 41 A+4 [right-arrow] C substitution. The intronic mutation causes splicing of exon 41, confirmed by sequencing of normal and shorter reverse transcriptase-PCR products. Reverse transcriptase-PCR using RNA from proband dermal fibroblasts and clonal cell lines

showed the mutant cDNA was about 15% of total [alpha]1(I) cDNA. The mutant transcript is translated; structurally abnormal [alpha] chains are demonstrated in the cell layer of proband fibroblasts by SDS-urea-PAGE. The proportion of mutant chains in the secreted procollagen was determined to be 10% by resistance to digestion with MMP-1, since chains lacking exon 41 are missing the vertebral collagenase cleavage site. Secreted proband collagen was used for analysis of kinetics of binding of [alpha]1(I) C-telopeptide using an optical biosensor. Telopeptide had slower association and faster dissociation from proband than from normal collagen. Purified proband pC-collagen was used to study fibril formation. The presence of the mutant molecules decreases the rate of fibril formation. The fibrils formed in the presence of 10-15% mutant molecules have strikingly increased length compared with normal collagen, but are well organized, as demonstrated by D-periodicity. These results suggest that some collagen molecules containing the mutant chain are incorporated into fibrils and that the absence of the telopeptide binding region from even a small portion of the monomers interferes with fibril growth. Both abnormal fibrils and slower remodeling may contribute to the severe phenotype.

Kawaguchi, T., K. Osatomi, et al. (2002). "Mechanism for Fatty Acid "Sparing" Effect on Glucose-induced Transcription. REGULATION OF CARBOHYDRATE-RESPONSIVE ELEMENT-BINDING PROTEIN BY AMP-ACTIVATED PROTEIN KINASE." J. Biol. Chem. **277**(6): 3829-3835.

http://www.jbc.org/cgi/content/abstract/277/6/3829

Carbohydrate-responsive element-binding protein (ChREBP) is a new transcription factor that binds to the carbohydrate-responsive element of the L-type pyruvate kinase gene (L-PK). The aim of this study was to investigate the mechanism by which feeding high fat diets results in decreased activity of ChREBP in the liver (Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., Arnot, D., and Uyeda, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9116-9121). We cloned the rat liver ChREBP gene for use throughout this study. Acetate, octanoate, and palmitate inhibited the glucose-induced activation of L-PK transcription in ChREBP-overexpressed hepatocytes. In these hepatocytes, the cytosolic AMP concentration increased 30-fold and AMP-activated protein kinase activity was activated 2-fold. Similarly to the fatty acids, 5-amino-4-imidazolecarboxamide ribotide, a specific activator of AMP-activated protein kinase (AMPK) also inhibited the L-PK transcription activity in ChREBP-overexpressed hepatocytes. Using as a substrate a truncated ChREBP consisting of the C-terminal region, we demonstrated that phosphorylation by AMPK resulted in inactivation of the DNA binding activity. AMPK specifically phosphorylated Ser568 of ChREBP. A S568A mutant of the ChREBP gene showed tight DNA binding and lost its fatty acid sensitivity, whereas a S568D mutant showed weak DNA binding and inhibited L-PK transcription activity even in the absence of fatty acid. These results strongly suggested that the fatty acid inhibition of glucose-induced L-PK transcription resulted from AMPK phosphorylation of ChREBP at Ser568, which inactivated the DNA binding activity. AMPK was activated by the increased AMP that was generated by the fatty acid activation.

Kim, S., C. Domon-Dell, et al. (2004). "Down-regulation of the Tumor Suppressor PTEN by the Tumor Necrosis Factor-{alpha}/Nuclear Factor-{kappa}B (NF-{kappa}B)-inducing Kinase/NF-{kappa}B Pathway Is Linked to a Default I{kappa}B-{alpha} Autoregulatory Loop." J. Biol. Chem. **279**(6): 4285-4291.

http://www.jbc.org/cgi/content/abstract/279/6/4285

The PTEN (phosphatase and tensin homolog deleted on chromosome ten) tumor suppressor gene affects multiple cellular processes including cell growth, proliferation, and cell migration by antagonizing phosphatidylinositol 3-kinase (PI3K). However, mechanisms by which PTEN

expression is regulated have not been studied extensively. Similar to PTEN, tumor necrosis factor-{alpha} (TNF-{alpha}) affects a wide spectrum of diseases including inflammatory processes and cancer by acting as a mediator of apoptosis, inflammation, and immunity. In this study, we show that treatment of cancer cell lines with TNF-{alpha} decreases PTEN expression. In addition, overexpression of TNF-{alpha} downstream signaling targets, nuclear factor-{kappa}B (NF-{kappa}B)-inducing kinase (NIK) and p65 nuclear factor NF-{kappa}B, lowers PTEN expression, suggesting that TNF-{alpha}-induced down-regulation of PTEN is mediated through a TNF-{alpha}/NIK/NF-{kappa}B pathway. Down-regulation of PTEN by NIK/NF-{kappa}B results in activation of the PI3K/Akt pathway and augmentation of TNF-{alpha}-induced PI3K/Akt stimulation. Importantly, we demonstrate that this effect is associated with a lack of an inhibitor of {kappa}B (I{kappa}B)-{alpha} autoregulatory loop. Moreover, these findings suggest the interaction between PI3K/Akt and NF-{kappa}B via transcriptional regulation of PTEN and offer one possible explanation for increased tumorigenesis in systems in which NF-{kappa}B is chronically activated. In such a tumor system, these findings suggest a positive feedback loop whereby Akt activation of NF-{kappa}B further stimulates Akt via down-regulation of the PI3K inhibitor PTEN.

Jeanson, L. and J.-F. Mouscadet (2002). "Ku Represses the HIV-1 Transcription. IDENTIFICATION OF A PUTATIVE Ku BINDING SITE HOMOLOGOUS TO THE MOUSE MAMMARY TUMOR VIRUS NRE1 SEQUENCE IN THE HIV-1 LONG TERMINAL REPEAT." J. Biol. Chem. **277**(7): 4918-4924.

http://www.jbc.org/cgi/content/abstract/277/7/4918

Ku has been implicated in nuclear processes, including DNA break repair, transcription, V(D)J recombination, and telomere maintenance. Its mode of action involves two distinct mechanisms: one in which a nonspecific binding occurs to DNA ends and a second that involves a specific binding to negative regulatory elements involved in transcription repression. Such elements were identified in mouse mammary tumor virus and human T cell leukemia virus retroviruses. The purpose of this study was to investigate a role for Ku in the regulation of human immunodeficiency virus (HIV)-1 transcription. First, HIV-1 LTR activity was studied in CHO-K1 cells and in CH0-derived xrs-6 cells, which are devoid of Ku80. LTR-driven expression of a reporter gene was significantly increased in xrs-6 cells. This enhancement was suppressed after re-expression of Ku80. Second, transcription of HIV-1 was followed in U1 human cells that were depleted in Ku by using a Ku80 antisense RNA. Ku depletion led to a increase of both HIV-1 mRNA synthesis and viral production compared with the parent cells. These results demonstrate that Ku acts as a transcriptional repressor of HIV-1 expression. Finally, a putative Ku-specific binding site was identified within the negative regulatory region of the HIV-1 long terminal repeat, which may account for this repression of transcription.

Richer, J. K., B. M. Jacobsen, et al. (2002). "Differential Gene Regulation by the Two Progesterone Receptor Isoforms in Human Breast Cancer Cells." J. Biol. Chem. **277**(7): 5209-5218.

http://www.jbc.org/cgi/content/abstract/277/7/5209

The PR-A and PR-B isoforms of progesterone receptors (PR) have different physiological functions, and their ratio varies widely in breast cancers. To determine whether the two PR regulate different genes, we used human breast cancer cell lines engineered to express one or the other isoform. Cells were treated with progesterone in triplicate, time-separated experiments, allowing statistical analyses of microarray gene expression data. Of 94 progesterone-regulated genes, 65 are uniquely regulated by PR-B, 4 uniquely by PR-A, and only 25 by both. Almost half the genes encode proteins that are membrane-bound or involved in membrane-initiated signaling.

We also find an important set of progesterone-regulated genes involved in mammary gland development and/or implicated in breast cancer. This first, large scale study of PR gene regulation has important implications for the measurement of PR in breast cancers and for the many clinical uses of synthetic progestins. It suggests that it is important to distinguish between the two isoforms in breast cancers and that isoform-specific genes can be used to screen for ligands that selectively modulate the activity of PR-A or PR-B. Additionally, use of natural target genes, rather than "consensus" response elements, for transcription studies should improve our understanding of steroid hormone action.

Winn, R. A., L. Marek, et al. (2005). "Restoration of Wnt-7a expression reverses non-small cell lung cancer cell transformation through frizzled-9 mediated growth inhibition and promotion of cellular differentiation." J. Biol. Chem.: M409392200.

http://www.jbc.org/cgi/content/abstract/M409392200v1

The Wnt signaling pathway is critical in normal development and mutation of specific components is frequently observed in carcinomas of diverse origins. The potential involvement of this pathway in lung tumorigenesis, however, has not been established. In this study, analysis of multiple Wnt mRNAs in non-small cell lung cancer (NSCLC) cell lines and primary lung tumors revealed markedly decreased Wnt-7a expression when compared to normal short term bronchial epithelial cell lines and normal uninvolved lung tissue. Wnt-7a transfection in NSCLC cell lines reversed cellular transformation, decreased anchorage independent growth, and induced epithelial differentiation as demonstrated by soft agar and three dimensional cell culture assays in a subset of the NSCLC cell lines. The action of Wnt-7a correlated with the expression of the specific Wnt receptor Fzd-9, and transfection of Fzd-9 into a Wnt-7a-insensitive NSCLC cell line established Wnt-7a sensitivity. Moreover, Wnt-7a was present in Fzd-9 immunoprecipitates, indicating a direct interaction of Wnt-7a and Fzd-9. In NSCLC cells, Wnt-7a and Fzd-9 induced both cadherin and Sprouty 4 expression, and stimulated the JNK pathway, but not b-catenin/TCF-activity. In addition, transfection of gain-of-function JNK strongly inhibited anchorage-independent growth. Thus, this study demonstrates that Wnt-7a and Fzd-9 signaling through activation of the JNK pathway induces cadherin proteins and the receptor tyrosine kinase inhibitor, Sprouty 4, and represents a novel tumor suppressor pathway in lung cancer that is required for maintenance of epithelial differentiation and inhibition of transformed cell growth in a subset of human NSCLC cancers.

Abdollahi, A., D. Pisarcik, et al. (2003). "LOT1 (PLAGL1/ZAC1), the Candidate Tumor Suppressor Gene at Chromosome 6q24-25, Is Epigenetically Regulated in Cancer." J. Biol. Chem. **278**(8): 6041-6049.

http://www.jbc.org/cgi/content/abstract/278/8/6041

LOT1 is a zinc-finger nuclear transcription factor, which possesses anti-proliferative effects and is frequently silenced in ovarian and breast cancer cells. The LOT1 gene is localized at chromosome 6q24-25, a chromosomal region maternally imprinted and linked to growth retardation in several organs and progression of disease states such as transient neonatal diabetes mellitus. Toward understanding the molecular mechanism underlying the loss of LOT1 expression in cancer, we have characterized the genomic structure and analyzed its epigenetic regulation. Genome mapping of LOT1 in comparison with the other splice variants, namely ZAC1 and PLAGL1, revealed that its mRNA (~4.7 kb; GenBankTM accession number U72621) is potentially spliced using six exons spanning at least 70 kb of the human genome. 5'-RACE (rapid amplification of cDNA ends) data indicate the presence of at least two transcription start sites. We found that in vitro methylation of the LOT1 promoter causes a significant loss in its ability to drive

luciferase transcription. To determine the nature of in vivo methylation of LOT1, we used bisulfite-sequencing strategies on genomic DNA. We show that in the ovarian and breast cancer cell lines and/or tumors the 5'-CpG island of LOT1 is a differentially methylated region. In these cell lines the ratio of methylated to unmethylated CpG dinucleotides in this region ranged from 31 to 99% and the ovarian tumors have relatively higher cytosine methylation than normal tissues. Furthermore, we show that trichostatin A, a specific inhibitor of histone deacetylase, relieves transcriptional silencing of LOT1 mRNA in malignantly transformed cells. It appears that, unlike DNA methylation, histone deacetylation does not target the promoter, and rather it is indirect and may be elicited by a mechanism upstream of the LOT1 regulatory pathway. Taken together, the data suggest that expression of LOT1 is under the control of two epigenetic modifications and that, in the absence of loss of heterozygosity, the biallelic (two-hit) or maximal silencing of LOT1 requires both processes.

Boraston, A. B., E. Kwan, et al. (2003). "Recognition and Hydrolysis of Noncrystalline Cellulose." J. Biol. Chem. **278**(8): 6120-6127.

http://www.jbc.org/cgi/content/abstract/278/8/6120

Cellulase Cel5A from alkalophilic Bacillus sp. 1139 contains a family 17 carbohydrate-binding module (BspCBM17) and a family 28 CBM (BspCBM28) in tandem. The two modules have significantly similar amino acid sequences, but amino acid residues essential for binding are not conserved. BspCBM28 was obtained as a discrete polypeptide by engineering the cel5A gene. BspCBM17 could not be obtained as a discrete polypeptide, so a family 17 CBM from endoglucanase Cel5A of Clostridium cellulovorans, CcCBM17, was used to compare the binding characteristics of the two families of CBM. Both CcCBM17 and BspCBM28 recognized two classes of binding sites on amorphous cellulose: a high affinity site (Ka ~1 x 106 M[-]1) and a low affinity site (Ka ~2 x 104 M[-]1). They did not compete for binding to the high affinity sites, suggesting that they bound at different sites on the cellulose. A polypeptide, BspCBM17/CBM28, comprising the tandem CBMs from Cel5A, bound to amorphous cellulose with a significantly higher affinity than the sum of the affinities of CcCBM17 and BspCBM28, indicating cooperativity between the linked CBMs. Cel5A mutants were constructed that were defective in one or both of the CBMs. The mutants differed from the wild-type enzyme in the amounts and sizes of the soluble products produced from amorphous cellulose. This suggests that either the CBMs can modify the action of the catalytic module of Cel5A or that they target the enzyme to areas of the cellulose that differ in susceptibility to hydrolysis.

Gencic, S. and D. A. Grahame (2003). "Nickel in Subunit beta of the Acetyl-CoA Decarbonylase/Synthase Multienzyme Complex in Methanogens. CATALYTIC PROPERTIES AND EVIDENCE FOR A BINUCLEAR Ni-Ni SITE." J. Biol. Chem. **278**(8): 6101-6110.

http://www.jbc.org/cgi/content/abstract/278/8/6101

The acetyl-CoA decarbonylase/synthase (ACDS) complex catalyzes the central reaction of acetyl C-C bond cleavage in methanogens growing on acetate and is also responsible for synthesis of acetyl units during growth on C-1 substrates. The ACDS [beta] subunit contains nickel and an Fe/S center and reacts with acetyl-CoA forming an acetyl-enzyme intermediate presumably directly involved in acetyl C-C bond activation. To investigate the role of nickel in this process two forms of the Methanosarcina thermophila [beta] subunit were overexpressed in anaerobically grown Escherichia coli. Both contained an Fe/S center but lacked nickel and were inactive in acetyl-enzyme formation in redox-dependent acetyltransferase assays. However, high activity developed during incubation with NiCl2. The native and nickel-reconstituted proteins both contained iron and nickel in a 2:1 ratio, with insignificant levels of other metals, including copper.

Binding of nickel elicited marked changes in the UV-visible spectrum, with intense charge transfer bands indicating multiple thiolate ligation to nickel. The kinetics of nickel incorporation matched the time course for enzyme activation. Other divalent metal ions could not substitute for nickel in yielding catalytic activity. Acetyl-CoA was formed in reactions with CoA, CO, and methylcobalamin, directly demonstrating C-C bond activation by the [beta] subunit in the absence of other ACDS subunits. Nickel was indispensable in this process too and was needed to form a characteristic EPR-detectable enzyme-carbonyl adduct in reactions with CO. In contrast to enzyme activation, EPR signal formation did not require addition of reducing agent, indicating indirect catalytic involvement of the paramagnetic species. Site-directed mutagenesis indicated that Cys-278 and Cys-280 coordinate nickel, with Cys-189 essential for Fe/S cluster formation. The results are consistent with an Ni2[Fe4S4] arrangement at the active site. A mechanism for C-C bond activation is proposed that includes a specific role for the Fe4S4 center and accounts for the absolute requirement for nickel.

Nair, R. and C. Shaha (2003). "Diethylstilbestrol Induces Rat Spermatogenic Cell Apoptosis in Vivo through Increased Expression of Spermatogenic Cell Fas/FasL System." J. Biol. Chem. **278**(8): 6470-6481.

http://www.jbc.org/cgi/content/abstract/278/8/6470

The significant role that estrogens play in spermatogenesis has opened up an exciting area of research in male reproductive biology. The realization that estrogens are essential for proper maintenance of spermatogenesis, as well as growing evidence pointing to the deleterious effects of estrogen-like chemicals on male reproductive health, has made it imperative to dissect the role estrogens play in the male. Using a model estrogen, diethylstilbestrol (DES), to induce spermatogenic cell apoptosis in vivo in the male rat, we provide a new insight into an estrogen-dependent regulation of the Fas-FasL system specifically in spermatogenic cells. We show a distinct increase in Fas-FasL expression in spermatogenic cells upon exposure to diethylstilbestrol. This increase is confined to the spermatid population, which correlates with increased apoptosis seen in the haploid cells. Testosterone supplementation is able to prevent DES-induced Fas-FasL up-regulation and apoptosis in the spermatogenic cells. DES-induced germ cell apoptosis does not occur in Fas-deficient lpr mice. One other important finding is that spermatogenic cells are type II cells, as the increase in Fas-FasL expression in the spermatogenic cells is followed by the cleavage of caspase-8 to its active form, following which Bax translocates to the mitochondria and precipitates the release of cytochrome c that is accompanied by a drop in mitochondrial potential. Subsequent to this, activation of caspase-9 occurs that in turn activates caspase-3 leading to the cleavage of poly(ADP-ribose) polymerase. Taken together, the data indicate that estrogen-like chemicals can precipitate apoptotic death in spermatogenic cells by increasing the expression of spermatogenic cell Fas-FasL, thus initiating apoptosis in the same lineage of cells through the activation of the apoptotic pathway chosen by type II cells.

Li, B., N. M. Nowak, et al. (2005). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF SECOND-SITE MUTATIONS THAT RESTORE FUNCTION TO A COUPLING-DEFICIENT MUTANT M3 RECEPTOR." J. Biol. Chem. **280**(7): 5664-5675.

http://www.jbc.org/cgi/content/abstract/280/7/5664

The M3 muscarinic receptor is a prototypical member of the class A family of G protein-coupled receptors (GPCRs). To gain insight into the structural mechanisms governing agonist-mediated M3 receptor activation, we recently developed a genetically modified yeast strain

(Saccharomyces cerevisiae) which allows the efficient screening of large libraries of mutant M3 receptors to identify mutant receptors with altered/novel functional properties. Class A GPCRs contain a highly conserved Asp residue located in transmembrane domain II (TM II; corresponding to Asp-113 in the rat M3 muscarinic receptor) which is of fundamental importance for receptor activation. As observed previously with other GPCRs analyzed in mammalian expression systems, the D113N point mutation abolished agonist-induced receptor/G protein coupling in yeast. We then subjected the D113N mutant M3 receptor to PCR-based random mutagenesis followed by a yeast genetic screen to recover point mutations that can restore G protein coupling to the D113N mutant receptor. A large scale screening effort led to the identification of three such second-site suppressor mutations, R165W, R165M, and Y250D. When expressed in the wild-type receptor background, these three point mutations did not lead to an increase in basal activity and reduced the efficiency of receptor/G protein coupling. Similar results were obtained when the various mutant receptors were expressed and analyzed in transfected mammalian cells (COS-7 cells). Interestingly, like Asp-113, Arg-165 and Tyr-250, which are located at the cytoplasmic ends of TM III and TM V, respectively, are also highly conserved among class A GPCRs. Our data suggest a conformational link between the highly conserved Asp-113, Arg-165, and Tyr-250 residues which is critical for receptor activation.

Mishra, D. P. and C. Shaha (2005). "Estrogen-induced Spermatogenic Cell Apoptosis Occurs via the Mitochondrial Pathway: ROLE OF SUPEROXIDE AND NITRIC OXIDE." J. Biol. Chem. **280**(7): 6181-6196.

http://www.jbc.org/cgi/content/abstract/280/7/6181

The detrimental effects of estrogen on testicular function provide a conceptual basis to examine the speculative link between increased exposure to estrogens and spermatogenic cell death. Using an in vitro model, we provide an understanding of the events leading to estrogen-induced apoptosis in cells of spermatogenic lineage. Early events associated with estrogen exposure were up-regulation of FasL and increased generation of H2O2, superoxide, and nitric oxide. The ability of anti-FasL antibodies to prevent several downstream biochemical changes and cell death induced by 17{beta}-estradiol substantiates the involvement of the cell death receptor pathway. Evidence for the amplification of the death-inducing signals through mitochondria was obtained from the transient mitochondrial hyperpolarization observed after estradiol exposure resulting in cytochrome c release. A combination of nitric oxide and superoxide but not H2O2 was responsible for the mitochondrial hyperpolarization. Mn(III) tetrakis(4-benzoic acid)porphyrin chloride, an intracellular peroxynitrite scavenger, was able to reduce mitochondrial hyperpolarization and cell death. Although nitric oxide augmentation occurred through an increase in the expression of inducible nitric-oxide synthase, superoxide up-regulation was a product of estradiol metabolism. All of the above changes were mediated through an estrogen receptor-based mechanism because tamoxifen, the estrogen receptor modulator, was able to rescue the cells from estrogen-induced alterations. This study establishes the importance of the independent capability of cells of the spermatogenic lineage to respond to estrogens and most importantly suggests that low dose estrogens can potentially cause severe spermatogenic cellular dysfunction leading to impaired fertility even without interference of the hypothalamo-hypophyseal axis.

Ho, R. H., B. F. Leake, et al. (2004). "Ethnicity-dependent Polymorphism in Na+-taurocholate Cotransporting Polypeptide (SLC10A1) Reveals a Domain Critical for Bile Acid Substrate Recognition." J. Biol. Chem. **279**(8): 7213-7222.

http://www.jbc.org/cgi/content/abstract/279/8/7213

The key transporter responsible for hepatic uptake of bile acids from portal circulation is Na+-taurocholate cotransporting polypeptide (NTCP, SLC10A1). This transporter is thought to be critical for the maintenance of enterohepatic recirculation of bile acids and hepatocyte function. Therefore, functionally relevant polymorphisms in this transporter would be predicted to have an important impact on bile acid homeostasis/liver function. However, little is known regarding genetic heterogeneity in NTCP. In this study, we demonstrate the presence of multiple single nucleotide polymorphisms in NTCP in populations of European, African, Chinese, and Hispanic Americans. Specifically four nonsynonymous single nucleotide polymorphisms associated with a significant loss of transport function were identified. Cell surface biotinylation experiments indicated that the altered transport activity of T668C (Ile223 [-&gt] Thr), a variant seen only in African Americans, was due at least in part to decreased plasma membrane expression. Similar expression patterns were observed when the variant alleles were expressed in HepG2 cells, and plasma membrane expression was assessed using immunofluorescence confocal microscopy. Interestingly the C800T (Ser267 [-&gt] Phe) variant, seen only in Chinese Americans, exhibited a near complete loss of function for bile acid uptake yet fully normal transport function for the non-bile acid substrate estrone sulfate, suggesting this position may be part of a region in the transporter critical and specific for bile acid substrate recognition. Accordingly, our study indicates functionally important polymorphisms in NTCP exist and that the likelihood of being carriers of such polymorphisms is dependent on ethnicity.

Karchner, S. I., D. G. Franks, et al. (2002). "Regulatory Interactions among Three Members of the Vertebrate Aryl Hydrocarbon Receptor Family: AHR Repressor, AHR1, and AHR2." J. Biol. Chem. **277**(9): 6949-6959.

http://www.jbc.org/cgi/content/abstract/277/9/6949

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds occur via the aryl hydrocarbon receptor (AHR), a member of the basic helix-loop-helix-Per-ARNT-Sim homology (bHLH-PAS) protein superfamily. A single AHR gene has been identified in mammals, whereas many fish species, including the Atlantic killifish (Fundulus heteroclitus) possess two distinct AHR genes (AHR1 and a novel form, AHR2). A mouse bHLH-PAS protein closely related to AHR and designated AHR repressor (AHRR) is induced by 3-methylcholanthrene and represses the transcriptional activity of the AHR. To determine whether AHRR is the mammalian ortholog of fish AHR2 and to investigate the mechanisms by which AHRR regulates AHR function, we cloned an AHRR ortholog in F. heteroclitus with high sequence identity to the mouse and human AHRRs. Killifish AHRR encodes a 680-residue protein with a predicted molecular mass of 75.2 kDa. We show that in vitro expressed AHRR proteins from human, mouse, and killifish all fail to bind [3H]TCDD or [3H][beta]-naphthoflavone. In transient transfection experiments using a luciferase reporter gene under control of AHR response elements, killifish AHRR inhibited the TCDD-dependent transactivation function of both AHR1 and AHR2. AHRR mRNA is widely expressed in killifish tissues and is inducible by TCDD or polychlorinated biphenyls, but its expression is not altered in a population of fish exhibiting genetic resistance to these compounds. The F. heteroclitus AHRR promoter contains three putative AHR response elements. Both AHR1 and AHR2 activated transcription of luciferase driven by the AHRR promoter, and AHRR could repress its own promoter. Thus, AHRR is an evolutionarily conserved, TCDD-inducible repressor of AHR1 and AHR2 function. Phylogenetic analysis shows that AHRR, AHR1, and AHR2 are distinct genes, members of an AHR gene family; these three vertebrate AHR-like genes descended from a single invertebrate AHR.

Cabral, W. A., E. Makareeva, et al. (2005). "Mutations near amino end of alpha 1(I) collagen cause combined OI/EDS by interference with N-propeptide processing." J. Biol. Chem.: M414698200.

http://www.jbc.org/cgi/content/abstract/M414698200v1

Patients with OI/EDS form a distinct subset of osteogenesis imperfecta patients. In addition to skeletal fragility, they have characteristics of Ehlers-Danlos syndrome. We identified 7 children with types III or IV OI, plus severe large and small joint laxity and early progressive scoliosis. In each child with OI/EDS, we identified a mutation in the first 90 residues of the helical region of [alpha]1(I) collagen. These mutations prevent or delay removal of the procollagen N-propeptide by purified N-proteinase (ADAMTS-2) in vitro and in pericellular assays. The mutant pN-collagen which results is efficiently incorporated into matrix by cultured fibroblasts and osteoblasts and is prominently present in newly incorporated and immaturely crosslinked collagen. Dermal collagen fibrils have significantly reduced cross-sectional diameters, corroborating incorporation of pN-collagen into fibrils in vivo. Differential scanning calorimetry revealed that these mutant collagens are less stable than the corresponding procollagens, which is not seen with other type I collagen helical mutations. These mutations disrupt a distinct folding region of high thermal stability in the first 90 residues at the amino end of type I collagen and alter the secondary structure of the adjacent N-proteinase cleavage site. Thus, these OI/EDS collagen mutations are directly responsible for the bone fragility of OI and indirectly responsible for EDS symptoms, by interference with N-propeptide removal.

Benlloch, M., A. Ortega, et al. (2005). "Acceleration of Glutathione Efflux and Inhibition of {gamma}-Glutamyltranspeptidase Sensitize Metastatic B16 Melanoma Cells to Endothelium-induced Cytotoxicity." J. Biol. Chem. **280**(8): 6950-6959.

http://www.jbc.org/cgi/content/abstract/280/8/6950

Highly metastatic B16 melanoma (B16M)-F10 cells, as compared with the low metastatic B16M-F1 line, have higher GSH content and preferentially overexpress BCL-2. In addition to its anti-apoptotic properties, BCL-2 inhibits efflux of GSH from B16M-F10 cells and thereby may facilitate metastatic cell resistance against endothelium-induced oxidative/nitrosative stress. Thus, we investigated in B16M-F10 cells which molecular mechanisms channel GSH release and whether their modulation may influence metastatic activity. GSH efflux was abolished in multidrug resistance protein 1 knock-out (MRP-/-1) B16M-F10 transfected with the Bcl-2 gene or in MRP-/-1 B16M-F10 cells incubated with L-methionine, which indicates that GSH release from B16M-F10 cells is channeled through MRP1 and a BCL-2-dependent system (likely related to an L-methionine-sensitive GSH carrier previously detected in hepatocytes). The BCL-2-dependent system was identified as the cystic fibrosis transmembrane conductance regulator, since monoclonal antibodies against this ion channel or H-89 (a protein kinase A-selective inhibitor)-induced inhibition of cystic fibrosis transmembrane conductance regulator gene expression completely blocked the BCL-2-sensitive GSH release. By using a perifusion system that mimics in vivo conditions, we found that GSH depletion in metastatic cells can be achieved by using Bcl-2 antisense oligodeoxynucleotide- and verapamil (an MRP1 activator)-induced acceleration of GSH efflux, in combination with acivicin-induced inhibition of {gamma}-glutamyltranspeptidase (which limits GSH synthesis by preventing cysteine generation from extracellular GSH). When applied under in vivo conditions, this strategy increased tumor cytotoxicity (up to [~]90%) during B16M-F10 cell adhesion to the hepatic sinusoidal endothelium.

Rahman, A. S., J. Hothersall, et al. (2005). "Tandemly Duplicated Acyl Carrier Proteins, Which Increase Polyketide Antibiotic Production, Can Apparently Function Either in Parallel or in Series." J. Biol. Chem. **280**(8): 6399-6408.

http://www.jbc.org/cgi/content/abstract/280/8/6399

Polyketide biosynthesis involves the addition of subunits commonly derived from malonate or methylmalonate to a starter unit such as acetate. Type I polyketide synthases are multifunctional polypeptides that contain one or more modules, each of which normally contains all the enzymatic domains for a single round of extension and modification of the polyketide backbone. Acyl carrier proteins (ACP(s)) hold the extender unit to which the starter or growing chain is added. Normally there is one ACP for each ketosynthase module. However, there are an increasing number of known examples of tandemly repeated ACP domains, whose function is as yet unknown. For the doublet and triplet ACP domains in the biosynthetic pathway for the antibiotic mupirocin from Pseudomonas fluorescens NCIMB10586 we have inactivated ACP domains by inframe deletion and amino acid substitution of the active site serine. By deletion analysis each individual ACP from a cluster can provide a basic but reduced activity for the pathway. In the doublet cluster, substitution analysis indicates that the pathway may follow two parallel routes, one via each of the ACPs, thus increasing overall pathway flow. In the triplet cluster, substitution in ACP5 blocked the pathway. Thus ACP5 appears to be arranged "in series" to ACP6 and ACP7. Thus although both the doublet and triplet clusters increase antibiotic production, the mechanisms by which they do this appear to be different and depend specifically on the biosynthetic stage involved. The function of some ACPs may be determined by their location in the protein rather than absolute enzymic activity.

Kessler, N., H. Schuhmann, et al. (2004). "The Linear Pentadecapeptide Gramicidin Is Assembled by Four Multimodular Nonribosomal Peptide Synthetases That Comprise 16 Modules with 56 Catalytic Domains." J. Biol. Chem. **279**(9): 7413-7419.

http://www.jbc.org/cgi/content/abstract/279/9/7413

Linear gramicidin is a membrane channel forming pentadecapeptide that is produced via the nonribosomal pathway. It consists of 15 hydrophobic amino acids with alternating L- and D-configuration forming a {beta}-helix-like structure. It has an N-formylated valine and a C-terminal ethanolamine. Here we report cloning and sequencing of the entire biosynthetic gene cluster as well as initial biochemical analysis of a new reductase domain. The biosynthetic gene cluster was identified on two nonoverlapping fosmids and a 13-kilobase pair (kbp) interbridge fragment covering a region of 74 kbp. Four very large open reading frames, lgrA, lgrB, lgrC, and lgrD with 6.8, 15.5, 23.3, and 15.3 kbp, were identified and shown to encode nonribosomal peptide synthetases with two, four, six, and four modules, respectively. Within the 16 modules identified, seven epimerization domains in alternating positions were detected as well as a putative formylation domain fused to the first module LgrA and a putative reductase domain attached to the C-terminal module of LgrD. Analysis of the substrate specificity by phylogenetic studies using the residues of the substrate-binding pockets of all 16 adenylation domains revealed a good agreement of the substrate amino acids predicted with the sequence of linear gramicidin. Additional biochemical analysis of the three adenylation domains of modules 1, 2, and 3 confirmed the colinearity of this nonribosomal peptide synthetase assembly line. Module 16 was predicted to activate glycine, which would then, being the C-terminal residue of the peptide chain, be reduced by the adjacent reductase domain to give ethanolamine, thereby releasing the final product N-formyl-pentadecapeptide-ethanolamine. However, initial biochemical analysis of this reductase showed only a one-step reduction yielding the corresponding aldehyde in vitro.

Hijarrubia, M. J., J. F. Aparicio, et al. (2003). "Domain Structure Characterization of the Multifunctional alpha -Aminoadipate Reductase from Penicillium chrysogenum by Limited Proteolysis. ACTIVATION OF alpha -AMINOADIPATE DOES NOT REQUIRE THE PEPTIDYL CARRIER PROTEIN BOX OR THE REDUCTION DOMAIN." J. Biol. Chem. **278**(10): 8250-8256.

http://www.jbc.org/cgi/content/abstract/278/10/8250

The [alpha]-aminoadipate reductase ([alpha]-AAR) of Penicillium chrysogenum, an enzyme that activates the [alpha]-aminoadipic acid by forming an [alpha]-aminoadipyl adenylate and reduces the activated intermediate to [alpha]-aminoadipic semialdehyde, was purified to homogeneity by immunoaffinity techniques, and the kinetics for [alpha]-aminoadipic acid, ATP, and NADPH were determined. Sequencing of the N-terminal end confirmed the 10 first amino acids deduced from the nucleotide sequence. Its domain structure has been investigated using limited proteolysis and active site labeling. Trypsin and elastase were used to cleave the multienzyme, and the location of fragments within the primary structure was established by N-terminal sequence analysis. Initial proteolysis generated two fragments: an N-terminal fragment housing the adenylation and the peptidyl carrier protein (PCP) domains (116 kDa) and a second fragment containing most of the reductive domain (28 kDa). Under harsher conditions the adenylation domain (about 64 kDa) and the PCP domain (30 kDa) become separated. Time-dependent acylation of [alpha]-AAR and of fragments containing the adenylation domain with tritiated [alpha]-aminoadipate occurred in vitro in the absence of NADPH. Addition of NADPH to the labeled [alpha]-AAR released most of the radioactive substrate. A fragment containing the adenylation domain was labeled even in absence of the PCP box. The labeling of this fragment (lacking PCP) was always weaker than that observed in the di-domain (adenylating and PCP) fragment suggesting that the PCP domain plays a role in the stability of the acyl intermediate. Low intensity direct acylation of the PCP box has also been observed. A domain structure of this multienzyme is proposed.

Jackson-Hayes, L., S. Song, et al. (2003). "A Thyroid Hormone Response Unit Formed between the Promoter and First Intron of the Carnitine Palmitoyltransferase-Ialpha Gene Mediates the Liver-specific Induction by Thyroid Hormone." J. Biol. Chem. **278**(10): 7964-7972.

http://www.jbc.org/cgi/content/abstract/278/10/7964

Carnitine palmitoyltransferase-I (CPT-I) catalyzes the rate-controlling step of fatty acid oxidation. CPT-I converts long-chain fatty acyl-CoAs to acylcarnitines for translocation across the mitochondrial membrane. The mRNA levels and enzyme activity of the liver isoform, CPT-I[alpha], are greatly increased in the liver of hyperthyroid animals. Thyroid hormone (T3) stimulates CPT-I[alpha] transcription far more robustly in the liver than in non-hepatic tissues. We have shown that the thyroid hormone receptor (TR) binds to a thyroid hormone response element (TRE) located in the CPT-I[alpha] promoter. In addition, elements in the first intron participate in the T3 induction of CPT-I[alpha] gene expression, but the CPT-I[alpha] intron alone cannot confer a T3 response. We found that deletion of sequences in the first intron between +653 and +744 decreased the T3 induction of CPT-I[alpha]. Upstream stimulatory factor (USF) and CCAAT enhancer binding proteins (C/EBPs) bind to elements within this region, and these factors are required for the T3 response. The binding of TR and C/EBP to the CPT-I[alpha] gene in vivo was shown by the chromatin immunoprecipitation assay. We determined that TR can physically interact with USF-1, USF-2, and C/EBP[alpha]. Transgenic mice were created that carry CPT-I[alpha]-luciferase transgenes with or without the first intron of the CPT-I[alpha] gene. In these mouse lines, the first intron is required for T3 induction as well as high levels of hepatic expression. Our data indicate that the T3 stimulates CPT-I[alpha] gene expression in the liver through a T3 response unit consisting of the TRE in the promoter and additional factors, C/EBP and USF, bound in the first intron.

Pulliainen, A. T., S. Haataja, et al. (2003). "Molecular Basis of H2O2 Resistance Mediated by Streptococcal Dpr. DEMONSTRATION OF THE FUNCTIONAL INVOLVEMENT OF THE PUTATIVE FERROXIDASE CENTER BY SITE-DIRECTED MUTAGENESIS IN STREPTOCOCCUS SUIS." J. Biol. Chem. **278**(10): 7996-8005.

http://www.jbc.org/cgi/content/abstract/278/10/7996

H2O2 is an unavoidable cytotoxic by-product of aerobic life. Dpr, a recently discovered member of the Dps protein family, provides a means for catalase-negative bacteria to tolerate H2O2. Potentially, Dpr could bind free intracellular iron and thus inhibit the Fenton chemistry-catalyzed formation of toxic hydroxyl radicals (H2O2 + Fe2+ [right-arrow] {middle dot}OH + -OH + Fe3+). We explored the in vivo function of Dpr in the catalase- and NADH peroxidase-negative pig and human pathogen Streptococcus suis. We show that: (i) a Dpr allelic exchange knockout mutant was hypersensitive (~106-fold) to H2O2, (ii) Dpr incorporated iron in vivo, (iii) a putative ferroxidase center was present in Dpr, (iv) single amino acid substitutions D74A or E78A to the putative ferroxidase center abolished the in vivo iron incorporation, and (v) the H2O2 hypersensitive phenotype was complemented by wild-type Dpr or by a membrane-permeating iron chelator, but not by the site-mutated forms of Dpr. These results demonstrate that the putative ferroxidase center of Dpr is functionally active in iron incorporation and that the H2O2 resistance is mediated by Dpr in vivo by its iron binding activity.

Satake, H., H. Y. Chen, et al. (2003). "Genes Modulated by Expression of GD3 Synthase in Chinese Hamster Ovary Cells. EVIDENCE THAT THE Tis21 GENE IS INVOLVED IN THE INDUCTION OF GD3 9-O-ACETYLATION." J. Biol. Chem. **278**(10): 7942-7948.

http://www.jbc.org/cgi/content/abstract/278/10/7942

9-O-Acetylation is a common sialic acid modification, expressed in a developmentally regulated and tissue/cell type-specific manner. The relevant 9-O-acetyltransferase(s) have not been isolated or cloned; nor have mechanisms for their regulation been elucidated. We previously showed that transfection of the GD3 synthase (ST8Sia-I) gene into Chinese hamster ovary (CHO)-K1 cells gave expression of not only the disialoganglioside GD3 but also 9-O-acetyl-GD3. We now use differential display PCR between wild type CHO-K1 cells and clones stably expressing GD3 synthase (CHO-GD3 cells) to detect any increased expression of other genes and explore the possible induction of a 9-O-acetyltransferase. The four CHO mRNAs showing major up-regulation were homologous to VCAM-1, Tis21, the KC-protein-like protein, and a functionally unknown type II transmembrane protein. A moderate increase in expression of the FxC1 and SPR-1 genes was also seen. Interestingly, these are different from genes observed by others to be up-regulated after transfection of GD3 synthase into a neuroblastoma cell line. We also isolated a CHO-GD3 mutant lacking 9-O-acetyl-GD3 following chemical mutagenesis (CHO-GD3-OAc[-]). Analysis of the above differential display PCR-derived genes in these cells showed that expression of Tis21 was selectively reduced. Transfection of a mouse Tis21 cDNA into the CHO-GD3-OAc[-] mutant cells restored 9-O-acetyl-GD3 expression. Since the only major gangliosides expressed by CHO-GD3 cells are GD3 and 9-O-acetyl-GD3 (in addition to GM3, the predominant ganglioside type in wild-type CHO-K1 cells), we conclude that GD3 enhances its own 9-O-acetylation via induction of Tis21. This is the first known nuclear inducible factor for 9-O-acetylation and also the first proof that 9-O-acetylation can be directly regulated by GD3 synthase. Finally, transfection of CHO-GD3-OAc[-] mutant cells with ST6Gal-I induced 9-O-acetylation specifically on sialylated N-glycans, in a manner similar to wild-type cells. This indicates separate machineries for 9-O-acetylation on [alpha]2-8-linked sialic acids of gangliosides and on [alpha]2-6-linked sialic acids on N-glycans.

Hoffmann, L., S. Maury, et al. (2003). "Purification, Cloning, and Properties of an Acyltransferase Controlling Shikimate and Quinate Ester Intermediates in Phenylpropanoid Metabolism." J. Biol. Chem. **278**(1): 95-103.

http://www.jbc.org/cgi/content/abstract/278/1/95

A protein hydrolyzing hydroxycinnamoyl-CoA esters has been purified from tobacco stem extracts

by a series of high pressure liquid chromatography steps. The determination of its N-terminal amino acid sequence allowed design of primers permitting the corresponding cDNA to be cloned by PCR. Sequence analysis revealed that the tobacco gene belongs to a plant acyltransferase gene family, the members of which have various functions. The tobacco cDNA was expressed in bacterial cells as a recombinant protein fused to glutathione S-transferase. The fusion protein was affinity-purified and cleaved to yield the recombinant enzyme for use in the study of catalytic properties. The enzyme catalyzed the synthesis of shikimate and quinate esters shown recently to be substrates of the cytochrome P450 3-hydroxylase involved in phenylpropanoid biosynthesis. The enzyme has been named hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase. We show that p-coumaroyl-CoA and caffeoyl-CoA are the best acyl group donors and that the acyl group is transferred more efficiently to shikimate than to quinate. The enzyme also catalyzed the reverse reaction, i.e. the formation of caffeoyl-CoA from chlorogenate (5-O-caffeoyl quinate ester). Thus, hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase appears to control the biosynthesis and turnover of major plant phenolic compounds such as lignin and chlorogenic acid.

Ishisaki, A., H. Hayashi, et al. (2003). "Human Umbilical Vein Endothelium-derived Cells Retain Potential to Differentiate into Smooth Muscle-like Cells." J. Biol. Chem. **278**(2): 1303-1309.

http://www.jbc.org/cgi/content/abstract/278/2/1303

Mouse embryonic stem-derived cells were recently shown to differentiate into endothelial and smooth muscle cells. In the present study, we investigated whether human umbilical vein endothelium-derived cells retain the potential to differentiate into smooth muscle cells. Examination of biochemical markers, including basic calponin, SM22[alpha], prostaglandin E synthase, von Willebrand factor, and PECAM-1, as well as cell contractility, showed that whereas endothelium-derived cells cultured with fibroblast growth factor can be characterized as endothelial cells, when deprived of fibroblast growth factor, a significant fraction differentiates into smooth muscle-like cells. Reapplication of fibroblast growth factor reversed this differentiation. Activin A was up-regulated in fibroblast growth factor-deprived, endothelium-derived cells; moreover, the inhibitory effects of exogenous follistatin and overexpressed Smad7 on smooth muscle-like differentiation confirmed that the differentiation was driven by activin A signaling. These findings indicate that when deprived of fibroblast growth factor, human umbilical vein endothelium-derived cells are capable of differentiating into smooth muscle-like cells through activin A-induced, Smad-dependent signaling, and that maintenance of the endothelial cell phenotype and differentiation into smooth muscle-like cells are reciprocally controlled by fibroblast growth factor-1 and activin A.

Uchimura, K., K. Kadomatsu, et al. (2002). "Functional Analysis of the Chondroitin 6-Sulfotransferase Gene in Relation to Lymphocyte Subpopulations, Brain Development, and Oversulfated Chondroitin Sulfates." J. Biol. Chem. **277**(2): 1443-1450.

http://www.jbc.org/cgi/content/abstract/277/2/1443

Chondroitin 6-sulfotransferase (C6ST) catalyzes the transfer of sulfate to position 6 of the N-acetylgalactosamine residue of chondroitin. To obtain direct evidence regarding the function of C6ST and its product, chondroitin 6-sulfate, in vivo, we isolated the mouse C6ST gene (C6st) and generated mice deficient in this gene (C6st[-]/[-]) by embryonic stem cell technology. C6st[-]/[-] mice were born at approximately the expected frequency and were viable through adulthood. In the spleen of C6st[-]/[-] mice, the level of chondroitin 6-sulfate became almost undetectable. Analyses of these knockout mice provided insights into the biosynthesis of oversulfated chondroitin sulfates in mice; chondroitin sulfate D in the brain of null mice and the cartilage and

telencephalon of null embryos disappeared, whereas the chondroitin sulfate E level in the spleen and brain of the null mice was unchanged. Despite the disappearance of chondroitin sulfate D structure, brain development was normal in the C6st[-]/[-] mice. Further analysis revealed that the number of CD62L+CD44low T lymphocytes corresponding to naive T lymphocytes in the spleen of 5-6-week-old C6st[-]/[-] mice was significantly decreased, whereas those in other secondary lymphoid organs were unchanged. This finding suggested that chondroitin 6-sulfate plays a role in the maintenance of naive T lymphocytes in the spleen of young mice.

Pankonin, M. S., J. T. Gallagher, et al. (2005). "Specific Structural Features of Heparan Sulfate Proteoglycans Potentiate Neuregulin-1 Signaling." J. Biol. Chem. **280**(1): 383-388.

http://www.jbc.org/cgi/content/abstract/280/1/383

Neuregulins are a family of growth and differentiation factors that act through activation of cell-surface erbB receptor tyrosine kinases and have essential functions both during development and on the growth of cancer cells. One alternatively spliced neuregulin-1 form has a distinct heparin-binding immunoglobulin-like domain that enables it to adhere to heparan sulfate proteoglycans at key locations during development and substantially potentiates its activity. We examined the structural specificity needed for neuregulin-1-heparin interactions using a gel mobility shift assay together with an assay that measures the ability of specific oligosaccharides to block erbB receptor phosphorylation in L6 muscle cells. Whereas the N-sulfate group of heparin was most important, the 2-O-sulfate and 6-O-sulfate groups also contributed to neuregulin-1 binding in these two assays. Optimal binding to neuregulin-1 required eight or more heparin disaccharides; however, as few as two disaccharides were still able to bind neuregulin-1 to a lesser extent. The physiological importance of this specificity was shown both by chemical and siRNA treatment of cultured muscle cells. Pretreatment of muscle cells with chlorate that blocks all sulfation or with an siRNA that selectively blocks N-sulfation significantly reduced erbB receptor activation by neuregulin-1 but had no effect on the activity of neuregulin-1 that lacks the heparin-binding domain. These results suggest that the regulation of glycosaminoglycan sulfation is an important biological mechanism that can modulate both the localization and potentiation of neuregulin-1 signaling.

Budagian, V., E. Bulanova, et al. (2003). "Signaling through P2X7 Receptor in Human T Cells Involves p56lck, MAP Kinases, and Transcription Factors AP-1 and NF-kappa B." J. Biol. Chem. **278**(3): 1549-1560.

http://www.jbc.org/cgi/content/abstract/278/3/1549

ATP-gated ion channel P2X receptors are expressed on the surface of most immune cells and can trigger multiple cellular responses, such as membrane permeabilization, cytokine production, and cell proliferation or apoptosis. Despite broad distribution and pleiotropic activities, signaling pathways downstream of these ionotropic receptors are still poorly understood. Here, we describe intracellular signaling events in Jurkat cells treated with millimolar concentrations of extracellular ATP. Within minutes, ATP treatment resulted in the phosphorylation and activation of p56lck kinase, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase but not p38 kinase. These effects were wholly dependent upon the presence of extracellular Ca2+ ions in the culture medium. Nevertheless, calmodulin antagonist calmidazolium and CaM kinase inhibitor KN-93 both had no effect on the activation of p56lck and ERK, whereas a pretreatment of Jurkat cells with MAP kinase kinase inhibitor P098059 was able to abrogate phosphorylation of ERK. Further, expression of c-Jun and c-Fos proteins and activator protein (AP-1) DNA binding activity were enhanced in a time-dependent manner. In contrast, DNA binding activity of NF-[kappa]B was reduced. ATP failed to stimulate the phosphorylation of ERK and c-Jun N-terminal kinase

and activation of AP-1 in the p56lck-deficient isogenic T cell line JCaM1, suggesting a critical role for p56lck kinase in downstream signaling. Regarding the biological significance of the ATP-induced signaling events we show that although extracellular ATP was able to stimulate proliferation of both Jurkat and JCaM1 cells, an increase in interleukin-2 transcription was observed only in Jurkat cells. The nucleotide selectivity and pharmacological profile data supported the evidence that the ATP-induced effects in Jurkat cells were mediated through the P2X7 receptor. Taken together, these results demonstrate the ability of extracellular ATP to activate multiple downstream signaling events in a human T-lymphoblastoid cell line.

Yan, C., H. Wang, et al. (2003). "Repression of 92-kDa Type IV Collagenase Expression by MTA1 Is Mediated through Direct Interactions with the Promoter via a Mechanism, Which Is Both Dependent on and Independent of Histone Deacetylation." J. Biol. Chem. **278**(4): 2309-2316.

http://www.jbc.org/cgi/content/abstract/278/4/2309

Although the expression of the metastases-associated gene MTA1 correlates with tumor metastases, its role in regulating type IV collagenase expression is unknown. Enforced MTA1 expression in HT1080 cells reduced basal and 12-myristate 13-acetate-induced 92-kDa type IV collagenase (MMP-9) protein/mRNA levels. DNase I hypersensitivity and PstI accessibility assays revealed multiple regions of the MMP-9 promoter ([-]650/[-]450 and [-]120/+1), showing reduced hypersensitivity in the MTA1-expressing cells. Chromatin immunoprecipitation assays demonstrated MTA1 binding to the distal region, which spans several regulatory cis elements. Co-immunoprecipitation and chromatin immunoprecipitation assay experiments revealed histone deacetylase 2 (HDAC2)-MTA1 protein-protein interactions and the MTA1-dependent recruitment of HDAC2 to the distal MMP-9 promoter region, yielding diminished histone H3/H4 acetylation. However, HDAC2 binding and H3/H4 acetylation at the proximal MMP-9 region were unaffected by MTA1 expression. Furthermore, trichostatin treatment only partially relieved MTA1-repressed MMP-9 expression, indicating a HDAC-insensitive component possibly involv ing the nucleosome-remodeling Mi2 activity, which was recruited to the promoter by MTA1. In summary, (a) MMP-9 adds to a short list of MTA1-regulated genes, which so far only includes c-myc and pS2, and (b) MTA1 binds to the MMP-9 promoter, thereby repressing expression of this type IV collagenase via histone-dependent and independent mechanisms.

Iwasaki, H., K. Chiba, et al. (2002). "Molecular Characterization of the Starfish Inositol 1,4,5-Trisphosphate Receptor and Its Role during Oocyte Maturation and Fertilization." J. Biol. Chem. **277**(4): 2763-2772.

http://www.jbc.org/cgi/content/abstract/277/4/2763

The release of calcium ions (Ca2+) from their intracellular stores is essential for the fertilization of oocytes of various species. The calcium pools can be induced to release Ca2+ via two main types of calcium channel receptor: the inositol 1,4,5-trisphosphate receptor (IP3R) and the ryanodine receptor. Starfish oocytes have often been used to study intracellular calcium mobilization during oocyte maturation and fertilization, but how the intracellular calcium channels contribute to intracellular calcium mobilization has never been understood fully, because these molecules have not been identified and no specific inhibitors of these channels have ever been found. In this study, we utilized a novel IP3R antagonist, the "IP3 sponge," to investigate the role of IP3 during fertilization of the starfish oocyte. The IP3 sponge strongly and specifically competed with endogenous IP3R for binding to IP3. By injecting IP3 sponge into starfish oocyte, the increase in intracellular calcium and formation of the fertilization envelope were both dramatically blocked, although oocyte maturation was not blocked. To investigate the role of IP3R in the starfish oocyte more precisely, we cloned IP3R from the ovary of starfish, and the predicted

amino acid sequence indicated that the starfish IP3R has 58-68% identity to mammalian IP3R types 1, 2, and 3. We then raised antibodies that recognize starfish IP3R, and use of the antibodies to perform immunoblot analysis revealed that the level of expression of IP3R remained unchanged throughout oocyte maturation. An immunocytochemical study, however, revealed that the distribution of starfish IP3R changes during oocyte maturation.

Ponimaskin, E. G., M. Heine, et al. (2002). "The 5-Hydroxytryptamine(4a) Receptor Is Palmitoylated at Two Different Sites, and Acylation Is Critically Involved in Regulation of Receptor Constitutive Activity." J. Biol. Chem. **277**(4): 2534-2546.

http://www.jbc.org/cgi/content/abstract/277/4/2534

We have reported recently that the mouse 5-hydroxytryptamine(4a) (5-HT4(a)) receptor undergoes dynamic palmitoylation (Ponimaskin, E. G., Schmidt, M. F., Heine, M., Bickmeyer, U., and Richter, D. W. (2001) Biochem. J. 353, 627-663). In the present study, conserved cysteine residues 328/329 in the carboxyl terminus of the 5-HT4(a) receptor were identified as potential acylation sites. In contrast to other palmitoylated G-protein-coupled receptors, the additional cysteine residue 386 positioned close to the COOH-terminal end of the receptor was also found to be palmitoylated. Using pulse and pulse-chase labeling techniques, we demonstrated that palmitoylation of individual cysteines is a reversible process and that agonist stimulation of the 5-HT4(a) receptor independently increases the rate of palmitate turnover for both acylation sites. Analysis of acylation-deficient mutants revealed that non-palmitoylated 5-HT4(a) receptors were indistinguishable from the wild type in their ability to interact with Gs, to stimulate the adenylyl cyclase activity and to activate cyclic nucleotide-sensitive cation channels after agonist stimulation. The most distinctive finding of the present study was the ability of palmitoylation to modulate the agonist-independent constitutive 5-HT4(a) receptor activity. We demonstrated that mutation of the proximal palmitoylation site (Cys328 [right-arrow] Ser/Cys329 [right-arrow] Ser) significantly increases the capacity of receptors to convert from the inactive (R) to the active (R*) form in the absence of agonist. In contrast, the rate of isomerization from R to R* for the Cys386 [right-arrow] Ser as well as for the triple, non-palmitoylated mutant (Cys328 [right-arrow] Ser/Cys329 [right-arrow] Ser/Cys386 [right-arrow]Ser) was similar to that obtained for the wild type.

Tremper-Wells, B. and M. L. Vallano (2005). "Nuclear Calpain Regulates Ca2+-dependent Signaling via Proteolysis of Nuclear Ca2+/Calmodulin-dependent Protein Kinase Type IV in Cultured Neurons." J. Biol. Chem. **280**(3): 2165-2175.

http://www.jbc.org/cgi/content/abstract/280/3/2165

Accumulating evidence indicates that calpains can reside in or translocate to the cell nucleus, but their functions in this compartment remain poorly understood. Dissociated cultures of cerebellar granule cells (GCs) demonstrate improved long-term survival when their growth medium is supplemented with depolarizing agents that stimulate Ca2+ influx and activate calmodulin-dependent signaling cascades, notably 20 mM KCl. We previously observed Ca2+-dependent down-regulation of Ca2+/calmodulin-dependent protein kinase (CaMK) type IV, which was attenuated by calpain inhibitors, in GCs supplemented with 20 mM KCl (Tremper-Wells, B., Mathur, A., Beaman-Hall, C. M., and Vallano, M. L. (2002) J. Neurochem. 81, 314-324). CaMKIV is highly enriched in the nucleus and thought to be critical for improved survival. Here, we demonstrate by immunolocalization/confocal microscopy and subcellular fractionation that the regulatory and catalytic subunits of m-calpain are enriched in GC nuclei, including GCs grown in medium containing 5 mM KCl. Calpain-mediated proteolysis of CaMKIV is selective, as several other nuclear and non-nuclear calpain substrates were not degraded under chronic depolarizing

culture conditions. Depolarization and Ca2+-dependent down-regulation of CaMKIV were associated with significant alterations in other components of the Ca2+-CaMKIV signaling cascade: the ratio of phosphorylated to total cAMP response element-binding protein (a downstream CaMKIV substrate) was reduced by [~]10-fold, and the amount of CaMK kinase (an upstream activator of CaMKIV) protein and mRNA was significantly reduced. We hypothesize that calpain-mediated CaMKIV proteolysis is an autoregulatory feedback response to sustained activation of a Ca2+-CaMKIV signaling pathway, resulting from growth of cultures in medium containing 25 mM KCl. This study establishes nuclear m-calpain as a regulator of CaMKIV and associated signaling molecules under conditions of sustained Ca2+ influx.

Laybutt, D. R., M. Glandt, et al. (2003). "Critical Reduction in beta -Cell Mass Results in Two Distinct Outcomes over Time. ADAPTATION WITH IMPAIRED GLUCOSE TOLERANCE OR DECOMPENSATED DIABETES." J. Biol. Chem. **278**(5): 2997-3005.

http://www.jbc.org/cgi/content/abstract/278/5/2997

We have proposed that hyperglycemia-induced dedifferentiation of [beta]-cells is a critical factor for the loss of insulin secretory function in diabetes. Here we examined the effects of the duration of hyperglycemia on gene expression in islets of partially pancreatectomized (Px) rats. Islets were isolated, and mRNA was extracted from rats 4 and 14 weeks after Px or sham Px surgery. Px rats developed different degrees of hyperglycemia; low hyperglycemia was assigned to Px rats with fed blood glucose levels less than 150 mg/dl, and high hyperglycemia was assigned above 150 mg/dl. [beta]-Cell hypertrophy was present at both 4 and 14 weeks. At the same time points, high hyperglycemia rats showed a global alteration in gene expression with decreased mRNA for insulin, IAPP, islet-associated transcription factors (pancreatic and duodenal homeobox-1, BETA2/NeuroD, Nkx6.1, and hepatocyte nuclear factor 1[alpha]), [beta]-cell metabolic enzymes (glucose transporter 2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, and pyruvate carboxylase), and ion channels/pumps (Kir6.2, VDCC[beta], and sarcoplasmic reticulum Ca2+-ATPase 3). Conversely, genes normally suppressed in [beta]-cells, such as lactate dehydrogenase-A, hexokinase I, glucose-6-phosphatase, stress genes (heme oxygenase-1, A20, and Fas), and the transcription factor c-Myc, were markedly increased. In contrast, gene expression in low hyperglycemia rats was only minimally changed at 4 weeks but significantly changed at 14 weeks, indicating that even low levels of hyperglycemia induce [beta]-cell dedifferentiation over time. In addition, whereas 2 weeks of correction of hyperglycemia completely reverses the changes in gene expression of Px rats at 4 weeks, the changes at 14 weeks were only partially reversed, indicating that the phenotype becomes resistant to reversal in the long term. In conclusion, chronic hyperglycemia induces a progressive loss of [beta]-cell phenotype with decreased expression of [beta]-cell-associated genes and increased expression of normally suppressed genes, these changes being present with even minimal levels of hyperglycemia. Thus, both the severity and duration of hyperglycemia appear to contribute to the deterioration of the [beta]-cell phenotype found in diabetes.

Ayabe, T., H. Wulff, et al. (2002). "Modulation of Mouse Paneth Cell alpha -Defensin Secretion by mIKCa1, a Ca2+-activated, Intermediate Conductance Potassium Channel." J. Biol. Chem. **277**(5): 3793-3800.

http://www.jbc.org/cgi/content/abstract/277/5/3793

Paneth cells in small intestinal crypts secrete microbicidal [alpha]-defensins in response to bacteria and bacterial antigens (Ayabe, T., Satchell, D. P., Wilson, C. L., Parks, W. C., Selsted, M. E., and Ouellette, A. J. (2000) Nat. Immunol. 1, 113-138). We now report that the Ca2+-activated K+ channel mIKCa1 modulates mouse Paneth cell secretion. mIKCa1 cDNA clones

identified in a mouse small intestinal crypt library by hybridization to human IKCa1 cDNA probes were isolated, and DNA sequence analysis showed that they were identical to mIKCa1 cDNAs isolated from erythroid cells and liver. The genomic organization was found to be conserved between mouse and human IKCa1 as shown by comparisons of the respective cDNA and genomic sequences. Reverse transcriptase-PCR experiments using nested primers amplified mIKCa1 from the lower half of bisected crypts and from single Paneth cells, but not from the upper half of bisected crypts, villus epithelium, or undifferentiated crypt epithelial cells, suggesting a lineage-specific role for mIKCa1 in mouse small bowel epithelium. The cloned mIKCa1 channel was calcium-activated and was blocked by ten structurally diverse peptide and nonpeptide inhibitors with potencies spanning 9 orders of magnitude and indistinguishable from that of the human homologue. Consistent with channel blockade, charybdotoxin, clotrimazole, and the highly selective IKCa1 inhibitors, TRAM-34 and TRAM-39, inhibited (~50%) Paneth cell secretion stimulated by bacteria or bacterial lipopolysaccharide, measured both as bactericidal activity and secreted cryptdin protein, but the inactive analog, TRAM-7, did not block secretion. These results demonstrate that mIKCa1 is modulator of Paneth cell [alpha]-defensin secretion and disclose an involvement in mucosal defense of the intestinal epithelium against ingested bacterial pathogens.

Kaneto, H., K. Suzuma, et al. (2002). "Involvement of Protein Kinase C beta 2 in c-myc Induction by High Glucose in Pancreatic beta -Cells." J. Biol. Chem. **277**(5): 3680-3685.

http://www.jbc.org/cgi/content/abstract/277/5/3680

The expression of the basic helix-loop-helix transcription factor c-Myc is induced in pancreatic islets of several different diabetic model animals and is possibly involved in suppression of the insulin gene transcription. In this study, we found that activity of protein kinase C is increased by high glucose, preceding the induction of c-myc expression and that PKC [beta]2 specifically regulates c-myc expression in pancreatic [beta]-cells. Since PKC [alpha], [beta]2, [delta], [epsilon], and [zeta] were expressed in rat pancreatic islets, we prepared each wild type (WT) and dominant negative type (DN) PKC isoform ([alpha], [beta]2, [delta], [epsilon], and [zeta])-expressing adenovirus to examine the effect of each PKC isoform on c-myc expression. In isolated rat pancreatic islets, adenovirus-mediated overexpression of WT PKC [beta]2, but not other PKC isoforms, markedly increased c-myc expression. Moreover, c-myc induction by high glucose was suppressed by adenovirus-mediated overexpression of DN PKC [beta]2 but not by other DN PKC isoforms. Finally, adenovirus-mediated overexpression of WT PKC [beta]2, but not of other PKC isoforms, leads to suppression of the insulin gene transcription in pancreatic islets. These results suggest that at least some of the reduction of insulin gene transcription found in the diabetic state is mediated by PKC [beta]2 regulation of c-myc expression.

Min, S. H., R. C. M. Simmen, et al. (2002). "Altered Levels of Growth-related and Novel Gene Transcripts in Reproductive and Other Tissues of Female Mice Overexpressing Spermidine/Spermine N1-Acetyltransferase (SSAT)." J. Biol. Chem. **277**(5): 3647-3657.

http://www.jbc.org/cgi/content/abstract/277/5/3647

Overexpression of SSAT (polyamine catabolic enzyme) in female mice results in impaired ovarian folliculogenesis and uterine hypoplasia. To identify the molecular basis for this, the gene expression profiles in uterus and ovary and for comparison, liver and kidney, from non-transgenic (NT) and SSAT transgenic (ST) mice were compared. The mRNA abundance for lipoprotein lipase and glyceraldehyde-3-phosphate dehydrogenase was elevated in all four ST (>NT) tissues. The translation initiation factor-3 subunit 5 mRNA, and transcripts related to endogenous murine leukemia provirus (MLV-related) and murine retrovirus-related sequences (MuRRS) were decreased in ST tissues. A novel calmodulin-related mRNA was strongly induced in ST liver and

kidney. SSAT overexpression was associated with increased levels of IGF-binding protein-2 (IGFBP-2) in the uterus and ovary, and a reduction in IGFBP-3 mRNA levels in the uterus. Exogenous spermidine and spermine elevated endogenous IGFBP-2 and SSAT mRNA abundance, whereas, putrescine stimulated IGFBP-2 mRNA abundance and transfected IGFBP-2 gene promoter activity in human (Hec-1-A) uterine cells. Sp1 and BTEB1 mRNAs that encode transcription factors for the IGFBP-2 gene also were induced in some ST tissues. The data suggest that SSAT and polyamines are important for the control of molecular pathways underlying reproductive tract tissue growth, phenotype, and function.

Tagami, S., J.-i. Inokuchi, et al. (2002). "Ganglioside GM3 Participates in the Pathological Conditions of Insulin Resistance." J. Biol. Chem. **277**(5): 3085-3092.

http://www.jbc.org/cgi/content/abstract/277/5/3085

Gangliosides are known as modulators of transmembrane signaling by regulating various receptor functions. We have found that insulin resistance induced by tumor necrosis factor-[alpha] (TNF-[alpha]) in 3T3-L1 adipocytes was accompanied by increased GM3 ganglioside expression caused by elevating GM3 synthase activity and its mRNA. We also demonstrated that TNF-[alpha] simultaneously produced insulin resistance by uncoupling insulin receptor activity toward insulin receptor substrate-1 (IRS-1) and suppressing insulin-sensitive glucose transport. Pharmacological depletion of GM3 in adipocytes by an inhibitor of glucosylceramide synthase prevented the TNF-[alpha]-induced defect in insulin-dependent tyrosine phosphorylation of IRS-1 and also counteracted the TNF-[alpha]-induced serine phosphorylation of IRS-1. Moreover, when the adipocytes were incubated with exogenous GM3, suppression of tyrosine phosphorylation of insulin receptor and IRS-1 and glucose uptake in response to insulin stimulation was observed, demonstrating that GM3 itself is able to mimic the effects of TNF on insulin signaling. We used the obese Zucker fa/fa rat and ob/ob mouse, which are known to overproduce TNF-[alpha] mRNA in adipose tissues, as typical models of insulin resistance. We found that the levels of GM3 synthase mRNA in adipose tissues of these animals were significantly higher than in their lean counterparts. Taken together, the increased synthesis of cellular GM3 by TNF may participate in the pathological conditions of insulin resistance in type 2 diabetes.

Cecchetto, G., S. Amillis, et al. (2004). "The AzgA Purine Transporter of Aspergillus nidulans: CHARACTERIZATION OF A PROTEIN BELONGING TO A NEW PHYLOGENETIC CLUSTER." J. Biol. Chem. **279**(5): 3132-3141.

http://www.jbc.org/cgi/content/abstract/279/5/3132

The azgA gene of Aspergillus nidulans encodes a hypoxanthine-adenine-guanine transporter. It has been cloned by a novel transposon methodology. The null phenotype of azgA was defined by a number of mutations, including a large deletion. In mycelia, the azgA gene is, like other genes of purine catabolism, induced by uric acid and repressed by ammonium. Its transcription depends on the pathway-specific UaY zinc binuclear cluster protein and the broad domain AreA GATA factor. AzgA is not closely related to any other characterized membrane protein, but many close homologues of unknown function are present in fungi, plants, and prokaryotes but not metazoa. Two of three data bases and the phylogeny presented in this article places proteins of this family in a cluster clearly separated (but perhaps phylogenetically related) from the NAT family that includes other eukaryotic and prokaryotic nucleobase transporters. Thus AzgA is the first characterized member of this family or subfamily of membrane proteins.

Lemercier, G., B. Espiau, et al. (2004). "A Pyrophosphatase Regulating Polyphosphate Metabolism in Acidocalcisomes Is Essential for Trypanosoma brucei Virulence in Mice." J. Biol. Chem. **279**(5): 3420-3425.

http://www.jbc.org/cgi/content/abstract/279/5/3420

We report the functional characterization of a soluble pyrophosphatase (TbVSP1), which localizes to acidocalcisomes, a vesicular acidic compartment of Trypanosoma brucei. Depending on the pH and the cofactors Mg2+ or Zn2+, both present in the compartment, the enzyme hydrolyzes either inorganic pyrophosphate (PPi) (kcat = 385 s-1) or tripolyP (polyP3) and polyphosphate (polyP) of 28 residues (polyP28) with kcat values of 52 and 3.5 s-1, respectively. An unusual N-terminal domain of 160 amino acids, containing a putative calcium EF-hand-binding domain, is involved in protein oligomerization. Using double-stranded RNA interference methodology, we produced an inducible bloodstream form (BF) deficient in the TbVSP1 protein (BFiVSP1). The long-chain polyP levels of these mutants were reduced by 60%. Their phenotypes revealed a deficient polyP metabolism, as indicated by their defective response to phosphate starvation and hyposmotic stress. BFiVSP1 did not cause acute virulent infection in mice, demonstrating that TbVSP1 is essential for growth of bloodstream forms in the mammalian host.

Lindvall, H., P. Nevsten, et al. (2004). "A Novel Hormone-sensitive Lipase Isoform Expressed in Pancreatic {beta}-Cells." J. Biol. Chem. **279**(5): 3828-3836.

http://www.jbc.org/cgi/content/abstract/279/5/3828

Hormone-sensitive lipase (HSL) is a key enzyme in fatty acid mobilization in many cell types. Two isoforms of HSL are known to date, namely HSLadi (84 kDa in rat) and HSLtes (130 kDa in rat). These are encoded by the same gene, with exons 1-9 encoding the parts that are common to both and an additional 5'-exon encoding the additional amino acids in HSLtes. HSL of various tissues, among these the islet of Langerhans, is larger than HSLadi, but not as large as HSLtes, indicating that there may be other 5'-coding exons. Here we describe the molecular basis for a novel 89-kDa HSL isoform that is expressed in {beta}-cells, adipocytes, adrenal glands, and ovaries in the rat and that is encoded by exons 1-9 and exon A, which is spliced to exon 1 and thereby introducing an upstream start codon. The additional 5'-base pairs encode a 43-amino acid peptide, which is highly positively charged. Conglomerates of HSL molecules are in close association with the secretory granules of the {beta}-cell, as determined by immunoelectron microscopy with antibodies targeting two separate regions of HSL. We have also determined that the human genomic sequence upstream of exon A has promoter activity in INS-1 cells as well as glucose sensing capability, mediating an increase in expression at high glucose concentration. The minimal promoter is present within 170 bp from the transcriptional start site and maximal glucose responsiveness is conferred by sequence within 850 bp from the transcriptional start site.

Papoucheva, E., A. Dumuis, et al. (2004). "The 5-Hydroxytryptamine(1A) Receptor Is Stably Palmitoylated, and Acylation Is Critical for Communication of Receptor with Gi Protein." J. Biol. Chem. **279**(5): 3280-3291.

http://www.jbc.org/cgi/content/abstract/279/5/3280

In the present study, we verified that the mouse 5-hydroxytryptamine(1A) (5-HT1A) receptor is modified by palmitic acid, which is covalently attached to the protein through a thioester-type bond. Palmitoylation efficiency was not modulated by receptor stimulation with agonists. Block of protein synthesis by cycloheximide resulted in a significant reduction of receptor acylation,

suggesting that palmitoylation occurs early after synthesis of the 5-HT1A receptor. Furthermore, pulse-chase experiments demonstrated that fatty acids are stably attached to the receptor. Two conserved cysteine residues 417 and 420 located in the proximal C-terminal domain were identified as acylation sites by site-directed mutagenesis. To address the functional role of 5-HT1A receptor acylation, we have analyzed the ability of acylation-deficient mutants to interact with heterotrimeric Gi protein and to modulate downstream effectors. Replacement of individual cysteine residues (417 or 420) resulted in a significantly reduced coupling of receptor with Gi protein and impaired inhibition of adenylyl cyclase activity. When both palmitoylated cysteines were replaced, the communication of receptors with G{alpha}i subunits was completely abolished. Moreover, non-palmitoylated mutants were no longer able to inhibit forskolin-stimulated cAMP formation, indicating that palmitoylation of the 5-HT1A receptor is critical for the enabling of Gi protein coupling/effector signaling. The receptor-dependent activation of extracellular signal-regulated kinase was also affected by acylation-deficient mutants, suggesting the importance of receptor palmitoylation for the signaling through the G{beta}{gamma}-mediated pathway, in addition to the G{alpha}i-mediated signaling.

Danielli, A., F. C. Kafatos, et al. (2003). "Cloning and Characterization of Four Anopheles gambiae Serpin Isoforms, Differentially Induced in the Midgut by Plasmodium berghei Invasion." J. Biol. Chem. **278**(6): 4184-4193.

http://www.jbc.org/cgi/content/abstract/278/6/4184

The genomic locus SRPN10 of the malaria vector Anopheles gambiae codes for four alternatively spliced serine protease inhibitors of the serpin superfamily. The four 40- to 42-kDa isoforms differ only at their C terminus, which bears the reactive site loop, and exhibit protein sequence similarity with other insect serpins and mammalian serpins of the ovalbumin family. Inhibition experiments with recombinant purified SRPN10 serpins reveal distinct and specific inhibitory activity of three isoforms toward different proteases. All isoforms are mainly expressed in the midgut but also in pericardial cells and hemocytes of the mosquito. The cellular localization of SRPN10 serpins is nucleocytoplasmic in pericardial cells, in hemocytes and in a hemocyte-like mosquito cell line, but in the gut the proteins are mostly localized in the nucleus. Although the transcript levels of all SRPN10 isoforms are marginally affected by bacterial challenge, the transcripts of two isoforms (KRAL and RCM) are induced in female mosquitoes in response to midgut invasion by Plasmodium berghei ookinetes. The KRAL and RCM SRPN10 isoforms represent new potential markers to study the ookinete midgut invasion process in anopheline mosquitoes.

Modregger, J., A. A. Schmidt, et al. (2003). "Characterization of Endophilin B1b, a Brain-specific Membrane-associated Lysophosphatidic Acid Acyl Transferase with Properties Distinct from Endophilin A1." J. Biol. Chem. **278**(6): 4160-4167.

http://www.jbc.org/cgi/content/abstract/278/6/4160

We have characterized mammalian endophilin B1, a novel member of the endophilins and a representative of their B subgroup. The endophilins B show the same domain organization as the endophilins A, which contain an N-terminal domain responsible for lipid binding and lysophosphatidic acid acyl transferase activity, a central coiled-coil domain for oligomerization, a less conserved linker region, and a C-terminal Src homology 3 (SH3) domain. The endophilin B1 gene gives rise to at least three splice variants, endophilin B1a, which shows a widespread tissue distribution, and endophilins B1b and B1c, which appear to be brain-specific. Endophilin B1, like endophilins A, binds to palmitoyl-CoA, exhibits lysophosphatidic acid acyl transferase activity, and interacts with dynamin, amphiphysins 1 and 2, and huntingtin. However, in contrast to endophilins A, endophilin B1 does not bind to synaptojanin 1 and synapsin 1, and overexpression of its SH3

domain does not inhibit transferrin endocytosis. Consistent with this, immunofluorescence analysis of endophilin B1b transfected into fibroblasts shows an intracellular reticular staining, which in part overlaps with that of endogenous dynamin. Upon subcellular fractionation of brain and transfected fibroblasts, endophilin B1 is largely recovered in association with membranes. Together, our results suggest that the action of the endophilins is not confined to the formation of endocytic vesicles from the plasma membrane, with endophilin B1 being associated with, and presumably exerting a functional role at, intracellular membranes.

Benezra, M., N. Chevallier, et al. (2003). "BRCA1 Augments Transcription by the NF-{kappa}B Transcription Factor by Binding to the Rel Domain of the p65/RelA Subunit." J. Biol. Chem. **278**(29): 26333-26341.

http://www.jbc.org/cgi/content/abstract/278/29/26333

BRCA1 is a tumor suppressor gene mutated in cases of hereditary breast and ovarian cancer. BRCA1 protein is involved in apoptosis and growth/tumor suppression. In this study, we present evidence that p65/RelA, one of the two subunits of the transcription factor NF-{kappa}B, binds to the BRCA1 protein. Treatment of 293T cells with the cytokine tumor necrosis factor-{alpha} induces an interaction between endogenous p65/RelA and BRCA1. GST-protein affinity assay experiments reveal that the Rel homology domain of the p65/RelA subunit of NF-{kappa}B interacts with multiple sites within the N-terminal region of BRCA1. Transient transfection of BRCA1 significantly enhances the ability of the tumor necrosis factor-{alpha} or interleukin-1{beta} to activate transcription from the promoters of NF-{kappa}B target genes. Mutation of the NF-{kappa}B-binding sites in the NF-{kappa}B reporter blocks the effect of BRCA1 on transcription. Also the ability of BRCA1 to activate NF-{kappa}B target genes is inhibited by a super-stable inhibitor of NF-{kappa}B and by the chemical inhibitor SN-50. These data indicate that BRCA1 acts as a co-activator with NF-{kappa}B. In addition, we show that cells infected with an adenovirus expressing BRCA1 up-regulate the endogenous expression of NF-{kappa}B target genes Fas and interferon-{beta}. Together, this information suggests that BRCA1 may play a role in cell life-death decisions following cell stress by modulation of the activity of NF-{kappa}B.

Unnikrishnan, I., S. Miller, et al. (2003). "Multiple Positive and Negative Elements Involved in the Regulation of Expression of GSY1 in Saccharomyces cerevisiae." J. Biol. Chem. **278**(29): 26450-26457.

http://www.jbc.org/cgi/content/abstract/278/29/26450

GSY1 is one of the two genes encoding glycogen synthase in Saccharomyces cerevisiae. Both the GSY1 message and the protein levels increased as cells approached stationary phase. A combination of deletion analysis and site-directed mutagenesis revealed a complex promoter containing multiple positive and negative regulatory elements. Expression of GSY1 was dependent upon the presence of a TATA box and two stress response elements (STREs). Expression was repressed by Mig1, which mediates responses to glucose, and Rox1, which mediates responses to oxygen. Characterization of the GSY1 promoter also revealed a novel negative element. This element, N1, can repress expression driven by either an STRE or a heterologous element, the UAS of CYC1. Repression by N1 is dependent on the number of these elements that are present, but is independent of their orientation. N1 repressed expression when placed either upstream or downstream of the UAS, although the latter position is more effective. Gel shift analysis detected a factor that appears to bind to the N1 element. The complexity of the GSY1 promoter, which includes two STREs and three distinct negative elements, was surprising. This complexity may allow GSY1 to respond to a wide range of environmental stresses.

Chipuk, J. E., L. V. Stewart, et al. (2002). "Identification and Characterization of A Novel Rat Ov-Serpin Family Member, Trespin." J. Biol. Chem. **277**(29): 26412-26421.

http://www.jbc.org/cgi/content/abstract/277/29/26412

Serpins are responsible for regulating a variety of proteolytic processes through a unique irreversible suicide substrate mechanism. To discover novel genes regulated by transforming growth factor-[beta]1 (TGF-[beta]1), we performed differential display reverse transcriptase-PCR analysis of NRP-152 rat prostatic epithelial cells and cloned a novel rat serpin that is transcriptionally down-regulated by TGF-[beta] and hence named trespin (TGF-[beta]-repressible serine proteinase inhibitor (trespin). Trespin is a 397-amino acid member of the ov-serpin clade with a calculated molecular mass of 45.2 kDa and 72% amino acid sequence homology to human bomapin; however, trespin exhibits different tissue expression, cellular localization, and proteinase specificity compared with bomapin. Trespin mRNA is expressed in many tissues, including brain, heart, kidney, liver, lung, prostate, skin, spleen, and stomach. FLAG-trespin expressed in HEK293 cells is localized predominantly in the cytoplasm and is not constitutively secreted. The presence of an arginine at the P1 position of trespin's reactive site loop suggests that trespin inhibits trypsin-like proteinases. Accordingly, in vitro transcribed and translated trespin forms detergent-stable and thermostable complexes with plasmin and elastase but not subtilisin A, trypsin, chymotrypsin, thrombin, or papain. Trespin interacts with plasmin at a near 1:1 stoichiometry, and immunopurified mammal-expressed trespin inhibits plasmin in a dose-dependent manner. These data suggest that trespin is a novel and functional member of the rat ov-serpin family.

Gelebart, P., T. Kovacs, et al. (2002). "Expression of Endomembrane Calcium Pumps in Colon and Gastric Cancer Cells. INDUCTION OF SERCA3 EXPRESSION DURING DIFFERENTIATION." J. Biol. Chem. **277**(29): 26310-26320.

http://www.jbc.org/cgi/content/abstract/277/29/26310

Calcium mobilization from the endoplasmic reticulum (ER) into the cytosol is a key component of several signaling networks controlling tumor cell growth, differentiation, or apoptosis. Sarco/endoplasmic reticulum calcium transport ATPases (SERCA-type calcium pumps), enzymes that accumulate calcium in the ER, play an important role in these phenomena. We report that SERCA3 expression is significantly reduced or lost in colon carcinomas when compared with normal colonic epithelial cells, which express this enzyme at a high level. To study the involvement of SERCA enzymes in differentiation, in this work differentiation of colon and gastric cancer cell lines was initiated, and the change in the expression of SERCA isoenzymes as well as intracellular calcium levels were investigated. Treatment of the tumor cells with butyrate or other established differentiation inducing agents resulted in a marked and specific induction of the expression of SERCA3, whereas the expression of the ubiquitous SERCA2 enzymes did not change significantly or was reduced. A similar marked increase in SERCA3 expression was found during spontaneous differentiation of post-confluent Caco-2 cells, and this closely correlated with the induction of other known markers of differentiation. Analysis of the expression of the SERCA3 alternative splice isoforms revealed induction of all three known iso-SERCA3 variants (3a, 3b, and 3c). Butyrate treatment of the KATO-III gastric cancer cells led to higher resting cytosolic calcium concentrations and, in accordance with the lower calcium affinity of SERCA3, to diminished ER calcium content. These data taken together indicate a defect in SERCA3 expression in colon cancers as compared with normal colonic epithelium, show that the calcium homeostasis of the endoplasmic reticulum may be remodeled during cellular differentiation, and indicate that SERCA3 constitutes an interesting new differentiation marker that may prove useful for the analysis of the phenotype of gastrointestinal adenocarcinomas.

Malhotra, J. D., M. C. Koopmann, et al. (2002). "Structural Requirements for Interaction of Sodium Channel beta 1 Subunits with Ankyrin." J. Biol. Chem. **277**(29): 26681-26688.

http://www.jbc.org/cgi/content/abstract/277/29/26681

Sodium channel [beta] subunits modulate channel kinetic properties and cell surface expression levels and function as cell adhesion molecules. [beta]1 and [beta]2 participate in homophilic cell adhesion resulting in ankyrin recruitment to cell contact sites. We hypothesized that a tyrosine residue in the cytoplasmic domain of [beta]1 may be important for ankyrin recruitment and tested our hypothesis using [beta]1 mutants replacing Tyr181 with alanine ([beta]1Y181A), phenylalanine ([beta]1Y181F), or glutamate ([beta]1Y181E), or a truncated construct deleting all residues beyond Tyr181 ([beta]1L182STOP). Ankyrin recruitment was observed in [beta]1L182STOP, showing that residues Ile166-Tyr181 contain the major ankyrin recruiting activity of [beta]1. Ankyrin recruitment was abolished in [beta]1Y181E, suggesting that tyrosine phosphorylation of [beta]1 may inhibit [beta]1-ankyrin interactions. AnkyrinG and [beta]1 associate in rat brain membranes and in transfected cells expressing [beta]1 and ankyrinG in the absence of sodium channel [alpha] subunits. [beta]1 subunits are recognized by anti-phosphotyrosine antibodies following treatment of these cell lines with fibroblast growth factor. [beta]1 and ankryinG association is not detectable in cells following treatment with fibroblast growth factor. AnkyrinG and [beta]1Y181E do not associate even in the absence of fibroblast growth factor treatment. [beta]1 subunit-mediated cell adhesion and ankyrin recruitment may contribute to sodium channel placement at nodes of Ranvier. The phosphorylation state of [beta]1Y181 may be a critical regulatory step in these developmental processes.

Lu, K. P. and K. S. Ramos (2003). "Redox Regulation of a Novel L1Md-A2 Retrotransposon in Vascular Smooth Muscle Cells." J. Biol. Chem. **278**(30): 28201-28209.

http://www.jbc.org/cgi/content/abstract/278/30/28201

Activation and reintegration of retrotransposons into the genome is linked to several diseases in human and rodents, but mechanisms of gene activation remain largely unknown. Here we identify a novel gene of L1Md-A2 lineage in vascular smooth muscle cells and show that environmental hydrocarbons enhance gene expression and activate monomer-driven transcription via a redox-sensitive mechanism. Site-directed mutagenesis and progressive deletion analyses identified two antioxidant/electrophile response-like elements (5'-GTGACTCGAGC-3') within the A2/3 and A3 region. These elements mediated activation, with the A3 monomer playing an essential role in transactivation. This signaling pathway may contribute to gene instability during the course of atherogenesis.

Torres, B., G. Porras, et al. (2003). "Regulation of the mhp Cluster Responsible for 3-(3-Hydroxyphenyl)propionic Acid Degradation in Escherichia coli." J. Biol. Chem. **278**(30): 27575-27585.

http://www.jbc.org/cgi/content/abstract/278/30/27575

The mhp gene cluster from Escherichia coli constitutes a model system to study bacterial degradation of 3-(3-hydroxyphenyl)propionic acid (3HPP). In this work the regulation of the inducible mhp catabolic genes has been studied by genetic and biochemical approaches. The Pr and Pa promoters, which control the expression of the divergently transcribed mhpR regulatory

gene and mhp catabolic genes, respectively, show a peculiar arrangement leading to transcripts that are complementary at their 5'-ends. By using Pr-lacZ and Pa-lacZ translational fusions and gel retardation assays, we have shown that the mhpR gene product behaves as a 3HPP-dependent activator of the Pa promoter, being the expression from Pr constitutive and MhpR-independent. DNase I footprinting experiments and mutational analysis mapped an MhpR-protected region, centered at position -58 with respect to the Pa transcription start site, which is indispensable for MhpR binding and in vivo activation of the Pa promoter. Superimposed in the specific MhpR-mediated regulation of the Pa promoter, we have observed a strict catabolite repression control carried out by the cAMP receptor protein (CRP) that allows expression of the mhp catabolic genes when the preferred carbon source (glucose) is not available and 3HPP is present in the medium. Gel retardation assays revealed that the specific activator, MhpR, is essential for the binding of the second activator, CRP, to the Pa promoter. Such peculiar synergistic transcription activation has not yet been observed in other aromatic catabolic pathways, and the MhpR activator becomes the first member of the IclR family of transcriptional regulators that is indispensable for recruiting CRP to the target promoter.

Bennin, D. A., A. S. A. Don, et al. (2002). "Cyclin G2 Associates with Protein Phosphatase 2A Catalytic and Regulatory B' Subunits in Active Complexes and Induces Nuclear Aberrations and a G1/S Phase Cell Cycle Arrest." J. Biol. Chem. **277**(30): 27449-27467.

http://www.jbc.org/cgi/content/abstract/277/30/27449

Cyclin G2, together with cyclin G1 and cyclin I, defines a novel cyclin family expressed in terminally differentiated tissues including brain and muscle. Cyclin G2 expression is up-regulated as cells undergo cell cycle arrest or apoptosis in response to inhibitory stimuli independent of p53 (Horne, M., Donaldson, K., Goolsby, G., Tran, D., Mulheisen, M., Hell, J. and Wahl, A. (1997) J. Biol. Chem. 272, 12650-12661). We tested the hypothesis that cyclin G2 may be a negative regulator of cell cycle progression and found that ectopic expression of cyclin G2 induces the formation of aberrant nuclei and cell cycle arrest in HEK293 and Chinese hamster ovary cells. Cyclin G2 is primarily partitioned to a detergent-resistant compartment, suggesting an association with cytoskeletal elements. We determined that cyclin G2 and its homolog cyclin G1 directly interact with the catalytic subunit of protein phosphatase 2A (PP2A). An okadaic acid-sensitive (<2 nM) phosphatase activity coprecipitates with endogenous and ectopic cyclin G2. We found that cyclin G2 also associates with various PP2A B' regulatory subunits, as previously shown for cyclin G1. The PP2A/A subunit is not detectable in cyclin G2-PP2A-B'-C complexes. Notably, cyclin G2 colocalizes with both PP2A/C and B' subunits in detergent-resistant cellular compartments, suggesting that these complexes form in living cells. The ability of cyclin G2 to inhibit cell cycle progression correlates with its ability to bind PP2A/B' and C subunits. Together, our findings suggest that cyclin G2-PP2A complexes inhibit cell cycle progression.

Gu, B. J., R. Sluyter, et al. (2004). "An Arg307 to Gln Polymorphism within the ATP-binding Site Causes Loss of Function of the Human P2X7 Receptor." J. Biol. Chem. **279**(30): 31287-31295.

http://www.jbc.org/cgi/content/abstract/279/30/31287

The P2X7 receptor is a ligand-gated channel that is highly expressed on mononuclear cells of the immune system and that mediates ATP-induced apoptosis. Wide variations in the function of the P2X receptor have been observed, explained in part by 7loss-of-function polymorphisms that change Glu496 to Ala (E496A) and Ile568 to Asn (I568N). In this study, a third polymorphism, which substitutes an uncharged glutamine for the highly positively charged Arg307 (R307Q), has been found in heterozygous dosage in 12 of 420 subjects studied. P2X7 function was measured by ATP-induced fluxes of Rb+, Ba2+, and ethidium+ into peripheral blood monocytes or various

lymphocyte subsets and was either absent or markedly decreased. Transfection experiments showed that P2X7 carrying the R307Q mutation lacked either channel or pore function despite robust protein synthesis and surface expression of the receptor. The monoclonal antibody (clone L4) that binds to the extracellular domain of wild type P2X7 and blocks P2X7 function failed to bind to the R307Q mutant receptor. Differentiation of monocytes to macrophages up-regulated P2X7 function in cells heterozygous for the R307Q to a value 10-40% of that for wild type macrophages. However, macrophages from a subject who was double heterozygous for R307Q/I568N remained totally non-functional for P2X7, and lymphocytes from the same subject also lacked ATP-stimulated phospholipase D activity. These data identify a third loss-of-function polymorphism affecting the human P2X7 receptor, and since the affected Arg307 is homologous to those amino acids essential for ATP binding to P2X1 and P2X2, it is likely that this polymorphism abolishes the binding of ATP to the extracellular domain of P2X7.

Sorger, D., K. Athenstaedt, et al. (2004). "A Yeast Strain Lacking Lipid Particles Bears a Defect in Ergosterol Formation." J. Biol. Chem. **279**(30): 31190-31196.

http://www.jbc.org/cgi/content/abstract/279/30/31190

Lipid particles of the yeast Saccharomyces cerevisiae are storage compartments for triacylglycerols (TAG) and steryl esters (STE). Four gene products, namely the TAG synthases Dga1p and Lro1p, and the STE synthases Are1p and Are2p contribute to storage lipid synthesis. A yeast strain lacking the four respective genes is devoid of lipid particles thus providing a valuable tool to study the physiological role of storage lipids and lipid particles. Using a dga1lro1are1are2 quadruple mutant transformed with plasmids bearing inducible DGA1, LRO1, or ARE2 we demonstrate that TAG synthesis contributes more efficiently to lipid particle proliferation than synthesis of STE. Moreover, we show that proteins typically located to lipid particles in wild type such as Erg1p, Erg6p, Erg7p, and Ayr1p are refined to microsomal fractions of the dga1lro1are1are2 quadruple mutant. This result confirms the close relationship between lipid particles and endoplasmic reticulum. Most interestingly, the amount of the squalene epoxidase Erg1p, which is dually located in lipid particles and endoplasmic reticulum of wild type, is decreased in the quadruple mutant, whereas amounts of other lipid particle proteins tested were not reduced. This decrease is not caused by down-regulation of ERG1 transcription but by the low stability of Erg1p in the quadruple mutant. Because a similar effect was also observed in are1are2 mutants this finding can be mainly attributed to the lack of STE. The quadruple mutant, however, was more sensitive to terbinafine, an inhibitor of Erg1p, than the are1are2 strain suggesting that the presence of TAG and/or intact lipid particles has an additional protective effect. In a strain lacking the two STE synthases, Are1p and Are2p, incorporation of ergosterol into the plasma membrane was reduced, although the total cellular amount of free ergosterol was higher in the mutant than in wild type. Thus, an esterification/deacylation mechanism appears to contribute to the supply of ergosterol to the plasma membrane.

Beaulieu, N., S. Morin, et al. (2002). "An Essential Role for DNA Methyltransferase DNMT3B in Cancer Cell Survival." J. Biol. Chem. **277**(31): 28176-28181.

http://www.jbc.org/cgi/content/abstract/277/31/28176

Abnormal methylation and associated silencing of tumor suppressor genes is a common feature of many types of cancers. The observation of persistent methylation in human cancer cells lacking the maintenance methyltransferase DNMT1 suggests the involvement of other DNA methyltransferases in gene silencing in cancer. To test this hypothesis, we have evaluated methylation and gene expression in cancer cells specifically depleted of DNMT3A or DNMT3B, de novo methyltransferases that are expressed in adult tissues. Here we have shown that

depletion of DNMT3B, but not DNMT3A, induced apoptosis of human cancer cells but not normal cells. DNMT3B depletion reactivated methylation-silenced gene expression but did not induce global or juxtacentromeric satellite demethylation as did specific depletion of DNMT1. Furthermore, the effect of DNMT3B depletion was rescued by exogenous expression of either of the splice variants DNMT3B2 or DNMT3B3 but not DNMT1. These results indicate that DNMT3B has significant site selectivity that is distinct from DNMT1, regulates aberrant gene silencing, and is essential for cancer cell survival.

Jurado, L. A., S. Song, et al. (2002). "Conserved Amino Acids within CCAAT Enhancer-binding Proteins (C/EBPalpha and beta) Regulate Phosphoenolpyruvate Carboxykinase (PEPCK) Gene Expression." J. Biol. Chem. **277**(31): 27606-27612.

http://www.jbc.org/cgi/content/abstract/277/31/27606

Thyroid hormone and cAMP stimulate transcription of the gene for phosphoenolpyruvate carboxykinase (PEPCK). CCAAT enhancer-binding proteins (C/EBP[alpha] and [beta]) are involved in multiple aspects of the nutritional, developmental and hormonal regulation of PEPCK gene expression. Previously, we have identified a thyroid hormone response element in the PEPCK promoter and demonstrated that C/EBP proteins bound to the P3(I) site are participants in the induction of PEPCK gene expression by thyroid hormone and cAMP. Here, we identify several peptide regions within the transactivation domain of C/EBP[alpha] that enhance the ability of T3 to stimulate gene transcription. We also demonstrate that several conserved amino acids in the transactivation domain of C/EBP[alpha] and C/EBP[beta] are required for the stimulation of basal gene expression and identify amino acids within C/EBP[beta] that participate in the cAMP induction of the PEPCK gene. Finally, we show that the CREB-binding protein (CBP) enhanced the induction of PEPCK gene transcription by thyroid hormone and that CBP is associated with the PEPCK gene in vivo. Our results indicate that both C/EBP proteins and CBP participate in the regulation of PEPCK gene transcription by thyroid hormone.

Kubota, H., R. W. Storms, et al. (2002). "Variant Forms of alpha -Fetoprotein Transcripts Expressed in Human Hematopoietic Progenitors. IMPLICATIONS FOR THEIR DEVELOPMENTAL POTENTIAL TOWARDS ENDODERM." J. Biol. Chem. **277**(31): 27629-27635.

http://www.jbc.org/cgi/content/abstract/277/31/27629

Hematopoietic stem cells have been identified as multipotent cells that give rise to all adult hematopoietic lineages. Although the hematopoietic lineage is derived from the mesodermal germ layer in the embryo, recent data suggest that bone marrow cells with an antigenic profile consistent with that of hematopoietic stem cells can also differentiate to cell types of the endodermal lineages, such as hepatocytes. However, the molecular mechanisms associated with these events are entirely unknown. For decades, [alpha]-fetoprotein (AFP) has been used as a differentiation marker for endodermal cells, because it was thought that the transcription of AFP mRNA is tightly regulated in a developmental and tissue-specific process. In this report we describe two new variant forms of AFP transcripts in human hematopoietic progenitors that are not expressed in mature cells. The variant AFP (vAFP) cDNA sequences isolated from a multipotent hematopoietic cell line, K562, revealed that the vAFP differed from the authentic transcript, consisting of 15 exons, by replacing exon 1 of AFP with one or two exons located in the 5'-untranslated region of the AFP gene. In addition to the K562 cell line, vAFP transcripts were detected in normal bone marrow, thymus, and brain but were not detected in normal spleen, intestine, liver, or the hepatocellular carcinoma cell line, HepG2. This suggests expression in normal hematopoietic progenitors. This hypothesis was confirmed by the finding that CD34+Lin[-] hematopoietic progenitor cells purified from cord blood by flow cytometric sorting also expressed

the variant transcripts. These results suggest that some hematopoietic progenitors are in a state that permits them to express certain types of transcripts that have been considered unique to endoderm.

Ohya, T., Y. Kawasaki, et al. (2002). "The DNA Polymerase Domain of polepsilon Is Required for Rapid, Efficient, and Highly Accurate Chromosomal DNA Replication, Telomere Length Maintenance, and Normal Cell Senescence in Saccharomyces cerevisiae." J. Biol. Chem. **277**(31): 28099-28108.

http://www.jbc.org/cgi/content/abstract/277/31/28099

Saccharomyces cerevisiae POL2 encodes the catalytic subunit of DNA polymerase [epsilon]. This study investigates the cellular functions performed by the polymerase domain of Pol2p and its role in DNA metabolism. The pol2-16 mutation has a deletion in the catalytic domain of DNA polymerase [epsilon] that eliminates its polymerase and exonuclease activities. It is a viable mutant, which displays temperature sensitivity for growth and a defect in elongation step of chromosomal DNA replication even at permissive temperatures. This mutation is synthetic lethal in combination with temperature-sensitive mutants or the 3'- to 5'-exonuclease-deficient mutant of DNA polymerase [delta] in a haploid cell. These results suggest that the catalytic activity of DNA polymerase [epsilon] participates in the same pathway as DNA polymerase [delta], and this is consistent with the observation that DNA polymerases [delta] and [epsilon] colocalize in some punctate foci on yeast chromatids during S phase. The pol2-16 mutant senesces more rapidly than wild type strain and also has shorter telomeres. These results indicate that the DNA polymerase domain of Pol2p is required for rapid, efficient, and highly accurate chromosomal DNA replication in yeast.

Inacio, A., A. L. Silva, et al. (2004). "Nonsense Mutations in Close Proximity to the Initiation Codon Fail to Trigger Full Nonsense-mediated mRNA Decay." J. Biol. Chem. **279**(31): 32170-32180.

http://www.jbc.org/cgi/content/abstract/279/31/32170

Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that degrades mRNAs containing premature translation termination codons. In mammalian cells, a termination codon is ordinarily recognized as "premature" if it is located greater than 50-54 nucleotides 5' to the final exon-exon junction. We have described a set of naturally occurring human {beta}-globin gene mutations that apparently contradict this rule. The corresponding {beta}-thalassemia genes contain nonsense mutations within exon 1, and yet their encoded mRNAs accumulate to levels approaching wild-type {beta}-globin ({beta}WT) mRNA. In the present report we demonstrate that the stabilities of these mRNAs with nonsense mutations in exon 1 are intermediate between {beta}WT mRNA and {beta}-globin mRNA carrying a prototype NMD-sensitive mutation in exon 2 (codon 39 nonsense; {beta}39). Functional analyses of these mRNAs with 5'-proximal nonsense mutations demonstrate that their relative resistance to NMD does not reflect abnormal RNA splicing or translation re-initiation and is independent of promoter identity and erythroid specificity. Instead, the proximity of the nonsense codon to the translation initiation AUG constitutes a major determinant of NMD. Positioning a termination mutation at the 5' terminus of the coding region blunts mRNA destabilization, and this effect is dominant to the "50-54 nt boundary rule." These observations impact on current models of NMD.

Wang, X.-D., J. Shou, et al. (2004). "Notch1-expressing Cells Are Indispensable for Prostatic Branching

Morphogenesis during Development and Re-growth Following Castration and Androgen Replacement." J. Biol. Chem. **279**(23): 24733-24744.

http://www.jbc.org/cgi/content/abstract/279/23/24733

Notch expression is frequently associated with progenitor cells, and its function is crucial for development. Our recent work showing that Notch1 is selectively expressed in basal epithelial cells of the prostate and higher Notch1 expression during development suggests that Notch1-expressing cells may define progenitor cells in the prostate. To test this hypothesis, we have generated a transgenic mouse line in which the Notch1-expressing cells can be ablated in a controlled manner. Specific targeting was achieved by expressing the bacterial nitroreductase, an enzyme that catalyzes its substrate into a cytotoxin capable of inducing apoptosis, under the Notch1 promoter. Cell death in transgenic prostate was confirmed by histological analyses including terminal dUTP nick-end labeling and caspase 3 immunocytochemical staining. We evaluated the consequences of ablation of Notch1-expressing cells in two systems, organ culture of early postnatal prostates and re-growth of prostate in castrated mice triggered by hormone replacement. Our data show that elimination of Notch1-expressing cells inhibited the branching morphogenesis, growth, and differentiation of early postnatal prostate in culture and impaired prostate re-growth triggered by hormone replacement in castrated mice. Furthermore, we found that Notch1 expression following castration and hormone replacement was concomitant with known basal cell markers p63 and cytokeratin 14 and was high in the proliferative human prostate epithelial cells. Taken together, these data suggest that Notch1-expressing cells define the progenitor cells in the prostatic epithelial cell lineage, which are indispensable for prostatic development and re-growth.

Lim, I. A., D. D. Hall, et al. (2002). "Selectivity and Promiscuity of the First and Second PDZ Domains of PSD-95 and Synapse-associated Protein 102." J. Biol. Chem. **277**(24): 21697-21711.

http://www.jbc.org/cgi/content/abstract/277/24/21697

PDZ domains typically interact with the very carboxyl terminus of their binding partners. Type 1 PDZ domains usually require valine, leucine, or isoleucine at the very COOH-terminal (P0) position, and serine or threonine 2 residues upstream at P[-]2. We quantitatively defined the contributions of carboxyl-terminal residues to binding selectivity of the prototypic interactions of the PDZ domains of postsynaptic density protein 95 (PSD-95) and its homolog synapse-associated protein 90 (SAP102) with the NR2b subunit of the N-methyl-D-aspartate-type glutamate receptor. Our studies indicate that all of the last five residues of NR2b contribute to the binding selectivity. Prominent were a requirement for glutamate or glutamine at P[-]3 and for valine at P0 for high affinity binding and a preference for threonine over serine at P[-]2, in the context of the last 11 residues of the NR2b COOH terminus. This analysis predicts a COOH-terminal (E/Q)(S/T)XV consensus sequence for the strongest binding to the first two PDZ domains of PSD-95 and SAP102. A search of the human genome sequences for proteins with a COOH-terminal (E/Q)(S/T)XV motif yielded 50 proteins, many of which have not been previously identified as PSD-95 or SAP102 binding partners. Two of these proteins, brain-specific angiogenesis inhibitor 1 and protein kinase C[alpha], co-immunoprecipitated with PSD-95 and SAP102 from rat brain extracts.

Marchesini, N., W. Osta, et al. (2004). "Role for Mammalian Neutral Sphingomyelinase 2 in Confluence-induced Growth Arrest of MCF7 Cells." J. Biol. Chem. **279**(24): 25101-25111.

http://www.jbc.org/cgi/content/abstract/279/24/25101

Recently, we reported that neutral sphingomyelinase 2 (nSMase2) functions as a bona fide neutral sphingomyelinase and that overexpression of nSMase2 in MCF7 breast cancer cells caused a decrease in cell growth (Marchesini, N., Luberto, C., and Hannun, Y. A. (2003) J. Biol. Chem. 278, 13775-13783). In this study, the role of endogenous nSMase2 in regulating growth arrest was investigated. The results show that endogenous nSMase2 mRNA was up-regulated [~]5-fold when MCF7 cells became growth-arrested at confluence, and total neutral SMase activity was increased by 119 {+/-} 41% with respect to control. Cell cycle analysis showed that up-regulation of endogenous nSMase2 correlated with G0/G1 cell cycle arrest and an increase in total ceramide levels (2.4-fold). Analysis of ceramide species showed that confluence caused selective increases in very long chain ceramide C24:1 (370 {+/-} 54%) and C24:0 (266 {+/-} 81%) during arrest. The role of endogenous nSMase2 in growth regulation and ceramide metabolism was investigated using short interfering RNA (siRNA)-mediated loss-of-function analysis. Down-regulation of nSMase2 with specific siRNA increased the cell population of cells in S phase of the cell cycle by 59 {+/-} 14% and selectively reverted the effects of growth arrest on the increase in levels of very long chain ceramides. Mechanistically, confluence arrest also induced hypophosphorylation of the retinoblastoma protein (6-fold) and induction of p21WAF1 (3-fold). Down-regulation of nSMase2 with siRNA largely prevented the dephosphorylation of the retinoblastoma protein and the induction of p21WAF1, providing a link between the action of nSMase2 and key regulators of cell cycle progression. Moreover, studies on nSMase2 localization in MCF7 cells showed that nSMase2 distributed throughout the cells in subconfluent, proliferating cultures. In contrast, nSMase2 became nearly exclusively located at the plasma membrane in confluent, contact-inhibited cells. Hence, we demonstrate for the first time that nSMase2 functions as a growth suppressor in MCF7 cells, linking confluence to the G0/G1 cell cycle check point.

Greenwood, I. A., L. J. Miller, et al. (2002). "The Large Conductance Potassium Channel beta -Subunit Can Interact with and Modulate the Functional Properties of a Calcium-activated Chloride Channel, CLCA1." J. Biol. Chem. **277**(25): 22119-22122.

http://www.jbc.org/cgi/content/abstract/277/25/22119

We have recently compared the biophysical and pharmacological properties of native Ca2+-activated Cl[-] currents in murine portal vein with mCLCA1 channels cloned from murine portal vein myocytes (Britton, F. C., Ohya, S., Horowitz, B., and Greenwood, I. A. (2002) J. Physiol. (Lond.) 539, 107-117). These channels shared a similar relative permeability to various anions, but the expressed channel current lacked the marked time dependence of the native current. In addition, the expressed channel showed a lower Ca2+ sensitivity than the native channel. As non-pore-forming regulatory [beta]-subunits alter the kinetics and increase the Ca2+ sensitivity of Ca2+-dependent K+ channels (BK channels) we investigated whether co-expression of [beta]-subunits with CLCA1 would alter the kinetics/Ca2+ sensitivity of mCLCA1. Internal dialysis of human embryonic kidney cells stably expressing CLCA1 with 500 nM Ca2+ evoked a significantly larger current when the [beta]-subunit KCNMB1 was co-expressed. In a small number of co-transfected cells marked time dependence to the activation kinetics was observed. Interaction studies using the mammalian two-hybrid technique demonstrated a physical association between CLCA1 and KCNMB1 when co-expressed in human embryonic kidney cells. These data suggest that activation of CLCA1 can be modified by accessory subunits.

Ma, X., J. Hu, et al. (2002). "Mutational Analysis of Human Thioredoxin Reductase 1. EFFECTS ON p53-MEDIATED GENE EXPRESSION AND INTERFERON AND RETINOIC ACID-INDUCED CELL DEATH." J. Biol. Chem. **277**(25): 22460-22468.

http://www.jbc.org/cgi/content/abstract/277/25/22460

The interferon (IFN)-[beta] and all-trans-retinoic acid combination suppresses tumor growth by inducing apoptosis in several tumor cell lines. A genetic technique permitted the isolation of human thioredoxin reductase (TR) as a critical regulator of IFN/all-trans-retinoic acid-induced cell death. Our recent studies have shown that TR1:thioredoxin 1-regulated cell death is effected in part through the activation of p53-dependent responses. To understand its death regulatory function, we have performed a mutational analysis of TR. Human TR1 has three major structural domains, the FAD binding domain, the NADPH binding domain, and an interface domain (ID). Here, we show that the deletion of the C-terminal interface domain results in a constitutive activation of TR-dependent death responses and promotes p53-dependent gene expression. TR mutant without the ID still retains its dependence on thioredoxin for promoting these responses. Thus, our data suggest that TR-ID acts as a regulatory domain.

Warren, C. E., A. Krizus, et al. (2002). "The Caenorhabditis elegans Gene, gly-2, Can Rescue the N-Acetylglucosaminyltransferase V Mutation of Lec4 Cells." J. Biol. Chem. **277**(25): 22829-22838.

http://www.jbc.org/cgi/content/abstract/277/25/22829

UDP-N-acetylglucosamine:[alpha]-6-D-mannoside [beta]-1,6-N-acetylglucosaminyltransferase V (GlcNAc-TV) is a regulator of polylactosamine-containing N-glycans and is causally involved in T cell regulation and tumor metastasis. The Caenorhabditis elegans genome contains a single orthologous gene, gly-2, that is transcribed and encodes a 669-residue type II membrane protein that is 36.7% identical to mammalian GlcNAc-TV (Mgat-5). Recombinant GLY-2 possessed GlcNAc-TV activity when assayed in vitro, and protein truncations demonstrated that the N-terminal boundary of the catalytic domain is Ile-138. gly-2 complemented the Phaseolus vulgaris leucoagglutinin binding defect of Chinese hamster ovary Lec4 cells, whereas GLY-2(L116R), an equivalent mutation to that which causes the Lec4A phenotype, could not. We conclude that the worm gene is functionally interchangeable with the mammalian form. GlcNAc-TV activity was detected in wild-type animals but not those homozygous for a deletion allele of gly-2. Activity was restored in mutant animals by an extrachromosomal array that encompassed the gly-2 gene. Green fluorescent protein reporter transgenes driven by the gly-2 promoter were expressed by developing embryos from the late comma stage onward, present in a complex subset of neurons in larvae and, in addition, the spermathecal and pharyngeal-intestinal valves and certain vulval cells of adults. However, no overt phenotypes were observed in animals homozygous for deletion alleles of gly-2.

Johnson, D. E. and C. C. Richardson (2003). "A Covalent Linkage between the Gene 5 DNA Polymerase of Bacteriophage T7 and Escherichia coli Thioredoxin, the Processivity Factor: FATE OF THIOREDOXIN DURING DNA SYNTHESIS." J. Biol. Chem. **278**(26): 23762-23772.

http://www.jbc.org/cgi/content/abstract/278/26/23762

Gene 5 protein (gp5) of bacteriophage T7 is a non-processive DNA polymerase, which acquires high processivity by binding to Escherichia coli thioredoxin. The gene 5 protein-thioredoxin complex (gp5/trx) polymerizes thousands of nucleotides before dissociating from a primer-template. We have engineered a disulfide linkage between the gene 5 protein and thioredoxin within the binding surface of the two proteins. The polymerase activity of the covalently linked complex (gp5-S-S-trx) is similar to that of gp5/trx on poly(dA)/oligo(dT). However, gp5-S-S-trx has only one third the polymerase activity of gp5/trx on single-stranded M13 DNA. gp5-S-S-trx has difficulty polymerizing nucleotides through sites of secondary structure on M13 DNA and stalls at these sites, resulting in lower processivity. However, gp5-S-S-trx has an identical processivity and rate of elongation when E. coli single-stranded DNA-binding protein (SSB protein) is used to remove secondary structure from M13 DNA. Upon completing synthesis on a DNA template

lacking secondary structure, both complexes recycle intact, without dissociation of the processivity factor, to initiate synthesis on a new DNA template. However, a complex stalled at secondary structure becomes unstable, and both subunits dissociate from each other as the polymerase prematurely releases from M13 DNA.

Osada, M., S. Imaoka, et al. (2002). "NADPH-Cytochrome P-450 Reductase in the Plasma Membrane Modulates the Activation of Hypoxia-inducible Factor 1." J. Biol. Chem. **277**(26): 23367-23373.

http://www.jbc.org/cgi/content/abstract/277/26/23367

Hypoxia induces a group of physiologically important genes that include erythropoietin (EPO) and vascular endothelial growth factor (VEGF). Hypoxia-inducible factor 1 (HIF-1) was identified as a hypoxia-activated transcription factor; however, the molecular mechanisms that underlie hypoxia signal transduction in mammalian cells remain undefined. In this study, we found that a flavoprotein, NADPH-P450 reductase (NPR), could regulate the induction of EPO mRNA under hypoxic conditions. Hypoxic EPO mRNA induction in Hep3B cells was inhibited by diphenyleneiodonium chloride, which is an inhibitor of NADPH-dependent enzymes. NPR antisense cDNA was transfected into Hep3B cells, and NPR-deficient hepatocyte cells (NPR[-] cells) were established. NPR[-] cells lacked EPO induction under hypoxia, and HIF-1[alpha] in NPR[-] cells did not respond to either transcriptional activation or translocation to the nucleus based on electrophoretic mobility shift assays and reporter gene assay including hypoxia response element. In contrast, NPR overexpression in Hep3B cells enhanced the DNA binding activity of HIF-1[alpha] by luciferase reporter gene assay. A study with HeLa S3 cells produced the same results. Furthermore, anti-NPR IgG inhibited EPO induction. EPO induction inhibited by diphenyleneiodonium chloride was recovered by bovine serum albumin-NADPH (a covalent binding complex of bovine serum albumin and NADPH) as well as NADPH. These results suggested that NPR located at the plasma membrane regulates EPO expression in hypoxia, including HIF-1 activation and translocation. We further studied the expression of NPR and VEGF mRNAs in human tumor tissues and found that the NPR mRNA levels were correlated with the VEGF mRNA levels, suggesting that NPR might be an important factor in the hypoxic induction of genes such as VEGF in vivo.

Wehage, E., J. Eisfeld, et al. (2002). "Activation of the Cation Channel Long Transient Receptor Potential Channel 2 (LTRPC2) by Hydrogen Peroxide. A SPLICE VARIANT REVEALS A MODE OF ACTIVATION INDEPENDENT OF ADP-RIBOSE." J. Biol. Chem. **277**(26): 23150-23156.

http://www.jbc.org/cgi/content/abstract/277/26/23150

LTRPC2 is a cation channel recently reported to be activated by adenosine diphosphate-ribose (ADP-ribose) and NAD. Since ADP-ribose can be formed from NAD and NAD is elevated during oxidative stress, we studied whole cell currents and increases in the intercellular free calcium concentration ([Ca2+]i) in long transient receptor potential channel 2 (LTRPC2)-transfected HEK 293 cells after stimulation with hydrogen peroxide (H2O2). Cation currents carried by monovalent cations and Ca2+ were induced by H2O2 (5 mM in the bath solution) as well as by intracellular ADP-ribose (0.3 mM in the pipette solution) but not by NAD (1 mM). H2O2-induced currents developed slowly after a characteristic delay of 3-6 min and receded after wash-out of H2O2. [Ca2+]i was rapidly increased by H2O2 in LTRPC2-transfected cells as well as in control cells; however, in LTRPC2-transfected cells, H2O2 evoked a second delayed rise in [Ca2+]i. A splice variant of LTRPC2 with a deletion in the C terminus (amino acids 1292-1325) was identified in neutrophil granulocytes. This variant was stimulated by H2O2 as the wild type. However, it did not respond to ADP-ribose. We conclude that activation of LTRPC2 by H2O2 is independent of ADP-ribose and that LTRPC2 may mediate the influx of Na+ and Ca2+ during oxidative stress, such as

the respiratory burst in granulocytes.

Yasukawa, K., D. Sawamura, et al. (2002). "Dominant and Recessive Compound Heterozygous Mutations in Epidermolysis Bullosa Simplex Demonstrate the Role of the Stutter Region in Keratin Intermediate Filament Assembly." J. Biol. Chem. **277**(26): 23670-23674.

http://www.jbc.org/cgi/content/abstract/277/26/23670

Keratin intermediate filaments are important cytoskeletal structural proteins involved in maintaining cell shape and function. Mutations in the epidermal keratin genes, keratin 5 or keratin 14 lead to the disruption of keratin filament assembly, resulting in an autosomal dominant inherited blistering skin disease, epidermolysis bullosa simplex (EBS). We investigated a large EBS kindred who exhibited a markedly heterogeneous clinical presentation and detected two distinct keratin 5 mutations in the proband, the most severely affected. One missense mutation (E170K) in the highly conserved helix initiation peptide sequence of the 1A rod domain was found in all the affected family members. In contrast, the other missense mutation (E418K) was found only in the proband. The E418K mutation was located in the stutter region, an interruption in the heptad repeat regularity, whose function as yet remains unclear. We hypothesized that this mutated stutter allele was clinically silent when combined with the wild type allele but aggravates the clinical severity of EBS caused by the E170K mutation on the other allele. To confirm this in vitro, we transfected mutant keratin 5 cDNA into cultured cells. Although only 12.7% of the cells transfected with the E170K mutation alone showed disrupted keratin filament aggregations, significantly more cells (30.0%) cotransfected with both E170K and E418K mutations demonstrated keratin aggregation ($p < 0.05$). These transfection assay results corresponded to the heterogeneous clinical findings of the EBS patient in this kindred. We have identified the first case of both compound heterozygous dominant (E170K) and recessive (E418K) mutations in any keratin gene and confirmed the significant involvement of the stutter region in the assembly and organization of the keratin intermediate filament network in vitro.

Bierl, C., B. Voetsch, et al. (2004). "Determinants of Human Plasma Glutathione Peroxidase (GPx-3) Expression." J. Biol. Chem. **279**(26): 26839-26845.

http://www.jbc.org/cgi/content/abstract/279/26/26839

Plasma glutathione peroxidase (GPx-3) is a selenocysteine-containing protein with antioxidant properties. GPx-3 deficiency has been associated with cardiovascular disease and stroke. The regulation of GPx-3 expression remains largely uncharacterized, however, and we studied its transcriptional and translational determinants in a cultured cell system. In transient transfections of a renal cell line (Caki-2), the published sequence cloned upstream of a luciferase reporter gene produced minimal activity (relative luminescence (RL) = 0.6 {+/-} 0.4). Rapid amplification of cDNA ends was used to identify a novel transcription start site that is located 233 bp downstream (3') of the published site and that produced a >25-fold increase in transcriptional activity (RL = 16.8 {+/-} 1.9; $p < 0.0001$). Analysis of the novel GPx-3 promoter identified Sp-1- and hypoxia-inducible factor-1-binding sites, as well as the redox-sensitive metal response element and antioxidant response element. Hypoxia was identified as a strong transcriptional regulator of GPx-3 expression, in part through the presence of the hypoxia-inducible factor-1-binding site, leading to an almost 3-fold increase in expression levels after 24 h compared with normoxic conditions (normalized RL = 3.5 {+/-} 0.3 versus 1.2 {+/-} 0.1; $p < 0.001$). We also investigated the role of the translational cofactors tRNASec, SECIS-binding protein-2, and SelD (selenophosphate synthetase D) in GPx-3 protein expression. tRNASec and SelD significantly enhanced GPx-3 expression, whereas SECIS-binding protein-2 showed a trend toward increased expression. These results demonstrate the presence of a novel functional transcription start site for the

human GPx-3 gene with a promoter regulated by hypoxia, and identify unique translational determinants of GPx-3 expression.

Rees, M. I., K. Harvey, et al. (2003). "Isoform Heterogeneity of the Human Gephyrin Gene (GPHN), Binding Domains to the Glycine Receptor, and Mutation Analysis in Hyperekplexia." J. Biol. Chem. **278**(27): 24688-24696.

http://www.jbc.org/cgi/content/abstract/278/27/24688

Gephyrin (GPHN) is an organizational protein that clusters and localizes the inhibitory glycine (GlyR) and GABAA receptors to the microtubular matrix of the neuronal postsynaptic membrane. Mice deficient in gephyrin develop a hereditary molybdenum cofactor deficiency and a neurological phenotype that mimics startle disease (hyperekplexia). This neuromotor disorder is associated with mutations in the GlyR {alpha}1 and {beta} subunit genes (GLRA1 and GLRB). Further genetic heterogeneity is suspected, and we hypothesized that patients lacking mutations in GLRA1 and GLRB might have mutations in the gephyrin gene (GPHN). In addition, we adopted a yeast two-hybrid screen, using the GlyR {beta} subunit intracellular loop as bait, in an attempt to identify further GlyR-interacting proteins implicated in hyperekplexia. Gephyrin cDNAs were isolated, and subsequent RT-PCR analysis from human tissues demonstrated the presence of five alternatively spliced GPHN exons concentrated in the central linker region of the gene. This region generated 11 distinct GPHN transcript isoforms, with 10 being specific to neuronal tissue. Mutation analysis of GPHN exons in hyperekplexia patients revealed a missense mutation (A28T) in one patient causing an amino acid substitution (N10Y). Functional testing demonstrated that GPHNN10Y does not disrupt GlyR-gephyrin interactions or collybistininduced cell-surface clustering. We provide evidence that GlyR-gephyrin binding is dependent on the presence of an intact C-terminal MoeA homology domain. Therefore, the N10Y mutation and alternative splicing of GPHN transcripts do not affect interactions with GlyRs but may affect other interactions with the cytoskeleton or gephyrin accessory proteins.

Adachi, H. and M. Tsujimoto (2002). "Characterization of the Human Gene Encoding the Scavenger Receptor Expressed by Endothelial Cell and Its Regulation by a Novel Transcription Factor, Endothelial Zinc Finger Protein-2." J. Biol. Chem. **277**(27): 24014-24021.

http://www.jbc.org/cgi/content/abstract/277/27/24014

The scavenger receptor expressed by endothelial cell (SREC), mediates the selective uptake of modified low density lipoprotein (LDL), such as acetylated LDL and oxidized LDL, into endothelial cells. The SREC gene spans 12 kilobase pairs and contains 11 exons. Analysis of full-length cDNA clones of SREC from a peripheral blood leukocyte cDNA library revealed that at least five alternatively spliced cDNAs were present, and two of them encoded soluble forms of SREC. The transcription start site of the SREC gene was mapped, and DNA sequence analysis revealed an Sp1 binding site in its proximal region. Deletion analysis of the 5'-flanking sequence revealed that sequence between base pairs [-]108 and [-]98 was critical for the promoter activity. This region contained half of an inverted repeat (IR) sequence with a triple nucleotide spacer (IR-3). A protected sequence between base pairs [-]268 and +17 was defined by in vitro DNase I footprinting analysis using human umbilical vein endothelial cell (HUVEC) nuclear extract. A novel transcription factor, endothelial zinc finger protein-2 (EZF-2), that binds to the 5'-flanking critical region of the SREC promoter activity was cloned from a HUVEC cDNA library employing a one-hybrid system. Whereas purified recombinant Sp1 alone produced similar protection in in vitro DNase I footprinting analysis, EZF-2 also bound to the 5'-flanking region SREC promoter. Co-transfection of SREC promoter and Sp1 or EZF-2 expression plasmids in HUVEC revealed that EZF-2 but not Sp1 increased SREC promoter activity. On the other hand, the mutation of either

the Sp1 motif or IR-3 motif resulted in a decrease in the promoter activity. These results suggest that whereas Sp1 is the major nuclear protein bound to the regulatory region of the promoter, both EZF-2 and Sp1 are responsible for its regulation.

Martin, V., R. Bredoux, et al. (2002). "Three Novel Sarco/endoplasmic Reticulum Ca2+-ATPase (SERCA) 3 Isoforms. EXPRESSION, REGULATION, AND FUNCTION OF THE MEMBERS OF THE SERCA3 FAMILY." J. Biol. Chem. **277**(27): 24442-24452.

http://www.jbc.org/cgi/content/abstract/277/27/24442

Sarco/endoplasmic reticulum Ca2+-ATPases (SERCAs) pump Ca2+ into the endoplasmic reticulum. Recently, three human SERCA3 (h3a-c) proteins and a previously unknown rat SERCA3 (r3b/c) mRNA have been described. Here, we (i) document two novel human SERCA3 splice variants h3d and h3e, (ii) provide data for the expression and mechanisms regulating the expression of all known SERCA3 variants (r3a, r3b/c, and h3a-e), and (iii) show functional characteristics of the SERCA3 isoforms. h3d and h3e are issued from the insertion of an additional penultimate exon 22 resulting in different carboxyl termini for these variants. Distinct distribution patterns of the SERCA3 gene products were observed in a series of cell lines of hematopoietic, epithelial, embryonic origin, and several cancerous types, as well as in panels of rat and human tissues. Hypertension and protein kinase C, calcineurin, or retinoic acid receptor signaling pathways were found to differently control rat and human splice variant expression, respectively. Stable overexpression of each variant was performed in human embryonic kidney 293 cells, and the SERCA3 isoforms were fully characterized. All SERCA3 isoforms were found to pump Ca2+ with similar affinities. However, they modulated the cytosolic Ca2+ concentration ([Ca2+]c) and the endoplasmic reticulum Ca2+ content ([Ca2+]er) in different manners. A newly generated polyclonal antibody and a pan-SERCA3 antibody proved the endogenous expression of the three novel SERCA3 proteins, h3d, h3e, and r3b/c. All these data suggest that the SERCA3 gene products have a more widespread role in cellular Ca2+ signaling than previously appreciated.

Mitoma, J., B. Petryniak, et al. (2003). "Extended Core 1 and Core 2 Branched O-Glycans Differentially Modulate Sialyl Lewis x-type L-selectin Ligand Activity." J. Biol. Chem. **278**(11): 9953-9961.

http://www.jbc.org/cgi/content/abstract/278/11/9953

It has been established that sialyl Lewis x in core 2 branched O-glycans serves as an E- and P-selectin ligand. Recently, it was discovered that 6-sulfosialyl Lewis x in extended core 1 O-glycans, NeuNAc[alpha]2[right-arrow]3Gal[beta]1[right-arrow]4(Fuc[alpha]1[right-arrow]3(sulfo[right-arrow]6))GlcNAc[beta]1[right-arrow] 3Gal[beta]1[right-arrow]3GalNAc[alpha]1[right-arrow]Ser/Thr, functions as an L-selectin ligand in high endothelial venules. Extended core 1 O-glycans can be synthesized when a core 1 extension enzyme is present. In this study, we first show that [beta]1,3-N-acetylglucosaminyltransferase-3 ([beta]3GlcNAcT-3) is almost exclusively responsible for core 1 extension among seven different [beta]3GlcNAcTs and thus acts on core 1 O-glycans attached to PSGL-1. We found that transcripts encoding [beta]3GlcNAcT-3 were expressed in human neutrophils and lymphocytes but that their levels were lower than those of transcripts encoding core 2 [beta]1,6-N-acetylglucosaminyltransferase I (Core2GlcNAcT-I). Neutrophils also expressed transcripts encoding fucosyltransferase VII (FucT-VII) and Core2GlcNAcT-I, whereas lymphocytes expressed only small amounts of transcripts encoding FucT-VII. To determine the roles of sialyl Lewis x in extended core 1 O-glycans, Chinese hamster ovary (CHO) cells were stably transfected to express PSGL-1, FucT-VII, and either [beta]3GlcNAcT-3 or Core2GlcNAcT-I. Glycan structural analyses disclosed that PSGL-1 expressed in these transfected cells carried

comparable amounts of sialyl Lewis x in extended core 1 and core 2 branched O-glycans. In a rolling assay, CHO cells expressing sialyl Lewis x in extended core 1 O-glycans supported a significant degree of shear-dependent tethering and rolling of neutrophils and lymphocytes, although less than CHO cells expressing sialyl Lewis x in core 2 branched O-glycans. These results indicate that sialyl Lewis x in extended core 1 O-glycans can function as an L-selectin ligand and is potentially involved in neutrophil adhesion on neutrophils bound to activated endothelial cells.

Ahmad, Z., M. Salim, et al. (2002). "Human Biliverdin Reductase Is a Leucine Zipper-like DNA-binding Protein and Functions in Transcriptional Activation of Heme Oxygenase-1 by Oxidative Stress." J. Biol. Chem. **277**(11): 9226-9232.

http://www.jbc.org/cgi/content/abstract/277/11/9226

Human biliverdin reductase (hBVR) is a serine/threonine kinase that catalyzes reduction of the heme oxygenase (HO) activity product, biliverdin, to bilirubin. A domain of biliverdin reductase (BVR) has primary structural features that resemble leucine zipper proteins. A heptad repeat of five leucines (L1-L5), a basic domain, and a conserved alanine characterize the domain. In hBVR, a lysine replaces L3. The secondary structure model of hBVR predicts an [alpha]-helix-turn-[beta]-sheet for this domain. hBVR translated by the rabbit reticulocyte lysate system appears on a nondenaturing gel as a single band with molecular mass of ~69 kDa. The protein on a denaturing gel separates into two anti-hBVR immunoreactive proteins of ~39.9 + 34.6 kDa. The dimeric form, but not purified hBVR, binds to a 100-mer DNA fragment corresponding to the mouse HO-1 (hsp32) promoter region encompassing two activator protein (AP-1) sites. The specificity of DNA binding is suggested by the following: (a) hBVR does not bind to the same DNA fragment with one or zero AP-1 sites; (b) a 56-bp random DNA with one AP-1 site does not form a complex with hBVR; (c) in vitro translated HO-1 does not interact with the 100-mer DNA fragment with two AP-1 sites; (d) mutation of Lys143, Leu150, or Leu157 blocks both the formation of the ~69-kDa specimens and hBVR DNA complex formation; and (e) purified preparations of hBVR or hHO-1 do not bind to DNA with two AP-1 sites. The potential significance of the AP-1 binding is suggested by the finding that the response of HO-1, in COS cells stably transfected with antisense hBVR, with 66% reduced BVR activity, to superoxide anion ([IMG]img001.gif" ALT="O₂">) formed by menadione is attenuated, whereas induction by heme is not affected. We propose a role for BVR in the signaling cascade for AP-1 complex activation necessary for HO-1 oxidative stress response.

Ko, Y.-G., K. Nishino, et al. (2005). "Stage-by-Stage Change in DNA Methylation Status of Dnmt1 Locus during Mouse Early Development." J. Biol. Chem. **280**(10): 9627-9634.

http://www.jbc.org/cgi/content/abstract/280/10/9627

Methylation of DNA is involved in tissue-specific gene control, and establishment of DNA methylation pattern in the genome is thought to be essential for embryonic development. Three isoforms of Dnmt1 (DNA methyltransferase 1) transcripts, Dnmt1s, Dnmt1o, and Dnmt1p, are produced by alternative usage of multiple first exons. Dnmt1s is expressed in somatic cells. Dnmt1p is found only in pachytene spermatocytes, whereas Dnmt1o is specific to oocytes and preimplantation embryos. Here we determined that there is a tissue-dependent differentially methylated region (T-DMR) in the 5' region of Dnmt1o but not in that of the Dnmt1s/1p. The methylation status of the Dnmt1o T-DMR was distinctively different in the oocyte from that in the sperm and adult somatic tissues and changed at each stage from fertilization to blastocyst stage, suggesting that active methylation and demethylation occur during preimplantation development. The T-DMR was highly methylated in somatic cells and embryonic stem cells. Analysis using

Dnmt-deficient embryonic stem cell lines revealed that Dnmt1, Dnmt3a, and Dnmt3b are each partially responsible for maintenance of methylation of Dnmt1o T-DMR. In particular, there are compensatory and cooperative roles between Dnmt3a and Dnmt3b. Thus, the regulatory region of Dnmt1o, but not of Dnmt1s/1p, appeared to be a target of DNA methylation. The present study also suggested that the DNA methylation status of the gene region dynamically changes during embryogenesis independently of the change in the bulk DNA methylation status.

Ramakrishnan, S. N., P. Lau, et al. (2005). "Rev-erb{beta} Regulates the Expression of Genes Involved in Lipid Absorption in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN ORPHAN NUCLEAR RECEPTORS AND MYOKINES." J. Biol. Chem. **280**(10): 8651-8659.

http://www.jbc.org/cgi/content/abstract/280/10/8651

Rev-erb{beta} is an orphan nuclear receptor that selectively blocks trans-activation mediated by the retinoic acid-related orphan receptor-{alpha} (ROR{alpha}). ROR{alpha} has been implicated in the regulation of high density lipoprotein cholesterol, lipid homeostasis, and inflammation. Reverb{beta} and ROR{alpha} are expressed in similar tissues, including skeletal muscle; however, the pathophysiological function of Rev-erb{beta} has remained obscure. We hypothesize from the similar expression patterns, target genes, and overlapping cognate sequences of these nuclear receptors that Rev-erb{beta} regulates lipid metabolism in skeletal muscle. This lean tissue accounts for >30% of total body weight and 50% of energy expenditure. Moreover, this metabolically demanding tissue is a primary site of glucose disposal, fatty acid oxidation, and cholesterol efflux. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. We utilize ectopic expression in skeletal muscle cells to understand the regulatory role of Rev-erb{beta} in this major mass peripheral tissue. Exogenous expression of a dominant negative version of mouse Rev-erb{beta} decreases the expression of many genes involved in fatty acid/lipid absorption (including Cd36, and Fabp-3 and -4). Interestingly, we observed a robust induction (>15-fold) in mRNA expression of interleukin-6, an "exercise-induced myokine" that regulates energy expenditure and inflammation. Furthermore, we observed the dramatic repression (>20-fold) of myostatin mRNA, another myokine that is a negative regulator of muscle hypertrophy and hyperplasia that impacts on body fat accumulation. This study implicates Rev-erb{beta} in the control of lipid and energy homoeostasis in skeletal muscle. In conclusion, we speculate that selective modulators of Rev-erb{beta} may have therapeutic utility in the treatment of dyslipidemia and regulation of muscle growth.

Cabral, W. A., M. V. Mertts, et al. (2003). "Type I Collagen Triplet Duplication Mutation in Lethal Osteogenesis Imperfecta Shifts Register of alpha Chains throughout the Helix and Disrupts Incorporation of Mutant Helices into Fibrils and Extracellular Matrix." J. Biol. Chem. **278**(12): 10006-10012.

http://www.jbc.org/cgi/content/abstract/278/12/10006

The majority of collagen mutations causing osteogenesis imperfecta (OI) are glycine substitutions that disrupt formation of the triple helix. A rare type of collagen mutation consists of a duplication or deletion of one or two Gly-X-Y triplets. These mutations shift the register of collagen chains with respect to each other in the helix but do not interrupt the triplet sequence, yet they have severe clinical consequences. We investigated the effect of shifting the register of the collagen helix by a single Gly-X-Y triplet on collagen assembly, stability, and incorporation into fibrils and matrix. These studies utilized a triplet duplication in COL1A1 exon 44 that occurred in the cDNA and gDNA of two siblings with lethal OI. The normal allele encodes three identical Gly-Ala-Hyp triplets at aa 868-876, whereas the mutant allele encodes four. The register shift delays helix formation, causing overmodification. Differential scanning calorimetry yielded a decrease in Tm of

2 {degrees}C for helices with one mutant chain and a 6 {degrees}C decrease in helices with two mutant chains. An in vitro binary co-processing assay of N-proteinase cleavage demonstrated that procollagen with the triplet duplication has slower N-propeptide cleavage than in normal controls or procollagen with pro[alpha]1(I) G832S, G898S, or G997S substitutions, showing that the register shift persists through the entire helix. The register shift disrupts incorporation of mutant collagen into fibrils and matrix. Proband fibrils formed inefficiently in vitro and contained only normal helices and helices with a single mutant chain. Helices with two mutant chains and a significant portion of helices with one mutant chain did not form fibrils. In matrix deposited by proband fibroblasts, mutant chains were abundant in the immaturely cross-linked fraction but constituted a minor fraction of maturely cross-linked chains. The profound effects of shifting the collagen triplet register on chain interactions in the helix and on fibril formation correlate with the severe clinical consequences.

Muradov, K. G., K. K. Boyd, et al. (2003). "The GAFa Domains of Rod cGMP-phosphodiesterase 6 Determine the Selectivity of the Enzyme Dimerization." J. Biol. Chem. **278**(12): 10594-10601.

http://www.jbc.org/cgi/content/abstract/278/12/10594

Retinal rod cGMP phosphodiesterase (PDE6 family) is the effector enzyme in the vertebrate visual transduction cascade. Unlike other known PDEs that form catalytic homodimers, the rod PDE6 catalytic core is a heterodimer composed of [alpha] and [beta] subunits. A system for efficient expression of rod PDE6 is not available. Therefore, to elucidate the structural basis for specific dimerization of rod PDE6, we constructed a series of chimeric proteins between PDE6[alpha][beta] and PDE5, which contain the N-terminal GAFa/GAFb domains, or portions thereof, of the rod enzyme. These chimeras were co-expressed in Sf9 cells in various combinations as His-, myc-, or FLAG-tagged proteins. Dimerization of chimeric PDEs was assessed using gel filtration and sucrose gradient centrifugation. The composition of formed dimeric enzymes was analyzed with Western blotting and immunoprecipitation. Consistent with the selectivity of PDE6 dimerization in vivo, efficient heterodimerization was observed between the GAF regions of PDE6[alpha] and PDE6[beta] with no significant homodimerization. In addition, PDE6[alpha] was able to form dimers with the cone PDE6[alpha]' subunit. Furthermore, our analysis indicated that the PDE6 GAFa domains contain major structural determinants for the affinity and selectivity of dimerization of PDE6 catalytic subunits. The key dimerization selectivity module of PDE6 has been localized to a small segment within the GAFa domains, PDE6[alpha]-59-74/PDE6[beta]-57-72. This study provides tools for the generation of the homodimeric [alpha][alpha] and [beta][beta] enzymes that will allow us to address the question of functional significance of the unique heterodimerization of rod PDE6.

Jiang, H., R. S. Peterson, et al. (2002). "A Requirement for the CD44 Cytoplasmic Domain for Hyaluronan Binding, Pericellular Matrix Assembly, and Receptor-mediated Endocytosis in COS-7 Cells." J. Biol. Chem. **277**(12): 10531-10538.

http://www.jbc.org/cgi/content/abstract/277/12/10531

CD44-negative COS-7 cells were transfected with expression constructs for CD44H (the predominant CD44 isoform), CD44E (epithelial isoform), or truncation mutant derivatives lacking the carboxyl-terminal 67 amino acids of the cytoplasmic domain, CD44H[Delta]67 and CD44E[Delta]67. The truncation mutant CD44H[Delta]67 is identical to a naturally occurring alternatively spliced "short tail" CD44 isoform (CD44st), which incorporates exon 19 in place of exon 20. CD44st lacks intracellular signaling motifs as well as protein domains necessary for interaction with cytoskeletal components. Transfection of COS-7 cells with each construct yielded equivalent levels of mRNA expression, whereas no CD44 expression was observed in parental,

nontransfected COS-7 cells. Western analysis and immunostaining of COS-7 transfectants confirmed CD44 protein expression of the truncation mutant derivatives. COS-7 cells transfected with CD44H or CD44E gained the capacity to bind fluorescein-conjugated HA (fl-HA) and assemble HA-dependent pericellular matrices in the presence of exogenously added HA and proteoglycan. In addition, the CD44H- and CD44E-transfected cells were able to internalize surface-bound fl-HA. COS-7 cells transfected with the vector alone or with either of the mutant CD44 isoforms, CD44H[Delta]67 or CD44E[Delta]67, did not exhibit the capacity to assemble pericellular matrices or to bind and internalize the fl-HA. Cotransfection of CD44[Delta]67 mutants together with CD44H reduced the size of the HA-dependent pericellular matrices. Transfection of bovine articular chondrocytes with CD44[Delta]67 also inhibited pericellular matrix assembly. Collectively, these results indicate an obligatory requirement for the CD44 receptor cytoplasmic domain for ligand (HA) binding, formation and retention of the pericellular matrix, as well as CD44-mediated endocytosis of HA. In addition, the results suggest a potential regulatory role for the differentially expressed alternatively spliced short tail CD44 isoform.

Levitin, F., A. Baruch, et al. (2005). "A Novel Protein Derived from the MUC1 Gene by Alternative Splicing and Frameshifting." J. Biol. Chem. **280**(11): 10655-10663.

http://www.jbc.org/cgi/content/abstract/280/11/10655

Genes that have been designated the name "MUC" code for proteins comprising mucin domains. These proteins may be involved in barrier and protective functions. The first such gene to be characterized and sequenced is the MUC1 gene. Here we report a novel small protein derived from the MUC1 gene by alternative splicing that does not contain the hallmark of mucin proteins, the mucin domain. This protein termed MUC1/ZD retains the same N-terminal MUC1 sequences as all of the other known MUC1 protein isoforms. The common N-terminal sequences comprise the signal peptide and a subsequent stretch of 30 amino acids. In contrast, the MUC1/ZD C-terminal 43 amino acids are novel and result from a reading frameshift engendered by a splicing event that forms MUC1/ZD. The expression of MUC1/ZD at the protein level in human tissues is demonstrated by Western blotting, immunohistochemistry, immunoprecipitation, and an ELISA. Utilization was made of affinity-purified MUC1/ZD-specific polyclonal antibodies as well as two different monoclonal antibodies that are monospecific for the MUC1/ZD protein. The MUC1/ZD protein is expressed in tissues as an oligomeric complex composed of monomers linked by disulfide bonds contributed by MUC1/ZD cysteine residues. MUC1/ZD protein expression did not parallel that of the tandem-repeat array-containing MUC1 protein. Results presented here demonstrate for the first time the expression of a novel MUC1 protein isoform MUC1/ZD, which is generated by an alternative splicing event that both deletes the tandem-repeat array and leads to a C-terminal reading frameshift.

Gardner, A. F., C. M. Joyce, et al. (2004). "Comparative Kinetics of Nucleotide Analog Incorporation by Vent DNA Polymerase." J. Biol. Chem. **279**(12): 11834-11842.

http://www.jbc.org/cgi/content/abstract/279/12/11834

Comparative kinetic and structural analyses of a variety of polymerases have revealed both common and divergent elements of nucleotide discrimination. Although the parameters for dNTP incorporation by the hyperthermophilic archaeal Family B Vent DNA polymerase are similar to those previously derived for Family A and B DNA polymerases, parameters for analog incorporation reveal alternative strategies for discrimination by this enzyme. Discrimination against ribonucleotides was characterized by a decrease in the affinity of NTP binding and a lower rate of phosphoryl transfer, whereas discrimination against ddNTPs was almost exclusively due to a slower rate of phosphodiester bond formation. Unlike Family A DNA polymerases,

incorporation of 9-[(2-hydroxyethoxy)methyl]X triphosphates (where X is adenine, cytosine, guanine, or thymine; acyNTPs) by Vent DNA polymerase was enhanced over ddNTPs via a 50-fold increase in phosphoryl transfer rate. Furthermore, a mutant with increased propensity for nucleotide analog incorporation (VentA488L DNA polymerase) had unaltered dNTP incorporation while displaying enhanced nucleotide analog binding affinity and rates of phosphoryl transfer. Based on kinetic data and available structural information from other DNA polymerases, we propose active site models for dNTP, ddNTP, and acyNTP selection by hyperthermophilic archaeal DNA polymerases to rationalize structural and functional differences between polymerases.

Kondapalli, J., A. S. Flozak, et al. (2004). "Laminar Shear Stress Differentially Modulates Gene Expression of p120 Catenin, Kaiso Transcription Factor, and Vascular Endothelial Cadherin in Human Coronary Artery Endothelial Cells." J. Biol. Chem. **279**(12): 11417-11424.

http://www.jbc.org/cgi/content/abstract/279/12/11417

We demonstrated previously that laminar shear stress (LSS) enhances human coronary artery endothelial cell (HCAEC) wound closure via a vascular endothelial cadherin (VE-cadherin)-dependent mechanism. VE-cadherin can interact with p120 catenin (p120ctn) to mediate cell locomotion and proliferation. In this study, we hypothesized that p120ctn and an interacting protein, Kaiso, a transcriptional factor with which p120ctn may interact, would be expressed differentially at the wound border and away from the wound border in HCAEC exposed to LSS. One of the major goals in this study was to assess the differential gene expression of p120ctn, Kaiso, and VE-cadherin in HCAEC at specific locations along the wound border to further our understanding of the molecular mechanisms involved in wound closure. We combined the technique of laser capture microdissection with quantitative real time PCR to compare p120ctn, Kaiso, and VE-cadherin mRNA expression in HCAEC at and away from the wound border under LSS. Total RNA was isolated from 200-1,000 laser-captured HCAEC and reverse transcribed into cDNA. Detection of p120ctn, Kaiso, and VE-cadherin mRNA was carried out using quantitative real time PCR. Normalization of cDNA templates was achieved by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) quantification. Quantitative real time PCR analysis revealed p120ctn:GAPDH ratios, Kaiso: GAPDH ratios, and VE-cadherin:GAPDH ratios, relative to static control for each set, of 0.99-4.18 (mean {+/-} S.E., 1.94 {+/-} 0.404), 1.0-5.24 (2.11 {+/-} 0.51), and 0.99-1.42 (1.09 {+/-} 0.09) after 3 h of LSS, respectively. With these techniques, we found that p120ctn and Kaiso transcripts were increased in laser-captured HCAEC at the wound border compared with HCAEC away from the wound border. In addition, differential expression of p120ctn and Kaiso mRNA was observed in HCAEC depending on how LSS was applied in relation to the wounding process. These techniques may have wide applicability for studying wound healing because gene expression of key adhesion molecules in HCAEC may now be determined from select regions of the endothelial wound border.

Brown, A. J., S. M. Goldsworthy, et al. (2003). "The Orphan G Protein-coupled Receptors GPR41 and GPR43 Are Activated by Propionate and Other Short Chain Carboxylic Acids." J. Biol. Chem. **278**(13): 11312-11319.

http://www.jbc.org/cgi/content/abstract/278/13/11312

GPR41 and GPR43 are related members of a homologous family of orphan G protein-coupled receptors that are tandemly encoded at a single chromosomal locus in both humans and mice. We identified the acetate anion as an agonist of human GPR43 during routine ligand bank screening in yeast. This activity was confirmed after transient transfection of GPR43 into mammalian cells using Ca2+ mobilization and [35S]guanosine 5'-O-(3-thiotriphosphate) binding

assays and by coexpression with GIRK G protein-regulated potassium channels in Xenopus laevis oocytes. Other short chain carboxylic acid anions such as formate, propionate, butyrate, and pentanoate also had agonist activity. GPR41 is related to GPR43 (52% similarity; 43% identity) and was activated by similar ligands but with differing specificity for carbon chain length, with pentanoate being the most potent agonist. A third family member, GPR42, is most likely a recent gene duplication of GPR41 and may be a pseudogene. GPR41 was expressed primarily in adipose tissue, whereas the highest levels of GPR43 were found in immune cells. The identity of the cognate physiological ligands for these receptors is not clear, although propionate is known to occur in vivo at high concentrations under certain pathophysiological conditions.

Yu, W., N. R. Murray, et al. (2003). "Role of Cyclooxygenase 2 in Protein Kinase C beta II-mediated Colon Carcinogenesis." J. Biol. Chem. **278**(13): 11167-11174.

http://www.jbc.org/cgi/content/abstract/278/13/11167

Elevated expression of protein kinase C [beta]II (PKC[beta]II) is an early promotive event in colon carcinogenesis (Gokmen-Polar, Y., Murray, N. R., Velasco, M. A., Gatalica, Z., and Fields, A. P. (2001) Cancer Res. 61, 1375-1381). Expression of PKC[beta]II in the colon of transgenic mice leads to hyperproliferation and increased susceptibility to colon carcinogenesis due, at least in part, to repression of transforming growth factor beta type II receptor (TGF-[beta]RII) expression (Murray, N. R., Davidson, L. A., Chapkin, R. S., Gustafson, W. C., Schattenberg, D. G., and Fields, A. P. (1999) J. Cell Biol., 145, 699-711). Here we report that PKC[beta]II induces the expression of cyclooxygenase type 2 (Cox-2) in rat intestinal epithelial (RIE) cells in vitro and in transgenic PKC[beta]II mice in vivo. Cox-2 mRNA increases more than 10-fold with corresponding increases in Cox-2 protein and PGE2 production in RIE/PKC[beta]II cells. PKC[beta]II activates the Cox-2 promoter by 2- to 3-fold and stabilizes Cox-2 mRNA by at least 4-fold. The selective Cox-2 inhibitor Celecoxib restores expression of TGF-[beta]RII both in vitro and in vivo and restores TGF[beta]-mediated transcription in RIE/PKC[beta]II cells. Likewise, the [omega]-3 fatty acid eicosapentaenoic acid (EPA), which inhibits PKC[beta]II activity and colon carcinogenesis, causes inhibition of Cox-2 protein expression, re-expression of TGF-[beta]RII, and restoration of TGF-[beta]1-mediated transcription in RIE/PKC[beta]II cells. Our data demonstrate that PKC[beta]II promotes colon cancer, at least in part, through induction of Cox-2, suppression of TGF-[beta] signaling, and establishment of a TGF-[beta]-resistant, hyperproliferative state in the colonic epithelium. Our data define a procarcinogenic PKC[beta]II [right-arrow] Cox-2 [right-arrow] TGF-[beta] signaling axis within the colonic epithelium, and provide a molecular mechanism by which dietary [omega]-3 fatty acids and nonsteroidal antiinflammatory agents such as Celecoxib suppress colon carcinogenesis.

Laybutt, D. R., A. Sharma, et al. (2002). "Genetic Regulation of Metabolic Pathways in beta -Cells Disrupted by Hyperglycemia." J. Biol. Chem. **277**(13): 10912-10921.

http://www.jbc.org/cgi/content/abstract/277/13/10912

In models of type 2 diabetes the expression of [beta]-cell genes is altered, but these changes have not fully explained the impairment in [beta]-cell function. We hypothesized that changes in [beta]-cell phenotype and global alterations in both carbohydrate and lipid pathways are likely to contribute to secretory abnormalities. Therefore, expression of genes involved in carbohydrate and lipid metabolism were analyzed in islets 4 weeks after 85-95% partial pancreatectomy (Px) when [beta]-cells have impaired glucose-induced insulin secretion and ATP synthesis. Px rats after 1 week developed mild to severe hyperglycemia that was stable for the next 3 weeks, whereas neither plasma triglyceride, non-esterified fatty acid, or islet triglyceride levels were altered. Expression of peroxisome proliferator-activated receptors (PPARs), with several target

genes, were reciprocally regulated; PPAR[alpha] was markedly reduced even at low level hyperglycemia, whereas PPAR[gamma] was progressively increased with increasing hyperglycemia. Uncoupling protein 2 (UCP-2) was increased as were other genes barely expressed in sham islets including lactate dehydrogenase-A (LDH-A), lactate (monocarboxylate) transporters, glucose-6-phosphatase, fructose-1,6-bisphosphatase, 12-lipoxygenase, and cyclooxygenase 2. On the other hand, the expression of [beta]-cell-associated genes, insulin, and GLUT2 were decreased. Treating Px rats with phlorizin normalized hyperglycemia without effecting plasma fatty acids and reversed the changes in gene expression implicating the importance of hyperglycemia per se in the loss of [beta]-cell phenotype. In addition, parallel changes were observed in [beta]-cell-enriched tissue dissected by laser capture microdissection from the central core of islets. In conclusion, chronic hyperglycemia leads to a critical loss of [beta]-cell differentiation with altered expression of genes involved in multiple metabolic pathways diversionary to normal [beta]-cell glucose metabolism. This global maladaptation in gene expression at the time of increased secretory demand may contribute to the [beta]-cell dysfunction found in diabetes.

Lin, S., Q. Shi, et al. (2002). "A Novel S-Adenosyl-L-methionine:Arsenic(III) Methyltransferase from Rat Liver Cytosol." J. Biol. Chem. **277**(13): 10795-10803.

http://www.jbc.org/cgi/content/abstract/277/13/10795

S-Adenosyl-L-methionine (AdoMet):arsenic(III) methyltransferase, purified from liver cytosol of adult male Fischer 344 rats, catalyzes transfer of a methyl group from AdoMet to trivalent arsenicals producing methylated and dimethylated arsenicals. The kinetics of production of methylated arsenicals in reaction mixtures containing enzyme, AdoMet, dithiothreitol, glutathione (GSH), and arsenite are consistent with a scheme in which monomethylated arsenical produced from arsenite is the substrate for a second methylation reaction that yields dimethylated arsenical. The mRNA for this protein predicts a 369-amino acid residue protein (molecular mass 41056) that contains common methyltransferase sequence motifs. Its sequence is similar to Cyt19, a putative methyltransferase, expressed in human and mouse tissues. Reverse transcription-polymerase chain reaction detects S-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA in rat tissues and in HepG2 cells, a human cell line that methylates arsenite and methylarsonous acid. S-Adenosyl-L-methionine:arsenic(III) methyltransferase mRNA is not detected in UROtsa cells, an immortalized human urothelial cell line that does not methylate arsenite. Because methylation of arsenic is a critical feature of its metabolism, characterization of this enzyme will improve our understanding of this metalloid's metabolism and its actions as a toxin and a carcinogen.

Yamagata, H., K. Yonesu, et al. (2002). "TGTCACA Motif Is a Novel cis-Regulatory Enhancer Element Involved in Fruit-specific Expression of the cucumisin Gene." J. Biol. Chem. **277**(13): 11582-11590.

http://www.jbc.org/cgi/content/abstract/277/13/11582

Cucumisin, a subtilisin-like serine protease, is expressed at high levels in the fruit of melon (Cucumis melo L.) and accumulates in the juice. We investigated roles of the promoter regions and DNA-protein interactions in fruit-specific expression of the cucumisin gene. In transient expression analysis, a chimeric gene construct containing a 1.2-kb cucumisin promoter fused to a [beta]-glucuronidase (GUS) reporter gene was expressed in fruit tissues at high levels, but the promoter activities in leaves and stems were very low. Deletion analysis indicated that a positive regulatory region is located between nucleotides [-]234 and [-]214 relative to the transcriptional initiation site. Gain-of-function experiments revealed that this 20-bp sequence conferred fruit specificity and contained a regulatory enhancer. Gel mobility shift experiments demonstrated the

presence of fruit nuclear factors that interact with the cucumisin promoter. A typical G-box (GACACGTGTC) present in the 20-bp sequence did not bind fruit protein, but two possible cis-elements, an I-box-like sequence (AGATATGATAAAA) and an odd base palindromic TGTCACA motif, were identified in the promoter region between positions [-]254 and [-]215. The I-box-like sequence bound more tightly to fruit nuclear protein than the TGTCACA motif. The I-box-like sequence functions as a negative regulatory element, and the TGTCACA motif is a novel enhancer element necessary for fruit-specific expression of the cucumisin gene. Specific nucleotides responsible for the binding of fruit nuclear protein in these two elements were also determined.

Dinadayala, P., A. Lemassu, et al. (2004). "Revisiting the Structure of the Anti-neoplastic Glucans of Mycobacterium bovis Bacille Calmette-Guerin: STRUCTURAL ANALYSIS OF THE EXTRACELLULAR AND BOILING WATER EXTRACT-DERIVED GLUCANS OF THE VACCINE SUBSTRAINS." J. Biol. Chem. **279**(13): 12369-12378.

http://www.jbc.org/cgi/content/abstract/279/13/12369

The attenuated strain of Mycobacterium bovis Bacille Calmette-Guerin (BCG), used worldwide to prevent tuberculosis and leprosy, is also clinically used as an immunotherapeutic agent against superficial bladder cancer. An anti-tumor polysaccharide has been isolated from the boiling water extract of the Tice substrain of BCG and tentatively characterized as consisting primarily of repeating units of 6-linked-glucosyl residues. Mycobacterium tuberculosis and other mycobacterial species produce a glycogen-like {alpha}-glucan composed of repeating units of 4-linked glucosyl residues substituted at some 6 positions by short oligoglucosyl units that also exhibits an anti-tumor activity. Therefore, the impression prevails that mycobacteria synthesize different types of anti-neoplastic glucans or, alternatively, the BCG substrains are singular in producing a unique type of glucan that may confer to them their immunotherapeutic property. The present study addresses this question through the comparative analysis of {alpha}-glucans purified from the extracellular materials and boiling water extracts of three vaccine substrains. The polysaccharides were purified, and their structural features were established by mono- and two-dimensional NMR spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of the enzymatic and chemical degradation products of the purified compounds. The glucans isolated by the two methods from the three substrains of BCG were shown to exhibit identical structural features shared with the glycogen-like {alpha}-glucan of M. tuberculosis and other mycobacteria. Incidentally, we observed an occasional release of dextrans from Sephadex columns that may explain the reported occurrence of 6-substituted {alpha}-glucans in mycobacteria.

Wang, G., A. Woods, et al. (2004). "RhoA/ROCK Signaling Suppresses Hypertrophic Chondrocyte Differentiation." J. Biol. Chem. **279**(13): 13205-13214.

http://www.jbc.org/cgi/content/abstract/279/13/13205

Coordinated proliferation and differentiation of growth plate chondrocytes is required for normal growth and development of the endochondral skeleton, but little is known about the intracellular signal transduction pathways regulating these processes. We have investigated the roles of the GTPase RhoA and its effector kinases ROCK1/2 in hypertrophic chondrocyte differentiation. RhoA, ROCK1, and ROCK2 are expressed throughout chondrogenic differentiation. RhoA overexpression in chondrogenic ATDC5 cells results in increased proliferation and a marked delay of hypertrophic differentiation, as shown by decreased induction of alkaline phosphatase activity, mineralization, and expression of the hypertrophic markers collagen X, bone sialoprotein, and matrix metalloproteinase 13. These effects are accompanied by activation of cyclin D1

transcription and repression of the collagen X promoter by RhoA. In contrast, inhibition of Rho/ROCK signaling by the pharmacological inhibitor Y27632 inhibits chondrocyte proliferation and accelerates hypertrophic differentiation. Dominant-negative RhoA also inhibits induction of the cyclin D1 promoter by parathyroid hormone-related peptide. Finally, Y27632 treatment partially rescues the effects of RhoA overexpression. In summary, we identify the RhoA/ROCK signaling pathway as a novel and important regulator of chondrocyte proliferation and differentiation.

Whiteman, S. C., A. Bianco, et al. (2003). "Human Rhinovirus Selectively Modulates Membranous and Soluble Forms of Its Intercellular Adhesion Molecule-1 (ICAM-1) Receptor to Promote Epithelial Cell Infectivity." J. Biol. Chem. **278**(14): 11954-11961.

http://www.jbc.org/cgi/content/abstract/278/14/11954

Human rhinoviruses are responsible for many upper respiratory tract infections. 90% of rhinoviruses utilize intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor, which also plays a critical role in recruitment of immune effector cells. Two forms of this receptor exist; membrane-bound (mICAM-1) and soluble ICAM-1 (sICAM-1). The soluble receptor may be produced independently from the membrane-bound form or it may be the product of proteolytic cleavage of mICAM-1. The ratio of airway epithelial cell expression of mICAM-1 to the sICAM-1 form may influence cell infectivity and outcome of rhinovirus infection. We therefore investigated the effect of rhinovirus on expression of both ICAM-1 receptors in normal human bronchial epithelial cells. We observed separate distinct messenger RNA transcripts coding for mICAM-1 and sICAM-1 in these cells, which were modulated by virus. Rhinovirus induced mICAM-1 expression on epithelial cells while simultaneously down-regulating sICAM-1 release, with consequent increase in target cell infectivity. The role of protein tyrosine kinases was investigated as a potential mechanistic pathway. Rhinovirus infection induced rapid phosphorylation of intracellular tyrosine kinase, which may be critical in up-regulation of mICAM-1. Elucidation of the underlying molecular mechanisms involved in differential modulation of both ICAM-1 receptors may lead to novel therapeutic strategies.

Olosz, F. and T. R. Malek (2002). "Structural Basis for Binding Multiple Ligands by the Common Cytokine Receptor gamma -Chain." J. Biol. Chem. **277**(14): 12047-12052.

http://www.jbc.org/cgi/content/abstract/277/14/12047

The common [gamma]-chain ([gamma]c) that functions both in ligand binding and signal transduction is a shared subunit of the multichain receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The structural basis by which the ectodomain of [gamma]c contributes to binding six distinct cytokines is only partially defined. In the present study, epitope mapping of antagonistic anti-[gamma]c monoclonal antibodies led to the identification of Asn-128 of mouse [gamma]c that represents another potential contact residue that is required for binding IL-2, IL-7, and IL-15 but not IL-4. In addition, Tyr-103, Cys-161, Cys-210, and Cys-211, previously identified to contribute to binding IL-2 and IL-7, were also found to be involved in binding IL-4 and IL-15. Collectively, these data favor a model in which [gamma]c utilizes a common mechanism for its interactions with multiple cytokines, and the binding sites are largely overlapping but not identical. Asn-128 and Tyr-103 likely act as contact residues whereas Cys-161, Cys-210, and Gly-211 may stabilize the structure of the proposed ligand-interacting surface formed by the two extracytoplasmic domains.

Jhandier, M. N., E. A. Kruglov, et al. (2005). "Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPDase2." J. Biol. Chem.: M412371200.

http://www.jbc.org/cgi/content/abstract/M412371200v1

Bile duct epithelia are the target of a number "cholangiopathies" characterized by disordered bile ductular proliferation. While mechanisms for bile ductular proliferation are unknown, recent evidence suggests that extracellular nucleotides regulate cell proliferation via activation of P2Y receptors. Portal fibroblasts may regulate bile duct epithelial P2Y receptors via expression of the ecto-nucleotidase NTPDase2. Thus, we tested the hypothesis that portal fibroblasts regulate bile duct epithelial proliferation via expression of NTPDase2. We generated a novel co-culture model of Mz-ChA-1 human cholangiocarcinoma cells and primary portal fibroblasts. Cell proliferation was measured by bromodeoxyuridine uptake. NTPDase2 expression was assessed by immunofluorescence and quantitative real-time RT-PCR. NTPDase2 expression in portal fibroblasts was blocked using siRNA. NTPDase2 overexpression in portal myofibroblasts isolated from bile duct ligated rats was achieved by cDNA transfection. Co-culture of Mz-ChA-1 cells with PF decreased their proliferation to 26% of control. Similar decreases in Mz-ChA-1 proliferation were induced by the soluble ecto-nucleotidase apyrase and the P2 receptor inhibitor suramin. The proliferation of Mz-ChA-1 cells returned to baseline when NTPDase2 expression in portal fibroblasts was inhibited using NTPDase2-specific siRNA. Untransfected portal myofibroblasts lacking NTPDase2 had no effect on Mz-ChA-1 proliferation, yet portal myofibroblasts transfected with NTPDase2 cDNA inhibited Mz-ChA-1 proliferation. We conclude that, portal fibroblasts inhibit bile ductular proliferation via expression of NTPDase2 and blockade of P2Y activation. Loss of NTPDase2 may mediate the bile ductular proliferation typical of obstructive cholestasis. This novel crosstalk signaling pathway may mediate pathologic alterations in bile ductular proliferation in other cholangiopathic conditions.

Koo, I. C. and R. S. Stephens (2003). "A Developmentally Regulated Two-component Signal Transduction System in Chlamydia." J. Biol. Chem. **278**(19): 17314-17319.

http://www.jbc.org/cgi/content/abstract/278/19/17314

Two-component systems allow bacteria to adapt to changing environmental conditions and may induce developmental changes necessary for survival. Chlamydia trachomatis alternates between two distinct developmental forms, each optimized for survival in a separate niche. Transcriptional regulation of development is not understood. The C. trachomatis genome sequence revealed a single pair of genes (ctcB-ctcC) predicted to encode proteins with sequence conservation to bacterial two-component systems. Sequence analysis revealed that the sensor kinase, CtcB, possessed an energy-sensing PAS domain and phosphorylation site. The response regulator, CtcC, had homology to [sigma]54 activators, possessing conserved receiver and ATPase domains and phosphorylation site, but lacked the C-terminal DNA-binding domain. ctcB and ctcC were expressed late in the developmental cycle, and both proteins were detected in EB lysates. Recombinant CtcB and CtcC were purified from denatured Escherichia coli inclusion bodies and refolded. CtcC was found to aggregate as dimers and tetramers in solution. In vitro phosphorylation assays showed that CtcB autophosphorylated in the presence of Mg2+, Mn2+, and Fe2+ and transferred the phosphoryl group in the presence of CtcC. Collectively, these results show that CtcB and CtcC function as a two-component system and are likely responsible for transcriptional regulation by [sigma]54 holoenzyme during late-stage chlamydial development.

Furusato, M., N. Sueyoshi, et al. (2002). "Molecular Cloning and Characterization of Sphingolipid Ceramide N-Deacylase from a Marine Bacterium, Shewanella alga G8." J. Biol. Chem. **277**(19): 17300-17307.

http://www.jbc.org/cgi/content/abstract/277/19/17300

Recently, lyso-sphingolipids have been identified as ligands for several orphan G protein-coupled receptors, although the molecular mechanism for their generation has yet to be clarified. Here, we report the molecular cloning of the enzyme, which catalyzes the generation of lyso-sphingolipids from various sphingolipids (sphingolipid ceramide N-deacylase). The 75-kDa enzyme was purified from the marine bacterium, Shewanella alga G8, and its gene was cloned from a G8 genomic library using sequences of the purified enzyme. The cloned enzyme was composed of 992 amino acids, including a signal sequence of 35 residues, and its molecular weight was estimated to be 109,843. Significant sequence similarities were found with an unknown protein of Streptomyces fradiae Y59 and a Lumbricus terrestris lectin but not other known functional proteins. The 106-kDa recombinant enzyme expressed in Escherichia coli hydrolyzed various glycosphingolipids and sphingomyelin, although it seems to be much less active than the native 75-kDa enzyme. In vitro translation using wheat germ extract revealed the activity of a 75-kDa deletion mutant lacking a C terminus to be much stronger than that of the full-length enzyme, suggesting that C-terminal processing is necessary for full activity.

Leite, M. F., K. Hirata, et al. (2002). "Molecular Basis for Pacemaker Cells in Epithelia." J. Biol. Chem. **277**(18): 16313-16323.

http://www.jbc.org/cgi/content/abstract/277/18/16313

Intercellular signaling is highly coordinated in excitable tissues such as heart, but the organization of intercellular signaling in epithelia is less clear. We examined Ca2+ signaling in hepatoma cells expressing the hepatocyte gap junction protein connexin32 (cx32) or the cardiac gap junction protein cx43, plus a fluorescently tagged V1a vasopressin receptor (V1aR). Release of inositol 1,4,5-trisphosphate (InsP3) in wild type cells increased Ca2+ in the injected cell but not in neighboring cells, while the Ca2+ signal spread to neighbors when gap junctions were expressed. Photorelease of caged Ca2+ rather than InsP3 resulted in a small increase in Ca2+ that did not spread to neighbors with or without gap junctions. However, photorelease of Ca2+ in cells stimulated with low concentrations of vasopressin resulted in a much larger increase in Ca2+, which spread to neighbors via gap junctions. Cells expressing tagged V1aR similarly had increased sensitivity to vasopressin, and could signal to neighbors via gap junctions. Higher concentrations of vasopressin elicited Ca2+ signals in all cells. In cx32 or cx43 but not in wild type cells, this signaling was synchronized and began in cells expressing the tagged V1aR. Thus, intercellular Ca2+ signals in epithelia are organized by three factors: 1) InsP3 must be generated in each cell to support a Ca2+ signal in that cell; 2) gap junctions are necessary to synchronize Ca2+ signals among cells; and 3) cells with relatively increased expression of hormone receptor will initiate Ca2+ signals and thus serve as pacemakers for their neighbors. Together, these factors may allow epithelia to act in an integrated, organ-level fashion rather than as a collection of isolated cells.

Vargas, M. R., M. Pehar, et al. (2005). "Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: Consequences for motor neuron survival." J. Biol. Chem.: M501920200.

http://www.jbc.org/cgi/content/abstract/M501920200v1

Fibroblast growth factor-1 (FGF-1) is highly expressed in motor neurons and can be released in response to sub-lethal cell injury. Because FGF-1 potently activates astroglia and exerts a direct neuroprotection after spinal cord injury or axotomy, we examined whether it regulated the

expression of inducible and cytoprotective heme oxygenase-1 (HO-1) enzyme in astrocytes. FGF-1 induced the expression of HO-1 in cultured rat spinal cord astrocytes, which was dependent on FGF receptor activation and prevented by cycloheximide. FGF-1 also induced Nrf2 mRNA and protein levels and prompted its nuclear translocation. HO-1 induction was abolished by transfection of astrocytes with a dominant-negative mutant Nrf2, indicating that FGF-1 regulates HO-1 expression through Nrf2. FGF-1 also modified the expression of other antioxidant genes regulated by Nrf2. Both Nrf2 and HO-1 levels were increased and co-localized with reactive astrocytes in the degenerating lumbar spinal cord of rats expressing the amyotrophic lateral sclerosis (ALS)-linked SOD1 G93A mutation. Overexpression of Nrf2 in astrocytes increased survival of co-cultured embryonic motor neurons and prevented motor neuron apoptosis mediated by nerve growth factor through p75 neurotrophin receptor. Taken together, these results emphasize the key role of astrocytes in determining motor neuron fate in ALS.

Zhao, K., M. Liu, et al. (2005). "The Global Transcriptional Response of Escherichia coli to Induced {sigma}32 Protein Involves {sigma}32 Regulon Activation Followed by Inactivation and Degradation of {sigma}32 in Vivo." J. Biol. Chem. **280**(18): 17758-17768.

http://www.jbc.org/cgi/content/abstract/280/18/17758

{sigma} 32 is the first alternative {sigma} factor discovered in Escherichia coli and can direct transcription of many genes in response to heat shock stress. To define the physiological role of {sigma}32, we have used transcription profiling experiments to identify, on a genome-wide basis, genes under the control of {sigma}32 in E. coli by moderate induction of a plasmid-borne rpoH gene under defined, steady-state growth conditions. Together with a bioinformatics approach, we successfully confirmed genes known previously to be directly under the control of {sigma}32 and also assigned many additional genes to the {sigma}32 regulon. In addition, to understand better the functional relevance of the increased amount of {sigma}32 to changes in the transcriptional level of {sigma}32-dependent genes, we measured the protein level of {sigma}32 both before and after induction by a newly developed quantitative Western blot method. At a normal constant growth temperature (37 {degrees}C), we found that the {sigma}32 protein level rapidly increased, plateaued, and then gradually decreased after induction, indicating {sigma}32 can be regulated by genes in its regulon and that the mechanisms of {sigma}32 synthesis, inactivation, and degradation are not strictly temperature-dependent. The decrease in the transcriptional level of {sigma}32-dependent genes occurs earlier than the decrease in full-length {sigma}32 in the wild type strain, and the decrease in the transcriptional level of {sigma}32-dependent genes is greatly diminished in a {Delta}DnaK strain, suggesting that DnaK can act as an anti-{sigma} factor to functionally inactivate {sigma}32 and thus reduce {sigma}32-dependent transcription in vivo.

Carrillo, C. D., E. Taboada, et al. (2004). "Genome-wide Expression Analyses of Campylobacter jejuni NCTC11168 Reveals Coordinate Regulation of Motility and Virulence by flhA." J. Biol. Chem. **279**(19): 20327-20338.

http://www.jbc.org/cgi/content/abstract/279/19/20327

We examined two variants of the genome-sequenced strain, Campylobacter jejuni NCTC11168, which show marked differences in their virulence properties including colonization of poultry, invasion of Caco-2 cells, and motility. Transcript profiles obtained from whole genome DNA microarrays and proteome analyses demonstrated that these differences are reflected in late flagellar structural components and in virulence factors including those involved in flagellar glycosylation and cytolethal distending toxin production. We identified putative {sigma}28 and {sigma}54 promoters for many of the affected genes and found that greater differences in expression were observed for {sigma}28-controlled genes. Inactivation of the gene encoding

{sigma}28, fliA, resulted in an unexpected increase in transcripts with {sigma}54 promoters, as well as decreased transcription of {sigma}28-regulated genes. This was unlike the transcription profile observed for the attenuated C. jejuni variant, suggesting that the reduced virulence of this organism was not entirely due to impaired function of {sigma}28. However, inactivation of flhA, an important component of the flagellar export apparatus, resulted in expression patterns similar to that of the attenuated variant. These findings indicate that the flagellar regulatory system plays an important role in campylobacter pathogenesis and that flhA is a key element involved in the coordinate regulation of late flagellar genes and of virulence factors in C. jejuni.

Cabral, A., D. F. Fischer, et al. (2003). "Distinct Functional Interactions of Human Skn-1 Isoforms with Ese-1 during Keratinocyte Terminal Differentiation." J. Biol. Chem. **278**(20): 17792-17799.

http://www.jbc.org/cgi/content/abstract/278/20/17792

Among the three major POU proteins expressed in human skin, Oct-1, Tst-1/Oct-6, and Skn-1/Oct-11, only the latter induced SPRR2A, a marker of keratinocyte terminal differentiation. In this study, we have identified three Skn-1 isoforms, which encode proteins with various N termini, generated by alternative promoter usage. These isotypes showed distinct expression patterns in various skin samples, internal squamous epithelia, and cultured human keratinocytes. Skn-1a and Skn-1d1 bound the SPRR2A octamer site with comparable affinity and functioned as transcriptional activators. Skn-1d2 did not affect SPRR2A expression. Skn-1a, the largest protein, functionally cooperated with Ese-1/Elf-3, an epithelial-specific transcription factor, previously implicated in SPRR2A induction. This cooperativity, which depended on an N-terminal pointed-like domain in Skn-1a, was not found for Skn-1d1. Actually, Skn-1d1 counteracted the cooperativity between Skn-1a and Ese-1. Apparently, the human Skn-1 locus encodes multifunctional protein isotypes, subjected to biochemical cross-talk, which are likely to play a major role in the fine-tuning of keratinocyte terminal differentiation.

Dai, E., H. Guan, et al. (2003). "Serp-1, a Viral Anti-inflammatory Serpin, Regulates Cellular Serine Proteinase and Serpin Responses to Vascular Injury." J. Biol. Chem. **278**(20): 18563-18572.

http://www.jbc.org/cgi/content/abstract/278/20/18563

Complex DNA viruses have tapped into cellular serpin responses that act as key regulatory steps in coagulation and inflammatory cascades. Serp-1 is one such viral serpin that effectively protects virus-infected tissues from host inflammatory responses. When given as purified protein, Serp-1 markedly inhibits vascular monocyte invasion and plaque growth in animal models. We have investigated mechanisms of viral serpin inhibition of vascular inflammatory responses. In vascular injury models, Serp-1 altered early cellular plasminogen activator (tissue plasminogen activator), inhibitor (PAI-1), and receptor (urokinase-type plasminogen activator) expression ($p < 0.01$). Serp-1, but not a reactive center loop mutant, up-regulated PAI-1 serpin expression in human endothelial cells. Treatment of endothelial cells with antibody to urokinase-type plasminogen activator and vitronectin blocked Serp-1-induced changes. Significantly, Serp-1 blocked intimal hyperplasia ($p < 0.0001$) after aortic allograft transplant ($p < 0.0001$) in PAI-1-deficient mice. Serp-1 also blocked plaque growth after aortic isograft transplant and after wire-induced injury ($p < 0.05$) in PAI-1-deficient mice indicating that increase in PAI-1 expression is not required for Serp-1 to block vasculopathy development. Serp-1 did not inhibit plaque growth in uPAR-deficient mice after aortic allograft transplant. We conclude that the poxviral serpin, Serp-1, attenuates vascular inflammatory responses to injury through a pathway mediated by native uPA receptors and vitronectin.

Zhang, Y. J., W. K. O'Neal, et al. (2002). "Identification of Dynein Heavy Chain 7 as an Inner Arm Component of Human Cilia That Is Synthesized but Not Assembled in a Case of Primary Ciliary Dyskinesia." J. Biol. Chem. **277**(20): 17906-17915.

http://www.jbc.org/cgi/content/abstract/277/20/17906

Although the basic structure of the axoneme has been highly conserved throughout evolution, the varied functions of specialized axonemes require differences in structure and regulation. Cilia lining the respiratory tract propel mucus along airway surfaces, providing a critical function to the defense mechanisms of the pulmonary system, yet little is known of their molecular structure. We have identified and cloned a dynein heavy chain that is a component of the inner dynein arm. Bronchial epithelial cells were obtained from normal donors and from a patient with primary ciliary dyskinesia (PCD) whose cilia demonstrated an absence of inner dynein arms by electron microscopy. Cilia from normal and PCD cells were compared by gel electrophoresis, and mass spectrometry was used to identify DNAH7 as a protein absent in PCD cilia. The full-length DNAH7 cDNA was cloned and shares 68% similarity with an inner arm dynein heavy chain from Drosophila. DNAH7 was induced during ciliated cell differentiation, and immunohistochemistry demonstrated the presence of DNAH7 in normal cilia. In cilia from PCD cells, DNAH7 was undetectable, whereas intracellular DNAH7 was clearly present. These studies identify DNAH7 as an inner arm component of human cilia that is synthesized but not assembled in a case of PCD.

Monslow, J., J. D. Williams, et al. (2004). "Identification and Analysis of the Promoter Region of the Human Hyaluronan Synthase 2 Gene." J. Biol. Chem. **279**(20): 20576-20581.

http://www.jbc.org/cgi/content/abstract/279/20/20576

Hyaluronan (HA) is a linear glycosaminoglycan of the vertebrate extracellular matrix that is synthesized at the plasma membrane by the HA synthase (HAS) enzymes HAS1, -2 and -3. The regulation of HA synthesis has been implicated in a variety of extracellular matrix-mediated and pathological processes, including renal fibrosis. We have recently described the genomic structures of each of the human HAS genes. In the present study, we analyzed the HAS2 promoter region. In 5'-rapid amplification of cDNA ends analysis of purified mRNA from human renal epithelial proximal tubular cells, we detected an extended sequence for HAS2 exon 1, relocating the transcription initiation site 130 nucleotides upstream of the reference HAS2 mRNA sequence, GenBankTM accession number NM_005328. A luciferase reporter gene assay of nested fragments spanning the 5' terminus of NM_005328 demonstrated the constitutive promoter activity of sequences directly upstream of the repositioned transcription initiation site but not of the newly designated exonic nucleotides. Using reverse transcription-PCR, expression of this extended HAS2 mRNA was demonstrated in a variety of human cell types, and orthologous sequences were detected in mouse and rat kidney. Alignment of human, murine, and equine genomic DNA sequences upstream of the repositioned HAS2 exon 1 provided evidence for the evolutionary conservation of specific transcription factor binding sites. The location of the HAS2 promoter will facilitate analysis of the transcriptional regulation of this gene in a variety of pathological contexts as well as in developmental models in which HAS2 null animals have an embryonic lethal phenotype.

Zimmermann, K., K. Ahrens, et al. (2002). "Targeted Disruption of the GAS41 Gene Encoding a Putative Transcription Factor Indicates That GAS41 Is Essential for Cell Viability." J. Biol. Chem. **277**(21): 18626-18631.

http://www.jbc.org/cgi/content/abstract/277/21/18626

The glioma-amplified sequence (GAS) 41 protein has been proposed to be a transcription factor. To investigate its functional role in vivo, we attempted to knock out the GAS41 gene by targeted disruption in the chicken pre-lymphoid cell line DT40. Heterozygous GAS41+/[-] cell lines generated by the first round of homologous recombination express approximately half the normal level of GAS41 mRNA. However, a homozygous GAS41[-]/[-] cell line with both GAS41 alleles disrupted was not obtained following the second round of transfection, indicating that the GAS41 gene is essential for cell viability. Indeed, homozygous GAS41[-]/[-] cell lines with two disrupted GAS41 alleles can be generated following substitution of the endogenous gene by stable integration of GAS41 cDNA controlled by a tetracycline-regulated CMV promoter. Inactivation of this promoter by tetracycline withdrawal results in rapid depletion of GAS41, causing a significant decrease in RNA synthesis and subsequently cell death. Thus, our results indicate that GAS41 is required for RNA transcription.

Cheng, S., H. Afif, et al. (2004). "Activation of Peroxisome Proliferator-activated Receptor {gamma} Inhibits Interleukin-1{beta}-induced Membrane-associated Prostaglandin E2 Synthase-1 Expression in Human Synovial Fibroblasts by Interfering with Egr-1." J. Biol. Chem. **279**(21): 22057-22065.

http://www.jbc.org/cgi/content/abstract/279/21/22057

Membrane-associated prostaglandin (PG) E2 synthase-1 (mPGES-1) catalyzes the conversion of PGH2 to PGE2, which contributes to many biological processes. Peroxisome proliferator-activated receptor {gamma} (PPAR{gamma}) is a ligand-activated transcription factor and plays an important role in growth, differentiation, and inflammation in different tissues. Here, we examined the effect of PPAR{gamma} ligands on interleukin-1{beta} (IL-1{beta})-induced mPGES-1 expression in human synovial fibroblasts. PPAR{gamma} ligands 15-deoxy-{Delta}12,14 prostaglandin J2 (15d-PGJ2) and the thiazolidinedione troglitazone (TRO), but not PPAR{alpha} ligand Wy14643, dose-dependently suppressed IL-1{beta}-induced PGE2 production, as well as mPGES-1 protein and mRNA expression. 15d-PGJ2 and TRO suppressed IL-1{beta}-induced activation of the mPGES-1 promoter. Overexpression of wild-type PPAR{gamma} further enhanced, whereas overexpression of a dominant negative PPAR{gamma} alleviated, the suppressive effect of both PPAR{gamma} ligands. Furthermore, pretreatment with an antagonist of PPAR{gamma}, GW9662, relieves the suppressive effect of PPAR{gamma} ligands on mPGES-1 protein expression, suggesting that the inhibition of mPGES-1 expression is mediated by PPAR{gamma}. We demonstrated that PPAR{gamma} ligands suppressed Egr-1-mediated induction of the activities of the mPGES-1 promoter and of a synthetic reporter construct containing three tandem repeats of an Egr-1 binding site. The suppressive effect of PPAR{gamma} ligands was enhanced in the presence of a PPAR{gamma} expression plasmid. Electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter showed that both 15d-PGJ2 and TRO suppressed IL-1{beta}-induced DNA-binding activity of Egr-1. These data define mPGES-1 and Egr-1 as novel targets of PPAR{gamma} and suggest that inhibition of mPGES-1 gene transcription may be one of the mechanisms by which PPAR{gamma} regulates inflammatory responses.

Collin, M. and V. A. Fischetti (2004). "A Novel Secreted Endoglycosidase from Enterococcus faecalis with Activity on Human Immunoglobulin G and Ribonuclease B." J. Biol. Chem. **279**(21): 22558-22570.

http://www.jbc.org/cgi/content/abstract/279/21/22558

The human pathogen Enterococcus faecalis can degrade the N-linked glycans of human RNase B to acquire nutrients, but no gene or protein has been associated with this activity. We identified an 88-kDa secreted protein, endoglycosidase (Endo) E, which is most likely responsible for this activity. EndoE, encoded by ndoE, consists of an {alpha}-domain with a family 18 glycosyl hydrolase motif and a {beta}-domain similar to family 20 glycosyl hydrolases. Phylogenetic analysis of EndoE indicates that the {alpha}-domain is related to human chitobiases, and the {beta}-domain is related to bacterial and human hexosaminidases. Recombinant expression of full-length EndoE or EndoE{alpha}, site-directed mutagenesis of the catalytic residues, mass spectroscopy, and homology modeling shows that EndoE{alpha} hydrolyzes the glycan on human RNase B, whereas EndoE{beta} hydrolyzes the conserved glycan on IgG. Denaturation experiments indicate that the chitinase activity on RNase B is not dependent on the tertiary structure, although it is on IgG. The ndoE gene and secreted EndoE are present in most E. faecalis but not in Enterococcus faecium isolates. Correspondingly, E. faecalis, but not E. faecium, degrades the glycan on RNase B during growth. Thus, we have identified a secreted enzyme from E. faecalis, EndoE, which by two distinct activities hydrolyzes the glycans on RNase B and IgG. Both activities could be important for the molecular pathogenesis and persistence of E. faecalis during human infections.

Nishino, K., N. Hattori, et al. (2004). "DNA Methylation-mediated Control of Sry Gene Expression in Mouse Gonadal Development." J. Biol. Chem. **279**(21): 22306-22313.

http://www.jbc.org/cgi/content/abstract/279/21/22306

DNA methylation at CpG sequences is involved in tissue-specific and developmentally regulated gene expression. The Sry (sex-determining region on the Y chromosome) gene encodes a master protein for initiating testis differentiation in mammals, and its expression is restricted to gonadal somatic cells at 10.5-12.5 days post-coitum (dpc) in the mouse. We found that in vitro methylation of the 5'-flanking region of the Sry gene caused suppression of reporter activity, implying that Sry gene expression could be regulated by DNA methylation-mediated gene silencing. Bisulfite restriction mapping and sodium bisulfite sequencing revealed that the 5'-flanking region of the Sry gene was hypermethylated in the 8.5-dpc embryos in which the Sry gene was not expressed. Importantly, this region was specifically hypomethylated in the gonad at 11.5 dpc, while the hypermethylated status was maintained in tissues that do not express the Sry gene. We concluded that expression of the Sry gene is under the control of an epigenetic mechanism mediated by DNA methylation.

Mansouri, A., L. D. Ridgway, et al. (2003). "Sustained Activation of JNK/p38 MAPK Pathways in Response to Cisplatin Leads to Fas Ligand Induction and Cell Death in Ovarian Carcinoma Cells." J. Biol. Chem. **278**(21): 19245-19256.

http://www.jbc.org/cgi/content/abstract/278/21/19245

The efficacy of cisplatin in cancer chemotherapy is limited by the development of resistance. Although the molecular mechanisms involved in chemoresistance are poorly understood, cellular response to cisplatin is known to involve activation of MAPK and other signal transduction pathways. An understanding of early signal transduction events in the response to cisplatin could be valuable for improving the efficacy of cancer therapy. We compared cisplatin-induced activation of three MAPKs, JNK, p38, and ERK, in a cisplatin-sensitive human ovarian carcinoma cell line (2008) and its resistant subclone (2008C13). The JNK and p38 pathways were activated differentially in response to cisplatin, with the cisplatin-sensitive cells showing prolonged activation (8-12 h) and the cisplatin-resistant cells showing only transient activation (1-3 h) of JNK and p38. In the sensitive cells, inhibition of cisplatin-induced JNK and p38 activation blocked

cisplatin-induced apoptosis; persistent activation of JNK resulted in hyperphosphorylation of the c-Jun transcription factor, which in turn stimulated the transcription of an immediate downstream target, the death inducer Fas ligand (FasL). Sequestration of FasL by incubation with a neutralizing anti-FasL antibody inhibited cisplatin-induced apoptosis. In contrast, chemoresistance in 2008C13 cells was associated with failure to up-regulate FasL. Moreover, in these cells, selective stimulation of the JNK/p38 MAPK pathways by adenovirus-mediated delivery of recombinant MKK7 or MKK3 led to sensitization to apoptosis through reactivating FasL expression. Thus, the JNK > c-Jun > FasL > Fas pathway plays an important role in mediating cisplatin-induced apoptosis in ovarian cancer cells, and the duration of JNK activation is critical in determining whether cells survive or undergo apoptosis.

Wright, G., J. J. Higgin, et al. (2003). "Activation of the Prolyl Hydroxylase Oxygen-sensor Results in Induction of GLUT1, Heme Oxygenase-1, and Nitric-oxide Synthase Proteins and Confers Protection from Metabolic Inhibition to Cardiomyocytes." J. Biol. Chem. **278**(22): 20235-20239.

http://www.jbc.org/cgi/content/abstract/278/22/20235

Recently an oxygen-sensing/transducing mechanism has been identified as a family of O2-dependent prolyl hydroxylase domain-containing enzymes (PHD). In normoxia, PHD hydroxylates a specific proline residue that directs the degradation of constitutively synthesized hypoxia-inducible factor-1{alpha}. During hypoxia, the cessation of hydroxylation of this proline results in less degradation and thus increases hypoxia-inducible factor-1{alpha} protein levels. In this study we have examined the consequences of activating the PHD oxygen-sensing pathway in cultured neonatal myocytes using ethyl-3,4 dihydroxybenzoate and dimethyloxalylglycine, inhibitors that, similar to hypoxia, inhibit this family of O2-dependent PHD enzymes. Increased glucose uptake and enhanced glycolytic metabolism are classical cellular responses to hypoxia. Ethyl-3,4 dihydroxybenzoate treatment of cardiomyocyte cultures for 24 h increased [3H]deoxy-4-glucose uptake concurrent with an induction of GLUT1 protein. In addition, ethyl-3,4 dihydroxybenzoate, dimethyloxalylglycine, and hypoxia treatments were found to induce protein levels of nitricoxide synthase-2 and heme oxygenase-1, two important cardioregulatory proteins whose expression in response to hypoxic conditions is poorly understood. In conjunction with these changes in gene expression, activation of the PHD oxygen-sensing mechanism was found to preserve myocyte viability in the face of metabolic inhibition with cyanide and 2-deoxyglucose. These results point to a key role for the PHD pathway in the phenotypic changes that are observed in a hypoxic myocyte and may suggest a strategy to pharmacologically induce protection in heart.

Dolgachev, V., M. S. Farooqui, et al. (2004). "De Novo Ceramide Accumulation Due to Inhibition of Its Conversion to Complex Sphingolipids in Apoptotic Photosensitized Cells." J. Biol. Chem. **279**(22): 23238-23249.

http://www.jbc.org/cgi/content/abstract/279/22/23238

The oxidative stress induced by photodynamic therapy (PDT) with the photosensitizer phthalocyanine 4 is accompanied by increases in ceramide mass. To assess the regulation of de novo sphingolipid metabolism during PDT-induced apoptosis, Jurkat human T lymphoma and Chinese hamster ovary cells were labeled with [14C]serine, a substrate of serine palmitoyltransferase (SPT), the enzyme catalyzing the initial step in the sphingolipid biosynthesis. A substantial elevation in [14C]ceramide with a concomitant decrease in [14C]sphingomyelin was detected. The labeling of [14C]ceramide was completely abrogated by the SPT inhibitor ISP-1. In addition, ISP-1 partly suppressed PDT-induced apoptosis. Pulse-chase experiments showed that the contribution of sphingomyelin degradation to PDT-initiated increase in de novo ceramide was absent or minor. PDT had no effect on either mRNA amounts of the SPT subunits LCB1 and

LCB2, LCB1 protein expression, or SPT activity in Jurkat cells. Moreover in Chinese hamster ovary cells LCB1 protein underwent substantial photodestruction, and SPT activity was profoundly inhibited after treatment. We next examined whether PDT affects conversion of ceramide to complex sphingolipids. Sphingomyelin synthase, as well as glucosylceramide synthase, was inactivated by PDT in both cell lines in a dose-dependent manner. These results are the first to show that in the absence of SPT up-regulation PDT induces accumulation of de novo ceramide by inhibiting its conversion to complex sphingolipids.

Bozinovski, S., J. E. Jones, et al. (2002). "Granulocyte/Macrophage-Colony-stimulating Factor (GM-CSF) Regulates Lung Innate Immunity to Lipopolysaccharide through Akt/Erk Activation of NFkappa B and AP-1 in Vivo." J. Biol. Chem. **277**(45): 42808-42814.

http://www.jbc.org/cgi/content/abstract/277/45/42808

The lung innate immune response to lipopolysaccharide (LPS) coordinates cellular inflammation, mediator, and protease release essential for host defense but deleterious in asthma, chronic obstructive pulmonary disease, and cystic fibrosis. In vitro, LPS signals to the transcription factors NF[kappa]B via TLR4, MyD88, and IL-1R-associated kinase (IRAK), to AP-1 by mitogen-activated protein (MAP) kinases, and via an alternate route in IRAK-deficient mice, but the in vivo lung signaling pathway(s) are not understood. We investigated the role of Akt and Erk1/2 as LPS intensely stimulates granulocyte/macrophage-colony-stimulating factor (GM-CSF) release, and neutralizing GM-CSF profoundly suppressed LPS-induced inflammation, suppressed expression and activity of lung proteases, significantly reduced GM-CSF and tumor necrosis factor [alpha] (TNF[alpha]) mRNA expression, and dampened nuclear localization of both NF[kappa]B (p50/65) and AP-1. LPS markedly activated Akt and Erk1/2, but not p38, in a GM-CSF-dependent manner in direct temporal association with NF[kappa]B and AP-1 activation. Pharmacological inhibition of Akt or Erk activation in LPS-treated tracheal explants ex vivo inhibited the release of GM-CSF. These data implicate GM-CSF-dependent activation of Akt in the amplification of this response and demonstrate the role of Erks rather than p38 in lung LPS inflammatory responses. Inhibition of GM-CSF may be of therapeutic benefit in inflammatory diseases in which LPS contributes to lung damage.

Young, N. M., J.-R. Brisson, et al. (2002). "Structure of the N-Linked Glycan Present on Multiple Glycoproteins in the Gram-negative Bacterium, Campylobacter jejuni." J. Biol. Chem. **277**(45): 42530-42539.

http://www.jbc.org/cgi/content/abstract/277/45/42530

Mass spectrometry investigations of partially purified Campylobacter jejuni protein PEB3 showed it to be partially modified with an Asn-linked glycan with a mass of 1406 Da and composed of one hexose, five N-acetylhexosamines and a species of mass 228 Da, consistent with a trideoxydiacetamidohexose. By means of soybean lectin affinity chromatography, a mixture of glycoproteins was obtained from a glycine extract, and two-dimensional gel proteomics analysis led to the identification of at least 22 glycoproteins, predominantly annotated as periplasmic proteins. Glycopeptides were prepared from the glycoprotein mixture by Pronase digestion and gel filtration. The structure of the glycan was determined by using nano-NMR techniques to be GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-[Glc[beta]1,3-]GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-GalNAc-[alpha]1,3-Bac-[beta]1,N-Asn-Xaa, where Bac is bacillosamine, 2,4-diacetamido-2,4,6-trideoxyglucopyranose. Protein glycosylation was abolished when the pglB gene was mutated, providing further evidence that the enzyme encoded by this gene is responsible for formation of the glycopeptide N-linkage. Comparison of the pgl locus with that of Neisseria meningitidis suggested that most of the homologous genes are probably involved in the biosynthesis of

bacillosamine.

Kang, H.-Y., K.-E. Huang, et al. (2002). "Differential Modulation of Androgen Receptor-mediated Transactivation by Smad3 and Tumor Suppressor Smad4." J. Biol. Chem. **277**(46): 43749-43756.

http://www.jbc.org/cgi/content/abstract/277/46/43749

Smad proteins have been demonstrated to be key components in the transforming growth factor [beta] signaling cascade. Here we demonstrate that Smad4, together with Smad3, can interact with the androgen receptor (AR) in the DNA-binding and ligand-binding domains, which may result in the modulation of 5[alpha]-dihydrotestosterone-induced AR transactivation. Interestingly, in the prostate PC3 and LNCaP cells, addition of Smad3 can enhance AR transactivation, and co-transfection of Smad3 and Smad4 can then repress AR transactivation in various androgen response element-promoter reporter assays as well as Northern blot and reverse transcription-PCR quantitation assays with prostate-specific antigen mRNA expression. In contrast, in the SW480{middle dot}C7 cells, lacking endogenous functional Smad4, the influence of Smad3 on AR transactivation is dependent on the various androgen response element-promoters. The influence of Smad3/Smad4 on the AR transactivation may involve the acetylation since the treatment of trichostatin A or sodium butyrate can reverse Smad3/Smad4-repressed AR transactivation and Smad3/Smad4 complex can also decrease the acetylation level of AR. Together, these results suggest that the interactions between AR, Smad3, and Smad4 may result in the differential regulation of the AR transactivation, which further strengthens their roles in the prostate cancer progression.

Zhang, R.-Z., P. Sabatelli, et al. (2002). "Effects on Collagen VI mRNA Stability and Microfibrillar Assembly of Three COL6A2 Mutations in Two Families with Ullrich Congenital Muscular Dystrophy." J. Biol. Chem. **277**(46): 43557-43564.

http://www.jbc.org/cgi/content/abstract/277/46/43557

We recently reported a severe deficiency in collagen type VI, resulting from recessive mutations of the COL6A2 gene, in patients with Ullrich congenital muscular dystrophy. Their parents, who are all carriers of one mutant allele, are unaffected, although heterozygous mutations in collagen VI caused Bethlem myopathy. Here we investigated the consequences of three COL6A2 mutations in fibroblasts from patients and their parents in two Ullrich families. All three mutations lead to nonsense-mediated mRNA decay. However, very low levels of undegraded mutant mRNA remained in patient B with compound heterozygous mutations at the distal part of the triple-helical domain, resulting in deposition of abnormal microfibrils that cannot form extensive networks. This observation suggests that the C-terminal globular domain is not essential for triple-helix formation but is critical for microfibrillar assembly. In all parents, the COL6A2 mRNA levels are reduced to 57-73% of the control, but long term collagen VI matrix depositions are comparable with that of the control. The almost complete absence of abnormal protein and near-normal accumulation of microfibrils in the parents may account for their lack of myopathic symptoms.

Fukuchi, M., A. Tabuchi, et al. (2004). "Activity-dependent Transcriptional Activation and mRNA Stabilization for Cumulative Expression of Pituitary Adenylate Cyclase-activating Polypeptide mRNA Controlled by Calcium and cAMP Signals in Neurons." J. Biol. Chem. **279**(46): 47856-47865.

http://www.jbc.org/cgi/content/abstract/279/46/47856

Although it has been established that an activity-dependent gene transcription is induced by the calcium (Ca2+) signals in neurons, it is unclear how the specific mRNA moieties are transiently accumulated in response to synaptic transmission which evokes multiple intracellular signals including Ca2+ and cAMP ones. The expression of pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide, is controlled by Ca2+ signals evoked via membrane depolarization in neurons, and, in cultured rat cortical neuronal cells, we found that the Ca2+ signal-mediated activation of the PACAP gene promoter was critically controlled by a single cAMP-response element (CRE) located at around -200, to which the CRE-binding protein predominantly bound. The Ca2+ signal-induced expression of PACAP mRNA was enhanced by forskolin, which evokes cAMP signals. In support, the PACAP gene promoter was synergistically enhanced by Ca2+ and cAMP signals through the CRE, accompanying a prolonged activation of extracellular signal-related protein kinase 1/2 and CRE-binding protein. On the other hand, sole administration of forskolin markedly reduced the cellular content of PACAP mRNA, which was restored by the addition of Ca2+ signals. We found that the stability of PACAP mRNA was increased in response to Ca2+ signals but not that of activity-regulated cytoskeleton-associated protein (Arc) mRNA, indicating an activity-dependent stabilization of specific mRNA species in neurons, which can antagonize the regulation mediated by cAMP signals. Thus, the transcriptional activation and mRNA stabilization are coordinately regulated by Ca2+ and cAMP signals for the cumulative expression of PACAP mRNA in neurons.

Lee, C.-G., T. W. Reichman, et al. (2004). "MLE Functions as a Transcriptional Regulator of the roX2 Gene." J. Biol. Chem. **279**(46): 47740-47745.

http://www.jbc.org/cgi/content/abstract/279/46/47740

Dosage compensation is a process that equalizes transcription activity between the sexes. In Drosophila, two non-coding RNA, roX1 and roX2, and at least six protein regulators, MSL-1, MSL-2, MSL-3, MLE, MOF, and JIL-1, have been identified as essential for dosage compensation. Although there is accumulating evidence of the intricate functional and physical interactions between protein and RNA regulators, little is known about how roX RNA expression and function are modulated in coordination with protein regulators. In this report, we have found that a relatively short (about 350 bp) upstream genomic region of the roX2 gene, Prox2, harbors an activity that drives transcription of the downstream gene. Our study has shown that MLE can stimulate the transcription activity of Prox2 and that MLE associates with Prox2 through direct interaction with a newly identified 54-bp repeat, Prox. Our observations suggest a novel mechanism by which roX2 RNA is regulated at the transcriptional level.

Dunty, J. M. and M. D. Schaller (2002). "The N Termini of Focal Adhesion Kinase Family Members Regulate Substrate Phosphorylation, Localization, and Cell Morphology." J. Biol. Chem. **277**(47): 45644-45654.

http://www.jbc.org/cgi/content/abstract/277/47/45644

The focal adhesion kinase (FAK) and cell adhesion kinase [beta] (CAK[beta], PYK2, CADTK, RAFTK) are highly homologous FAK family members, yet clearly have unique roles in the cell. Comparative analyses of FAK and CAK[beta] have revealed intriguing differences in their activities. These differences were investigated further through the characterization of a set of FAK/CAK[beta] chimeric kinases. CAK[beta] exhibited greater catalytic activity than FAK in vitro, providing a molecular basis for differential substrate phosphorylation by FAK and CAK[beta] in

vivo. Furthermore, the N terminus may regulate catalytic activity since chimeras containing the FAK N terminus and CAK[beta] catalytic domain exhibited a striking high level of catalytic activity and substrate phosphorylation. Unexpectedly, a modulatory role for the N termini in subcellular localization was also revealed. Chimeras containing the FAK N terminus and CAK[beta] C terminus localized to focal adhesions, whereas chimeras containing the N and C termini of CAK[beta] did not. Finally, prominent changes in cell morphology were induced upon expression of chimeras containing the CAK[beta] N terminus, which were not associated with apoptotic cell death, cell cycle progression delay, or changes in Rho activity. These results demonstrate novel regulatory roles for the N terminus of FAK family kinases.

Leavey, P. J., C. Gonzalez-Aller, et al. (2002). "A 29-kDa Protein Associated with p67phox Expresses Both Peroxiredoxin and Phospholipase A2 Activity and Enhances Superoxide Anion Production by a Cell-free System of NADPH Oxidase Activity." J. Biol. Chem. **277**(47): 45181-45187.

http://www.jbc.org/cgi/content/abstract/277/47/45181

Production of toxic oxygen metabolites provides a mechanism for microbicidal activity of the neutrophil. The NADPH oxidase enzyme system initiates the production of oxygen metabolites by reducing oxygen to form superoxide anion (O[IMG]img001.gif" ALT="<UP>₂</UP>">). With stimulation of the respiratory burst, cytosolic oxidase components, p47phox, p67phox, and Rac, translocate to the phagolysomal and plasma membranes where they form a complex with cytochrome b558 and express enzyme activity. A 29-kDa neutrophil protein (p29) was identified by co-immunoprecipitation with p67phox. N-terminal sequence analysis of p29 revealed homology to an open reading frame gene described in a myeloid leukemia cell line. A cDNA for p29 identical to the open reading frame protein was amplified from RNA of neutrophils. Significant interaction between p29 and p67phox was demonstrated using a yeast two-hybrid system. A recombinant (rh) p29 was expressed in Sf9 cells resulting in a protein with an apparent molecular weight of 34,000. The rh-p29 showed immunoreactivity with the original rabbit antiserum that detected p47phox and p67phox. In addition, rh-p29 exhibited PLA2 activity, which was Ca2+ independent, optimal at low pH, and preferential for phosphatidylcholine substrates. The recombinant protein protected glutathione synthetase and directly inactivated H2O2. By activity and sequence homology, rh-p29 can be classified as a peroxiredoxin. Finally, O[IMG]img001.gif" ALT="<UP>₂</UP>"> production by plasma membrane and recombinant cytosolic oxidase components in the SDS-activated, cell-free NADPH oxidase system were enhanced by rh-p29. This effect was not inhibited by PLA2 inhibitors. Thus, p29 is a novel protein that associates with p67 and has peroxiredoxin activity. This protein has a potential role in protecting the NADPH oxidase by inactivating H2O2 or altering signaling pathways affected by H2O2.

Okuda, T., M. Okamura, et al. (2002). "Single Nucleotide Polymorphism of the Human High Affinity Choline Transporter Alters Transport Rate." J. Biol. Chem. **277**(47): 45315-45322.

http://www.jbc.org/cgi/content/abstract/277/47/45315

High affinity choline uptake plays a critical role in the regulation of acetylcholine synthesis in cholinergic neurons. Recently, we succeeded in molecular cloning of the high affinity choline transporter (CHT1), which is specifically expressed in cholinergic neurons. Here we demonstrate the presence of functionally relevant, nonsynonymous single nucleotide polymorphism in the human CHT1 gene by comprehensive sequence analysis of the exons and the intron/exon boundaries including the transcription start site. The deduced amino acid change for the polymorphism is isoleucine to valine at amino acid 89 (I89V) located within the third

transmembrane domain of the protein. The allele frequency of I89V was 6% for Ashkenazi Jews. Functional assessment of the I89V transporter in mammalian cell lines revealed a 40-50% decrease in Vmax for choline uptake rate compared with the wild type, whereas there was no alteration in the apparent affinities for choline, sodium, chloride, and the specific inhibitor hemicholinum-3. There also was no change in the specific hemicholinum-3 binding activity. The decreased choline uptake was not associated with the surface expression level of the protein as assessed by biotinylation assay. These results suggest an impaired substrate translocation in the I89V transporter. The Caenorhabditis elegans ortholog of CHT1 has a valine residue at the corresponding position and a single replacement from valine to isoleucine caused a decrease in the choline uptake rate by 40%, suggesting that this hydrophobic residue is generally critical in the choline transport rate in CHT1. This polymorphism in the allelic CHT1 gene may represent a predisposing factor for cholinergic dysfunction.

Yang, T. and B. W. Poovaiah (2002). "A Calmodulin-binding/CGCG Box DNA-binding Protein Family Involved in Multiple Signaling Pathways in Plants." J. Biol. Chem. **277**(47): 45049-45058.

http://www.jbc.org/cgi/content/abstract/277/47/45049

We reported earlier that the tobacco early ethylene-responsive gene NtER1 encodes a calmodulin-binding protein (Yang, T., and Poovaiah, B. W. (2000) J. Biol. Chem. 275, 38467-38473). Here we demonstrate that there is one NtER1 homolog as well as five related genes in Arabidopsis. These six genes are rapidly and differentially induced by environmental signals such as temperature extremes, UVB, salt, and wounding; hormones such as ethylene and abscisic acid; and signal molecules such as methyl jasmonate, H2O2, and salicylic acid. Hence, they were designated as AtSR1-6 (Arabidopsis thaliana signal-responsive genes). Ca2+/calmodulin binds to all AtSRs, and their calmodulin-binding regions are located on a conserved basic amphiphilic [alpha]-helical motif in the C terminus. AtSR1 targets the nucleus and specifically recognizes a novel 6-bp CGCG box (A/C/G)CGCG(G/T/C). The multiple CGCG cis-elements are found in promoters of genes such as those involved in ethylene signaling, abscisic acid signaling, and light signal perception. The DNA-binding domain in AtSR1 is located on the N-terminal 146 bp where all AtSR1-related proteins share high similarity but have no similarity to other known DNA-binding proteins. The calmodulin-binding nuclear proteins isolated from wounded leaves exhibit specific CGCG box DNA binding activities. These results suggest that the AtSR gene family encodes a family of calmodulin-binding/DNA-binding proteins involved in multiple signal transduction pathways in plants.

Barlic, J., D. H. McDermott, et al. (2004). "Interleukin (IL)-15 and IL-2 Reciprocally Regulate Expression of the Chemokine Receptor CX3CR1 through Selective NFAT1- and NFAT2-dependent Mechanisms." J. Biol. Chem. **279**(47): 48520-48534.

http://www.jbc.org/cgi/content/abstract/279/47/48520

We have recently reported that interleukin (IL)-15 and IL-2, which signal through IL-2R{beta}{gamma}, oppositely regulate expression of the proinflammatory chemokine receptor CX3CR1. Here we delineate molecular mechanisms responsible for this paradox. By using a luciferase reporter plasmid, we identified a 433-bp region spanning the major transcriptional start point of human CX3CR1 that, when expressed in human peripheral blood mononuclear cells (PBMCs), possessed strong constitutive promoter activity. IL-2 and IL-15 treatment increased and abolished this activity, respectively, mimicking their effects on endogenous CX3CR1. IL-2 and IL-15 have been reported to also have opposite effects on the immunoregulatory transcription factor NFAT (nuclear factor of activated T cells), and the 433-bp region contains a {kappa}B-like NFAT site. The effects of IL-15 and IL-2 on both CX3CR1 reporter activity and endogenous CX3CR1

transcription in PBMCs were abolished by the NFAT inhibitors cyclosporin A and VIVIT. Moreover, mutation of the {kappa}B-like NFAT sequence markedly attenuated IL-2 and IL-15 modulation of CX3CR1 promoter-reporter activity in PBMCs. Furthermore, chromatin immunoprecipitation revealed that IL-15 promoted specific recruitment of NFAT1 but not NFAT2 to the CX3CR1 promoter, whereas IL-2 had the converse effect. This appears to be relevant in vivo because mouse CX3CR1 mRNA was expressed in both PBMCs and splenocytes from NFAT1-/- mice injected with recombinant IL-15 but was undetectable in cells from IL-15-injected NFAT1+/+ BALB/c mice; as predicted, IL-2 up-regulated cx3cr1 in both mouse strains to a similar extent. Thus, by pharmacologic, genetic, and biochemical criteria in vitro and in vivo, our results suggest that IL-15 and IL-2 oppositely regulate CX3CR1 gene expression by differentially recruiting NFAT1 and NFAT2 to a {kappa}B-like NFAT site within the CX3CR1 promoter. We propose that expression of CX3CR1 and possibly other immunoregulatory genes may be determined in part by the balance of NFAT1 and NFAT2 activity in leukocytes.

Patnaik, S. K., B. Potvin, et al. (2004). "LEC12 and LEC29 Gain-of-Function Chinese Hamster Ovary Mutants Reveal Mechanisms for Regulating VIM-2 Antigen Synthesis and E-selectin Binding." J. Biol. Chem. **279**(48): 49716-49726.

http://www.jbc.org/cgi/content/abstract/279/48/49716

LEC12 and LEC29 are two gain-of-function Chinese hamster ovary glycosylation mutants that express the Fut9 gene encoding {alpha}(1,3)fucosyltransferase IX ({alpha}(1,3) Fuc-TIX). Both mutants express the Lewis X (LeX) determinant Gal{beta}(1,4)[Fuc{alpha}(1,3)]GlcNAc, and LEC12, but not LEC29 cells, also express the VIM-2 antigen SA{alpha}(2,3)-Gal{beta}(1,4)GlcNAc{beta}(1,3)Gal{beta}(1,4)[Fuc{alpha}(1,3)]GlcNAc. Here we show that LEC29 cells transfected with a Fut9 cDNA express VIM-2, and thus LEC29 cells synthesize appropriate acceptors to generate the VIM-2 epitope. Semiquantitative reverse transcription-PCR showed that LEC12 has 10- to 20-fold less Fut9 gene transcripts than LEC29. However, Western analysis revealed that LEC12 has [~]20 times more Fut9 protein than LEC29. The latter finding was consistent with our previous observation that LEC12 has [~]40 times more in vitro {alpha}(1,3)Fuc-T activity than LEC29. The basis for the difference in Fut9 protein levels was found to lie in sequence differences in the 5'-untranslated regions (5'-UTR) of LEC12 and LEC29 Fut9 gene transcripts. Whereas reporter assays with the respective 5'-UTR regions linked to luciferase did not indicate a reduced translation efficiency caused by the LEC29 5'-UTR, transfected full-length LEC29 Fut9 cDNA or in vitro-synthesized full-length LEC29 Fut9 RNA gave less Fut9 protein than similar constructs with a LEC12 5'-UTR. This difference appears to be largely responsible for the reduced {alpha}(1,3)Fuc-TIX activity and lack of VIM-2 expression of LEC29 cells. This could be of physiological relevance, because LEC29 and parent Chinese hamster ovary cells transiently expressing a Fut9 cDNA were able to bind mouse E-selectin, although they did not express sialyl-LeX.

Robichaud, G. A., M. Nardini, et al. (2004). "Human Pax-5 C-terminal Isoforms Possess Distinct Transactivation Properties and Are Differentially Modulated in Normal and Malignant B Cells." J. Biol. Chem. **279**(48): 49956-49963.

http://www.jbc.org/cgi/content/abstract/279/48/49956

The transcription factor Pax-5 occupies a central role in B cell differentiation and has been implicated in the development of B cell lymphoma. The transcriptional activation function of Pax-5 requires an intact N-terminal DNA-binding domain and is strongly influenced by the C-terminal transactivation domain. We report the identification and characterization of five human Pax-5 isoforms, which occur through the alternative splicing of exons that encode for the C-terminal

transactivation domain. These isoforms arise from the inclusion or exclusion of exon 7, exon 8, and/or exon 9. Three of the Pax-5 isoforms generate novel protein sequences rich in proline, serine, and threonine amino acids that are the hallmarks of transactivation domains. The Pax-5 isoforms are expressed in peripheral blood mononuclear cells, cancerous and non-cancerous B cell lines, as well as in primary B cell lymphoma tissue. Electrophoretic mobility shift assays demonstrate that the isoforms possess specific DNA binding activity and recognize the PAX-5 consensus binding sites. In reporter assays using the CD19 promoter, the transactivation properties of the various isoforms were significantly influenced by the changes in the C-terminal protein sequence. Finally, we demonstrate, for the first time, that human Pax-5 isoform expression is modulated by specific signaling pathways in B lymphocytes.

Billich, A., F. Bornancin, et al. (2003). "Phosphorylation of the Immunomodulatory Drug FTY720 by Sphingosine Kinases." J. Biol. Chem. **278**(48): 47408-47415.

http://www.jbc.org/cgi/content/abstract/278/48/47408

The immunomodulatory drug FTY720 is phosphorylated in vivo, and the resulting FTY720 phosphate as a ligand for sphingosine-1-phosphate receptors is responsible for the unique biological effects of the compound. So far, phosphorylation of FTY720 by murine sphingosine kinase (SPHK) 1a had been documented. We found that, while FTY720 is also phosphorylated by human SPHK1, the human type 2 isoform phosphorylates the drug 30-fold more efficiently, because of a lower Km of FTY720 for SPHK2. Similarly, murine SPHK2 was more efficient than SPHK1a. Among splice variants of the human SPHKs, an N-terminally extended SPHK2 isoform was even more active than SPHK2 itself. Further SPHK superfamily members, namely ceramide kinase and a "SPHK-like" protein, failed to phosphorylate sphingosine and FTY720. Thus, only SPHK1 and 2 appear to be capable of phosphorylating FTY720. Using selective assay conditions, SPHK1 and 2 activities in murine tissues were measured. While activity of SPHK2 toward sphingosine was generally lower than of SPHK1, FTY720 phosphorylation was higher under conditions favoring SPHK2. In human endothelial cells, while activity of SPHK1 toward sphingosine was 2-fold higher than of SPHK2, FTY720 phosphorylation was 7-fold faster under SPHK2 assay conditions. Finally, FTY720 was poorly phosphorylated in human blood as compared with rodent blood, in line with the low activity of SPHK1 and in particular of SPHK2 in human blood. To conclude, both SPHK1 and 2 are capable of phosphorylating FTY720, but SPHK2 is quantitatively more important than SPHK1.

Jenkins, R. H., G. J. Thomas, et al. (2004). "Myofibroblastic Differentiation Leads to Hyaluronan Accumulation through Reduced Hyaluronan Turnover." J. Biol. Chem. **279**(40): 41453-41460.

http://www.jbc.org/cgi/content/abstract/279/40/41453

During the initiation and progression of fibrosis there is extensive differentiation of cells to a myofibroblastic phenotype. Because the synthesis of hyaluronan (HA) was recently linked to oncogenic epithelial-mesenchymal transformation, the present study investigated whether increased HA synthesis was also associated with myofibroblastic differentiation. HA synthesis and size were measured by incorporation of [3H]glucosamine, ion exchange, and size exclusion chromatography. Hyaluronan synthase (HAS) or hyaluronidase (HYAL) mRNA levels were assessed by reverse transcription-PCR. HYAL was detected by immunoblotting and the degradation of [3H]HA. Between 2- and 3-fold more HA appeared in the conditioned medium and became associated with the cells upon myofibroblastic differentiation. Inhibition of HAS and examination of HAS mRNA expression demonstrated that this was not the result of increased synthesis of HA or the induction of HAS 2. After differentiation, however, myofibroblasts metabolized exogenously supplied [3H]HA at a slower rate than fibroblasts and expressed lower

levels of both HYAL 1 and HYAL 2 mRNA. Immunoblotting revealed more HYAL 1 and 2 in the myofibroblast conditioned medium. After acidification, however, there was no difference in HA degradation. This suggests that much of the released HYAL is inactive and that the observed differences in HA degradation are caused by cell-associated rather than secreted activity. This was confirmed by immunohistochemical staining for HYAL 1 and HYAL 2. This finding indicates the potential importance of the HYAL enzymes in controlling fibrotic progression and contrasts HA synthesis as a mediator of oncogenic transformation with that of HA degradation controlling fibrogenic differentiation.

Smith, S. B., R. Gasa, et al. (2003). "Neurogenin3 and Hepatic Nuclear Factor 1 Cooperate in Activating Pancreatic Expression of Pax4." J. Biol. Chem. **278**(40): 38254-38259.

http://www.jbc.org/cgi/content/abstract/278/40/38254

During fetal development, paired/homeodomain transcription factor Pax4 controls the formation of the insulin-producing {beta} cells and the somatostatin-producing {delta} cells in the islets of Langerhans in the pancreas. Targeting of Pax4 expression to the islet lineage in the fetal pancreas depends on a short sequence located [~]2 kb upstream of the transcription initiation site of the PAX4 gene. This short sequence contains binding sites for homeodomain transcription factors PDX1 and hepatic nuclear factor (HNF)1, nuclear receptor HNF4{alpha}, and basic helix-loop-helix factor Neurogenin3. In the current study we demonstrate that the HNF1{alpha} and Neurogenin3 binding sites are critical for activity of the region through synergy between the two proteins. Synergy involves a physical interaction between the factors and requires the activation domains of both factors. Furthermore, exogenous expression of Neurogenin3 is sufficient to induce expression of the endogenous pax4 gene in the mouse pancreatic ductal cell line mPAC, which already expresses HNF1{alpha}, whereas expression of both Neurogenin3 and HNF1{alpha} are necessary to activate the pax4 gene in the fibroblast cell line NIH3T3. These data demonstrate how Neurogenin3 and HNF1{alpha} activate the pax4 gene during the cascade of gene expression events that control pancreatic endocrine cell development.

Besteiro, S., M. Biran, et al. (2002). "Succinate Secreted by Trypanosoma brucei Is Produced by a Novel and Unique Glycosomal Enzyme, NADH-dependent Fumarate Reductase." J. Biol. Chem. **277**(41): 38001-38012.

http://www.jbc.org/cgi/content/abstract/277/41/38001

In all trypanosomatids, including Trypanosoma brucei, glycolysis takes place in peroxisome-like organelles called glycosomes. These are closed compartments wherein the energy and redox (NAD+/NADH) balances need to be maintained. We have characterized a T. brucei gene called FRDg encoding a protein 35% identical to Saccharomyces cerevisiae fumarate reductases. Microsequencing of FRDg purified from glycosome preparations, immunofluorescence, and Western blot analyses clearly identified this enzyme as a glycosomal protein that is only expressed in the procyclic form of T. brucei but is present in all the other trypanosomatids studied, i.e. Trypanosoma congolense, Crithidia fasciculata and Leishmania amazonensis. The specific inactivation of FRDg gene expression by RNA interference showed that FRDg is responsible for the NADH-dependent fumarate reductase activity detected in glycosomal fractions and that at least 60% of the succinate secreted by the T. brucei procyclic form (in the presence of D-glucose as the sole carbon source) is produced in the glycosome by FRDg. We conclude that FRDg plays a key role in the energy metabolism by participating in the maintenance of the glycosomal NAD+/NADH balance. We have also detected a significant pyruvate kinase activity in the cytosol of the T. brucei procyclic cells that was not observed previously. Consequently, we propose a revised model of glucose metabolism in procyclic trypanosomes that may also be valid

for all other trypanosomatids except the T. brucei bloodstream form. Interestingly, H. Gest has hypothesized previously (Gest, H. (1980) FEMS Microbiol. Lett. 7, 73-77) that a soluble NADH-dependent fumarate reductase has been present in primitive organisms and evolved into the present day fumarate reductases, which are quinol-dependent. FRDg may have the characteristics of such an ancestral enzyme and is the only NADH-dependent fumarate reductase characterized to date.

Elfering, S. L., T. M. Sarkela, et al. (2002). "Biochemistry of Mitochondrial Nitric-oxide Synthase." J. Biol. Chem. **277**(41): 38079-38086.

http://www.jbc.org/cgi/content/abstract/277/41/38079

We reported that the generation of nitric oxide by mitochondria is catalyzed by a constitutive, mitochondrial nitric-oxide synthase (mtNOS). Given that this production may establish the basis for a novel regulatory pathway of energy metabolism, oxygen consumption, and oxygen free radical production, it becomes imperative to identify unequivocally and characterize this enzyme to provide a basis for its regulation. The mitochondrial localization of mtNOS was supported by following the hepatic distribution of mtNOS, immunoblotting submitochondrial fractions, and immunohistochemistry of liver tissues. mtNOS was identified as brain NOS[alpha] by various methods (mass spectrometry of proteolytic fragments, amino acid analysis, molecular weight, pI, and analysis of PCR fragments), excluding the occurrence of a novel isoform or other splice variants. Distribution of mtNOS transcript indicated its occurrence in liver, brain, heart, muscle, kidney, lung, testis, and spleen. In contrast to brain NOS, mtNOS has two post-translational modifications: acylation with myristic acid and phosphorylation at the C terminus. The former modification is a reversible and post-translational process, which may serve for subcellular targeting or membrane anchoring. The latter modification could be linked to enzymatic regulation. These results are discussed in terms of the role that nitric oxide may have in cellular bioenergetics.

Jackson, M., J. W. Baird, et al. (2002). "Cloning and Characterization of Ehox, a Novel Homeobox Gene Essential for Embryonic Stem Cell Differentiation." J. Biol. Chem. **277**(41): 38683-38692.

http://www.jbc.org/cgi/content/abstract/277/41/38683

We report here the identification and characterization of a novel paired-like homeobox-containing gene (Ehox). This gene, identified in embryonic stem (ES) cells, is differentially expressed during in vitro ES cell differentiation. We have assessed Ehox function using the ES cell in vitro differentiation system. This has involved molecular and biological analyses of the effects of sense or antisense Ehox expression (using episomal vectors) on ES cell differentiation. Analysis of antisense Ehox-expressing ES cells indicates that they are unable to express marker genes associated with hematopoietic, endothelial, or cardiac differentiation following removal of leukemia inhibitory factor. In contrast, overexpression of Ehox using the sense construct accelerated the appearance of these differentiation markers. ES cell self-renewal and differentiation assays reveal that inhibition of Ehox activity results in the maintenance of a stem cell phenotype in limiting concentrations of leukemia inhibitory factor and the almost complete impairment of the cardiomyocyte differentiation capacity of these cells. We therefore conclude that Ehox is a novel homeobox-containing gene that is essential for the earliest stages of murine ES cell differentiation.

Longo, K. A., J. A. Kennell, et al. (2002). "Wnt Signaling Protects 3T3-L1 Preadipocytes from Apoptosis through Induction of Insulin-like Growth Factors." J. Biol. Chem. **277**(41): 38239-38244.

http://www.jbc.org/cgi/content/abstract/277/41/38239

Ectopic expression of Wnt-1 in 3T3-L1 preadipocytes stabilizes [beta]-catenin, activates TCF-dependent gene transcription, and blocks adipogenesis. Here we report that upon serum withdrawal, Wnt-1 causes 3T3-L1 cells to resist apoptosis through a mechanism that is partially dependent on phosphatidylinositol 3-kinase. Although activation of Wnt signaling by inhibition of GSK-3 activity or ectopic expression of dominant stable [beta]-catenin blocks apoptosis, inhibition of Wnt signaling through expression of dominant negative TCF-4 increases apoptosis. Wnt-1 stimulates 3T3-L1 preadipocytes to secrete factors that increase PKB/Akt phosphorylation at levels comparable with treatment with 10% serum. With DNA microarrays, we identified several secreted antiapoptotic genes that are induced by Wnt-1, notably insulin-like growth factor I (IGF-I) and IGF-II. Consistent with IGFs mediating the antiapoptotic effects of Wnt-1 in preadipocytes, conditioned medium from Wnt-1 expressing 3T3-L1 cells was unable to promote protein kinase B phosphorylation after the addition of recombinant IGFBP-4. Thus, we demonstrated that Wnt-1 induces expression of antiapoptotic genes in 3T3-L1 preadipocytes such as IGF-I and IGF-II, which allows these cells to resist apoptosis in response to serum deprivation.

Vannahme, C., N. Smyth, et al. (2002). "Characterization of SMOC-1, a Novel Modular Calcium-binding Protein in Basement Membranes." J. Biol. Chem. **277**(41): 37977-37986.

http://www.jbc.org/cgi/content/abstract/277/41/37977

We have isolated the novel gene SMOC-1 that encodes a secreted modular protein containing an EF-hand calcium-binding domain homologous to that in BM-40. It further consists of two thyroglobulin-like domains, a follistatin-like domain and a novel domain. Recombinant expression in human cells showed that SMOC-1 is a glycoprotein with a calcium-dependent conformation. Results from Northern blots, reverse transcriptase-PCR, and immunoblots revealed a widespread expression in many tissues. Immunofluorescence studies with an antiserum directed against recombinant human SMOC-1 demonstrated a basement membrane localization of the protein and additionally its presence in other extracellular matrices. Immunogold electron microscopy confirmed the localization of SMOC-1 within basement membranes in kidney and skeletal muscle as well as its expression in the zona pellucida surrounding the oocyte.

Holmborn, K., J. Ledin, et al. (2004). "Heparan Sulfate Synthesized by Mouse Embryonic Stem Cells Deficient in NDST1 and NDST2 Is 6-O-Sulfated but Contains No N-Sulfate Groups." J. Biol. Chem. **279**(41): 42355-42358.

http://www.jbc.org/cgi/content/abstract/279/41/42355

Heparan sulfate structure differs significantly between various cell types and during different developmental stages. The diversity is created during biosynthesis by sulfotransferases, which add sulfate groups to the growing chain, and a C5-epimerase, which converts selected glucuronic acid residues to iduronic acid. All these modifications are believed to depend on initial glucosamine N-sulfation carried out by the enzyme glucosaminyl N-deacetylase/N-sulfotransferase (NDST). Here we report that heparan sulfate synthesized by mouse embryonic stem cells deficient in NDST1 and NDST2 completely lacks N-sulfation but still contains 6-O-sulfate groups, demonstrating that 6-O-sulfation can occur without prior N-sulfation. Reverse transcriptase-PCR analysis indicates that all three identified 6-O-sulfotransferases are expressed

by the cells, 6-O-sulfotransferase-1 being the dominating form. The 6-O-sulfated polysaccharide lacking N-sulfate groups also contains N-unsubstituted glucosamine units, raising questions about how these units are generated.

Gronlund, H., T. Bergman, et al. (2003). "Formation of Disulfide Bonds and Homodimers of the Major Cat Allergen Fel d 1 Equivalent to the Natural Allergen by Expression in Escherichia coli." J. Biol. Chem. **278**(41): 40144-40151.

http://www.jbc.org/cgi/content/abstract/278/41/40144

Dander from the domestic cat (Felis domesticus) is one of the most common causes of IgE-mediated allergy. Attempts to produce tetrameric folded major allergen Fel d 1 by recombinant methods with structural features similar to the natural allergen have been only partially successful. In this study, a recombinant folded Fel d 1 with molecular and biological properties similar to the natural counterpart was produced. A synthetic gene coding for direct fusion of the Fel d 1 chain 2 N-terminally to chain 1 was constructed by overlapping oligonucleotides in PCR. Escherichia coli expression resulted in a non-covalently associated homodimer with an apparent molecular mass of 30 kDa defined by size exclusion chromatography. Furthermore, each 19,177-Da subunit displayed a disulfide pattern identical to that found in the natural Fel d 1, i.e. Cys3(1) Cys73(2), Cys44(1)-Cys48(2), Cys70(1)-Cys7(2), as determined by electrospray mass spectrometry after tryptic digestion. Circular dichroism analysis showed identical folds of natural and recombinant Fel d 1. Furthermore, recombinant Fel d l reacted specifically with serum IgE, inducing expression of CD203c on basophils and lymphoproliferative responses in cat-allergic patients. The results show that the overall fold and immunological properties of the recombinant Fel d 1 are very similar to those of natural Fel d 1. Moreover, the recombinant Fel d 1 construct provides a tool for defining the three-dimensional structure of Fel d 1 and represents a reagent for diagnosis and allergen-specific immunotherapy of cat allergy.

Huynh, T. T., V. T. Huynh, et al. (2003). "Gene Knockdown of {gamma}-Glutamylcysteine Synthetase by RNAi in the Parasitic Protozoa Trypanosoma brucei Demonstrates That It Is an Essential Enzyme." J. Biol. Chem. **278**(41): 39794-39800.

http://www.jbc.org/cgi/content/abstract/278/41/39794

The parasitic protozoa Trypanosoma brucei utilizes a novel cofactor (trypanothione, T(SH)2), which is a conjugate of GSH and spermidine, to maintain cellular redox balance. {gamma}-Glutamylcysteine synthetase ({gamma}-GCS) catalyzes the first step in the biosynthesis of GSH. To evaluate the importance of thiol metabolism to the parasite, RNAi methods were used to knock down gene expression of {gamma}-GCS in procyclic T. brucei cells. Induction of {gamma}-GCS RNAi with tetracycline led to cell death within 4-6 days post-induction. Cell death was preceded by the depletion of the {gamma}-GCS protein and RNA and by the loss of the cellular pools of GSH and T(SH)2. The addition of GSH (80 {micro}M) to cell cultures rescued the RNAi cell death phenotype and restored the intracellular thiol pools to wild-type levels. Treatment of cells with buthionine sulfoximine (BSO), an enzyme-activated inhibitor of {gamma}-GCS, also resulted in cell death. However, the toxicity of the inhibitor was not reversed by GSH, suggesting that BSO has more than one cellular target. BSO depletes intracellular thiols to a similar extent as {gamma}-GCS RNAi; however, addition of GSH did not restore the pools of GSH and T(SH)2. These data suggest that BSO also acts to inhibit the transport of GSH or its peptide metabolites into the cell. The ability of BSO to inhibit both synthesis and transport of GSH likely makes it a more effective cytotoxic agent than an inhibitor with a single mode of action. Finally the potential for the T(SH)2 biosynthetic enzymes to be regulated in response to reduced thiol levels was studied. The expression levels of ornithine decarboxylase and of S-adenosylmethionine

decarboxylase, two essential enzymes in spermidine biosynthesis, remained constant in induced {gamma}-GCS RNAi cell lines.

Karbarz, M. J., S. R. Kalb, et al. (2003). "Expression Cloning and Biochemical Characterization of a Rhizobium leguminosarum Lipid A 1-Phosphatase." J. Biol. Chem. **278**(41): 39269-39279.

http://www.jbc.org/cgi/content/abstract/278/41/39269

Lipid A of Rhizobium leguminosarum, a nitrogen-fixing plant endosymbiont, displays several significant structural differences when compared with Escherichia coli. An especially striking feature of R. leguminosarum lipid A is that it lacks both the 1- and 4'-phosphate groups. Distinct lipid A phosphatases that attack either the 1 or the 4' positions have previously been identified in extracts of R. leguminosarum and Rhizobium etli but not Sinorhizobium meliloti or E. coli. Here we describe the identification of a hybrid cosmid (pMJK-1) containing a 25-kb R. leguminosarum 3841 DNA insert that directs the overexpression of the lipid A 1-phosphatase. Transfer of pMJK-1 into S. meliloti 1021 results in heterologous expression of 1-phosphatase activity, which is normally absent in extracts of strain 1021, and confers resistance to polymyxin. Sequencing of a 7-kb DNA fragment derived from the insert of pMJK-1 revealed the presence of a lipid phosphatase ortholog (designated LpxE). Expression of lpxE in E. coli behind the T7lac promoter results in the appearance of robust 1-phosphatase activity, which is normally absent in E. coli membranes. Matrix-assisted laser-desorption/time of flight and radiochemical analysis of the product generated in vitro from the model substrate lipid IVA confirms the selective removal of the 1-phosphate group. These findings show that lpxE is the structural gene for the 1-phosphatase. The availability of lpxE may facilitate the re-engineering of lipid A structures in diverse Gram-negative bacteria and allow assessment of the role of the 1-phosphatase in R. leguminosarum symbiosis with plants. Possible orthologs of LpxE are present in some intracellular human pathogens, including Francisella tularensis, Brucella melitensis, and Legionella pneumophila.

Mateo, J., S. Kreda, et al. (2003). "Requirement of Cys399 for Processing of the Human Ecto-ATPase (NTPDase2) and Its Implications for Determination of the Activities of Splice Variants of the Enzyme." J. Biol. Chem. **278**(41): 39960-39968.

http://www.jbc.org/cgi/content/abstract/278/41/39960

Ecto-ATPase (CD39L1) corresponds to the type 2 enzyme of the ecto-nucleoside triphosphate diphosphohydrolase family (E-NTPDase). We have isolated from human ECV304 cells three cDNAs with high homology with members of the E-NTPDase family that encode predicted proteins of 495, 472, and 450 amino acids. Sequencing of a genomic DNA clone confirmed that these three sequences correspond to splice variants of the human ecto-ATPase (NTPDase2{alpha},-2{beta}, and -2{gamma}). Although all three enzyme forms were expressed heterologously to similar levels in Chinese hamster ovary cells clone K-1 (CHO-K1) cells, only the 495-amino acid protein (NTPDase2{alpha} exhibited ecto-ATPase activity. Immunolocalization studies demonstrated that NTPDase2{alpha} is fully processed and trafficked to the plasma membrane, whereas the NTPDase2{beta} and -2{gamma} splice variants were retained in not fully glycosylated forms in the endoplasmic reticulum. The potential roles of two highly conserved residues, Cys399 and Asn443, in the activity and cellular trafficking of the ecto-ATPase were examined. Mutation of Cys399, which is absent in NTPDase2{beta} and -2{gamma}, produced a protein completely devoid of nucleotidase activity, while mutation of Asn443 to Asp resulted in substantial loss of activity. Neither the Cys399 nor Asn443 mutants were fully glycosylated, and both were retained in the endoplasmic reticulum. These results indicate that the lack of ecto-nucleotidase activity exhibited by NTPDase2{beta} and -2{gamma} and the C399S mutant, as well as the large reduction of activity in the N443D mutant are due to alterations in the

folding/maturation of these proteins.

Merckx, A., K. Le Roch, et al. (2003). "Identification and Initial Characterization of Three Novel Cyclin-related Proteins of the Human Malaria Parasite Plasmodium falciparum." J. Biol. Chem. **278**(41): 39839-39850.

http://www.jbc.org/cgi/content/abstract/278/41/39839

The molecular mechanisms regulating cell proliferation and development during the life cycle of malaria parasites remain to be elucidated. The peculiarities of the cell cycle organization during Plasmodium falciparum schizogony suggest that the modalities of cell cycle control in this organism may differ from those in other eukaryotes. Indeed, existing data concerning Plasmodium cell cycle regulators such as cyclin-dependent kinases reveal structural and functional properties that are divergent from those of their homologues in other systems. The work presented here lies in the context of the exploitation of the recently available P. falciparum genome sequence toward the characterization of putative cell cycle regulators. We describe the in silico identification of three open reading frames encoding proteins with maximal homology to various members of the cyclin family and demonstrate that the corresponding polypeptides are expressed in the erythrocytic stages of the infection. We present evidence that these proteins possess cyclin activity by demonstrating either their association with histone H1 kinase activity in parasite extracts or their ability to activate PfPK5, a P. falciparum cyclin-dependent kinase homologue, in vitro. Furthermore, we show that RINGO, a protein with no sequence homology to cyclins but that is nevertheless a strong activator of mammalian CDK1/2, is also a strong activator of PfPK5 in vitro. This raises the possibility that "cryptic" cell cycle regulators may be found among the 50% of the open reading frames in the P. falciparum genome that display no homology to any known proteins.

Ortega, A., P. Ferrer, et al. (2003). "Down-regulation of Glutathione and Bcl-2 Synthesis in Mouse B16 Melanoma Cells Avoids Their Survival during Interaction with the Vascular Endothelium." J. Biol. Chem. **278**(41): 39591-39599.

http://www.jbc.org/cgi/content/abstract/278/41/39591

B16 melanoma (B16M) cells with high GSH content show high metastatic activity. However, the molecular mechanisms linking GSH to metastatic cell survival are unclear. The possible relationship between GSH and the ability of Bcl-2 to prevent cell death was studied in B16M cells with high (F10) and low (F1) metastatic potential. Analysis of a Bcl-2 family of genes revealed that B16M-F10 cells, as compared with B16M-F1 cells, overexpressed preferentially Bcl-2 ([~]5.7-fold). Hepatic sinusoidal endothelium-induced B16M-F10 cytotoxicity in vitro increased from [~]19% (controls) to [~]97% in GSH-depleted B16M-F10 cells treated with an antisense Bcl-2 oligodeoxynucleotide (Bcl-2-AS). L-Buthionine (S,R)-sulfoximine-induced GSH depletion or Bcl-2-AS decreased the metastatic growth of B16M-F10 cells in the liver. However, the combination of L-buthionine (S,R)-sulfoximine and Bcl-2-AS abolished metastatic invasion. Bcl-2-overexpressing B16M-F1/Tet-Bcl-2 and B16M-F10/Tet-Bcl-2 cells, as compared with controls, showed an increase in GSH content, no change in the rate of GSH synthesis, and a decrease in GSH efflux. Thus, Bcl-2 overexpression may increase metastatic cell resistance against oxidative/nitrosative stress by inhibiting release of GSH. In addition, Bcl-2 availability regulates the mitochondrial GSH (mtGSH)-dependent opening of the permeability transition pore complex. Death in B16M-F10 cells was sharply activated at mtGSH levels below 30% of controls values. However, this critical threshold increased to [~]60% of control values in Bcl-2-AS-treated B16M-F10 cells. GSH ester-induced replenishment of mtGSH levels (even under conditions of cytosolic GSH depletion) prevented cell death. Our results indicate that survival of B16M cells with high metastatic potential

can be challenged by inhibiting their GSH and Bcl-2 synthesis.

Tanner, J. A., R. M. Watt, et al. (2003). "The Severe Acute Respiratory Syndrome (SARS) Coronavirus NTPase/Helicase Belongs to a Distinct Class of 5' to 3' Viral Helicases." J. Biol. Chem. **278**(41): 39578-39582.

http://www.jbc.org/cgi/content/abstract/278/41/39578

The putative NTPase/helicase protein from severe acute respiratory syndrome coronavirus (SARS-CoV) is postulated to play a number of crucial roles in the viral life cycle, making it an attractive target for anti-SARS therapy. We have cloned, expressed, and purified this protein as an N-terminal hexahistidine fusion in Escherichia coli and have characterized its helicase and NTPase activities. The enzyme unwinds double-stranded DNA, dependent on the presence of a 5' single-stranded overhang, indicating a 5'o 3' polarity of activity, a distinct characteristic of coronaviridae helicases. We provide the first quantitative analysis of the polynucleic acid binding and NTPase activities of a Nidovirus helicase, using a high throughput phosphate release assay that will be readily adaptable to the future testing of helicase inhibitors. All eight common NTPs and dNTPs were hydrolyzed by the SARS helicase in a magnesium-dependent reaction, stimulated by the presence of either single-stranded DNA or RNA. The enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates. Homopolynucleotides significantly stimulated the ATPase activity (15-25-fold) with the notable exception of poly(G) and poly(dG), which were non-stimulatory. We found a large variation in the apparent strength of binding of different homopolynucleotides, with dT24 binding over 10 times more strongly than dA24 as observed by the apparent Km.

Hermoso, M., C. M. Satterwhite, et al. (2002). "ClC-3 Is a Fundamental Molecular Component of Volume-sensitive Outwardly Rectifying Cl- Channels and Volume Regulation in HeLa Cells and Xenopus laevis Oocytes." J. Biol. Chem. **277**(42): 40066-40074.

http://www.jbc.org/cgi/content/abstract/277/42/40066

Volume-sensitive osmolyte and anion channels (VSOACs) are activated upon cell swelling in most vertebrate cells. Native VSOACs are believed to be a major pathway for regulatory volume decrease (RVD) through efflux of chloride and organic osmolytes. ClC-3 has been proposed to encode native VSOACs in Xenopus laevis oocytes and in some mammalian cells, including cardiac and vascular smooth muscle cells. The relationship between the ClC-3 chloride channel, the native volume-sensitive osmolyte and anion channel (VSOAC) currents, and cell volume regulation in HeLa cells and X. laevis oocytes was investigated using ClC-3 antisense. In situ hybridization in HeLa cells, semiquantitative and real-time PCR, and immunoblot studies in HeLa cells and X. laevis oocytes demonstrated the presence of ClC-3 mRNA and protein, respectively. Exposing both cell types to hypotonic solutions induced cell swelling and activated native VSOACs. Transient transfection of HeLa cells with ClC-3 antisense oligonucleotide or X. laevis oocytes injected with antisense cRNA abolished the native ClC-3 mRNA transcript and protein and significantly reduced the density of native VSOACs activated by hypotonically induced cell swelling. In addition, antisense against native ClC-3 significantly impaired the ability of HeLa cells and X. laevis oocytes to regulate their volume. These results suggest that ClC-3 is an important molecular component underlying VSOACs and the RVD process in HeLa cells and X. laevis oocytes.

Zheng, M. and P. J. McKeown-Longo (2002). "Regulation of HEF1 Expression and Phosphorylation by TGF-beta 1 and Cell Adhesion." J. Biol. Chem. **277**(42): 39599-39608.

http://www.jbc.org/cgi/content/abstract/277/42/39599

Transforming growth factor-[beta]1 (TGF-[beta]1) is a multipotential cytokine, which regulates remodeling of tissue extracellular matrix during early tumorigenesis and wound healing. Human enhancer of filamentation-1 (HEF1), a multifunctional docking protein, is involved in integrin-based signaling, which affects cell motility, growth, and apoptosis. Our studies reveal that TGF-[beta]1 is a potent inducer of HEF1 gene transcription in human dermal fibroblasts. TGF-[beta]1 promoted HEF1 expression in a dose-dependent manner and resulted in a 16-fold increase in HEF1 protein level. TGF-[beta]1 had no effect on the stability of either HEF1 protein or mRNA. The TGF-[beta]1-induced HEF1 expression was independent of cell adhesion and resistant to cytoskeleton disruption. TGF-[beta]1 increased levels of both p105 and p115 HEF1 in adherent fibroblasts. Digestion with specific phosphatases indicated that the p115HEF1 resulted from serine/threonine phosphorylation of p105HEF1. The appearance of the p115HEF1 as well as tyrosine phosphorylation of p105HEF1 required cell adhesion and/or an organized cytoskeleton. An in vitro kinase assay indicated that p105HEF1 was a substrate for Src. PP1, a specific Src kinase inhibitor, was able to block adhesion-dependent tyrosine phosphorylation of p105HEF1. These findings suggest that TGF-[beta]1 regulates HEF1 gene expression and that HEF1 phosphorylation is dependent on cell adhesion and Src kinase activity.

Kiefer, H. L. B., T. M. Hanley, et al. (2004). "Retinoic Acid Inhibition of Chromatin Remodeling at the Human Immunodeficiency Virus Type 1 Promoter: UNCOUPLING OF HISTONE ACETYLATION AND CHROMATIN REMODELING." J. Biol. Chem. **279**(42): 43604-43613.

http://www.jbc.org/cgi/content/abstract/279/42/43604

All-trans retinoic acid (RA) represses HIV-1 transcription and replication in cultured monocytic cells and in primary monocyte-derived macrophages. Here we examine the role of histone acetylation and chromatin remodeling in RA-mediated repression. RA pretreatment of latently infected U1 promonocytes inhibits HIV-1 expression in response to the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA). TSA is thought to activate HIV-1 transcription by inducing histone hyperacetylation within a regulatory nucleosome, nuc-1, positioned immediately downstream from the transcription start site. Acetylation of nuc-1 is thought to be a critical step in activation that precedes nuc-1 remodeling and, subsequently, transcriptional initiation. Here we demonstrate that TSA treatment induces H3 and H4 hyperacetylation and nuc-1 remodeling. Although RA pretreatment inhibits nuc-1 remodeling and HIV-1 transcription, it has no effect on histone acetylation. This suggests that acetylation and remodeling are not obligatorily coupled. We also show that growth of U1 cells in retinoid-deficient medium induces nuc-1 remodeling and HIV-1 expression but does not induce histone hyperacetylation. These findings suggest that remodeling, not histone hyperacetylation, is the limiting step in transcriptional activation in these cells. Together, these data suggest that RA signaling maintains the chromatin structure of the HIV-1 promoter in a transcriptionally non-permissive state that may contribute to the establishment of latency in monocyte/macrophages.

Mills, J. A., K. Motichka, et al. (2004). "Inactivation of the Mycobacterial Rhamnosyltransferase, Which Is Needed for the Formation of the Arabinogalactan-Peptidoglycan Linker, Leads to Irreversible Loss of Viability." J. Biol. Chem. **279**(42): 43540-43546.

http://www.jbc.org/cgi/content/abstract/279/42/43540

Temperature-sensitive mutant 2-20/32 of Mycobacterium smegmatis mc2155 was isolated and genetically complemented with a Mycobacterium tuberculosis H37Rv DNA fragment that contained a single open reading frame. This open reading frame is designated Rv3265c in the M. tuberculosis H37Rv genome. Rv3265c shows homology to the Escherichia coli gene wbbL, which encodes a dTDP-Rha:{alpha}-D-GlcNAc-pyrophosphate polyprenol, {alpha}-3-L-rhamnosyltransferase. In E. coli this enzyme is involved in O-antigen synthesis, but in mycobacteria it is required for the rhamnosyl-containing linker unit responsible for the attachment of the cell wall polymer mycolyl-arabinogalactan to the peptidoglycan. The M. tuberculosis wbbL homologue, encoded by Rv3265c, was shown to be capable of restoring an E. coli K12 strain containing an insertionally inactivated wbbL to O-antigen positive. Likewise, the E. coli wbbL gene allowed 2-20/32 to grow at higher non-permissive temperatures. The rhamnosyltransferase activity of M. tuberculosis WbbL was demonstrated in 2-20/32 as was the loss of this transferase activity in 2-20/32 at elevated temperatures. The wbbL of the temperature-sensitive mutant contained a single-base change that converted what was a proline in mc2155 to a serine residue. Exposure of 2-20/32 to higher non-permissive temperatures resulted in bacteria that could not be recovered at the lower permissive temperatures.

Yoshimura, Y., M. Tani, et al. (2004). "Molecular Cloning and Functional Analysis of Zebrafish Neutral Ceramidase." J. Biol. Chem. **279**(42): 44012-44022.

http://www.jbc.org/cgi/content/abstract/279/42/44012

Almost all observations on the functions of neutral ceramidase have been carried out at cellular levels but not at an individual level. Here, we report the molecular cloning of zebrafish neutral ceramidase (znCD) and its functional analysis during embryogenesis. We isolated a cDNA clone encoding znCD by 5' and 3' rapid amplification of cDNA ends-PCR. It possessed an open reading frame of 2,229 base pairs encoding 743 amino acids. A possible signal/anchor sequence near the N terminus and four potential O-glycosylation and eight potential N-glycosylation sites were found in the putative sequence. The enzyme activity at neutral pH increased markedly after transformation of Chinese hamster CHOP and zebrafish BRF41 cells with the cDNA. The overexpressed enzyme was found to be distributed in endoplasmic reticulum/Golgi compartments as well as the plasma membranes. The antisense morpholino oligonucleotide (AMO), which was designed based on the sequence of znCD mRNA, successfully blocked the translation of znCD in a wheat germ in vitro translation system. The knockdown of znCD with AMO led to an increase in the number of zebrafish embryos with severe morphological and cellular abnormalities such as abnormal morphogenesis in the head and tail, pericardiac edema, defect of blood cell circulation, and an increase of apoptotic cells, especially in the head and neural tube regions, at 36 h post-fertilization. The ceramide level in AMO-injected embryos increased significantly compared with that in control embryos. Simultaneous injection of both AMO and synthetic znCD mRNA into one-cell-stage embryos rescued znCD activity and blood cell circulation. These results indicate that znCD is essential for the metabolism of ceramide and the early development of zebrafish.

Gross, I., D. J. Morrison, et al. (2003). "The Receptor Tyrosine Kinase Regulator Sprouty1 Is a Target of the Tumor Suppressor WT1 and Important for Kidney Development." J. Biol. Chem. **278**(42): 41420-41430.

http://www.jbc.org/cgi/content/abstract/278/42/41420

WT1 encodes a transcription factor involved in kidney development and tumorigenesis. Using representational difference analysis, we identified a new set of WT1 targets, including a homologue of the Drosophila receptor tyrosine kinase regulator, sprouty. Sprouty1 was up-regulated in cell lines expressing wild-type but not mutant WT1. WT1 bound to the endogenous

sprouty1 promoter in vivo and directly regulated sprouty1 through an early growth response gene-1 binding site. Expression of Sprouty1 and WT1 overlapped in the developing metanephric mesenchyme, and Sprouty1, like WT1, plays a key role in the early steps of glomerulus formation. Disruption of Sprouty1 expression in embryonic kidney explants by antisense oligonucleotides reduced condensation of the metanephric mesenchyme, leading to a decreased number of glomeruli. In addition, sprouty1 was expressed in the ureteric tree and antisense-treated ureteric trees had cystic lumens. Therefore, sprouty1 represents a physiologically relevant target gene of WT1 during kidney development.

Lodhi, K. M., M. H. Ozdener, et al. (2003). "The Upstream Open Reading Frame Mediates Constitutive Effects on Translation of Cytochrome P-450c27 from the Seventh In-frame AUG Codon in Rat Liver." J. Biol. Chem. **278**(42): 40647-40657.

http://www.jbc.org/cgi/content/abstract/278/42/40647

The 2.3-kb mRNA that codes for cytochrome P-450c27 (CYP27) has an unexpectedly long 5'-untranslated region (UTR) that holds six AUGs, leading to several upstream open reading frames (uORFs). The initiation of translation from the seventh AUG forms a putative 55-kDa precursor, which is processed in mitochondria to form a 52-kDa mature protein. The first three AUGs form fully overlapping uORF1, uORF2, and uORF3 that are in-frame with the seventh AUG and next two form fully overlapping uORF4 and uORF5 that are out-of-frame with the seventh AUG. Although not recognized by the scanning ribosomes under normal conditions, the sixth in-frame AUG forms a putative 57-kDa extension of the main open reading frame. The purpose of this study was to identify the elements in the 5'-UTR that direct CYP27 mRNA translation exclusively from the seventh AUG. Expression of 5' deletion mutants in COS cells reveal that the intact 5'-UTR not only directs the initiation of translation from the seventh AUG but also acts as a negative regulator. A 2-kb deletion mutant that lacks uORF1 initiates translation equally from the sixth and the seventh AUGs, forming both 57- and 55-kDa precursor proteins with a 2-fold increase in rate of translation. However, induction in translation does not affect the levels of the mature 52-kDa form in mitochondria but causes accumulation of the precursor form in cytosol not seen in COS cells transfected with wild-type cDNA. Mutation of the stop codon that terminates uORF1 completely shifts the initiation of translation from the seventh to the first AUG, forming a 67-kDa precursor that is processed into a 52-kDa mature protein in mitochondria. Confirmation of the bicistronic nature of CYP27 mRNA by epitope mapping of uORF1 suggests that translation of CYP27 mRNA from the seventh AUG is directed and regulated by uORF1 expression.

Tuli, R., S. Tuli, et al. (2003). "Transforming Growth Factor-{beta}-mediated Chondrogenesis of Human Mesenchymal Progenitor Cells Involves N-cadherin and Mitogen-activated Protein Kinase and Wnt Signaling Cross-talk." J. Biol. Chem. **278**(42): 41227-41236.

http://www.jbc.org/cgi/content/abstract/278/42/41227

The multilineage differentiation potential of adult tissue-derived mesenchymal progenitor cells (MPCs), such as those from bone marrow and trabecular bone, makes them a useful model to investigate mechanisms regulating tissue development and regeneration, such as cartilage. Treatment with transforming growth factor-{beta} (TGF-{beta}) superfamily members is a key requirement for the in vitro chondrogenic differentiation of MPCs. Intracellular signaling cascades, particularly those involving the mitogen-activated protein (MAP) kinases, p38, ERK-1, and JNK, have been shown to be activated by TGF-{beta}s in promoting cartilage-specific gene expression. MPC chondrogenesis in vitro also requires high cell seeding density, reminiscent of the cellular condensation requirements for embryonic mesenchymal chondrogenesis, suggesting common chondro-regulatory mechanisms. Prompted by recent findings of the crucial role of the cell

adhesion protein, N-cadherin, and Wnt signaling in condensation and chondrogenesis, we have examined here their involvement, as well as MAP kinase signaling, in TGF-{beta}1-induced chondrogenesis of trabecular bone-derived MPCs. Our results showed that TGF-{beta}1 treatment initiates and maintains chondrogenesis of MPCs through the differential chondro-stimulatory activities of p38, ERK-1, and to a lesser extent, JNK. This regulation of MPC chondrogenic differentiation by the MAP kinases involves the modulation of N-cadherin expression levels, thereby likely controlling condensation-like cell-cell interaction and progression to chondrogenic differentiation, by the sequential up-regulation and progressive down-regulation of N-cadherin. TGF-{beta}1-mediated MAP kinase activation also controls WNT-7A gene expression and Wnt-mediated signaling through the intracellular {beta}-catenin-TCF pathway, which likely regulates N-cadherin expression and subsequent N-cadherin-mediated cell-adhesion complexes during the early steps of MPC chondrogenesis.

van der Slot, A. J., A.-M. Zuurmond, et al. (2003). "Identification of PLOD2 as Telopeptide Lysyl Hydroxylase, an Important Enzyme in Fibrosis." J. Biol. Chem. **278**(42): 40967-40972.

http://www.jbc.org/cgi/content/abstract/278/42/40967

The hallmark of fibrotic processes is an excessive accumulation of collagen. The deposited collagen shows an increase in pyridinoline cross-links, which are derived from hydroxylated lysine residues within the telopeptides. This change in cross-linking is related to irreversible accumulation of collagen in fibrotic tissues. The increase in pyridinoline cross-links is likely to be the result of increased activity of the enzyme responsible for the hydroxylation of the telopeptides (telopeptide lysyl hydroxylase, or TLH). Although the existence of TLH has been postulated, the gene encoding TLH has not been identified. By analyzing the genetic defect of Bruck syndrome, which is characterized by a pyridinoline deficiency in bone collagen, we found two missense mutations in exon 17 of PLOD2, thereby identifying PLOD2 as a putative TLH gene. Subsequently, we investigated fibroblasts derived from fibrotic skin of systemic sclerosis (SSc) patients and found that PLOD2 mRNA is highly increased indeed. Furthermore, increased pyridinoline cross-link levels were found in the matrix deposited by SSc fibroblasts, demonstrating a clear link between mRNA levels of the putative TLH gene (PLOD2) and the hydroxylation of lysine residues within the telopeptides. These data underscore the significance of PLOD2 in fibrotic processes.

Abbas, T., M. Olivier, et al. (2002). "Differential Activation of p53 by the Various Adducts of Mitomycin C." J. Biol. Chem. **277**(43): 40513-40519.

http://www.jbc.org/cgi/content/abstract/277/43/40513

Mitomycin C (MC) is a cytotoxic chemotherapeutic agent that causes DNA damage in the form of DNA cross-links as well as a variety of DNA monoadducts and is known to induce p53. The various DNA adducts formed upon treatment of mouse mammary tumor cells with MC as well as 10-decarbamoyl MC (DMC) and 2,7-diaminomitosene (2,7-DAM), the major MC metabolite, have been elucidated. The cytotoxicity of DMC parallels closely that of MC in a number of rodent cell lines tested, whereas 2,7-DAM is relatively noncytotoxic. In this study, we investigate the ability of MC, DMC, and 2,7-DAM to activate p53 at equidose concentrations by treating tissue culture cell lines with the three mitomycins. Whereas MC and DMC induced p53 protein levels and increased the levels of p21 and Gadd45 mRNA, 2,7-DAM did not. Furthermore, MC and DMC, but not 2,7-DAM, were able to induce apoptosis efficiently in ML-1 cells. Therefore the 2,7-DAM monoadducts were unable to activate the p53 pathway. Interestingly, DMC was able to initiate apoptosis via a p53-independent pathway whereas MC was not. This is the first finding that adducts of a multiadduct type DNA-damaging agent are differentially recognized by DNA damage

sensor pathways.

Fujii, I., Y. Yasuoka, et al. (2004). "Hydrolytic Polyketide Shortening by Ayg1p, a Novel Enzyme Involved in Fungal Melanin Biosynthesis." J. Biol. Chem. **279**(43): 44613-44620.

http://www.jbc.org/cgi/content/abstract/279/43/44613

The pentaketide 1,3,6,8-tetrahydroxynaphthalene (T4HN) is a key precursor of 1,8-dihydroxynaphthalene-melanin, an important virulence factor in pathogenic fungi, where T4HN is believed to be the direct product of pentaketide synthases. We showed recently the involvement of a novel protein, Ayg1p, in the formation of T4HN from the heptaketide precursor YWA1 in Aspergillus fumigatus. To investigate the mechanism of its enzymatic function, Ayg1p was purified from an Aspergillus oryzae strain that overexpressed the ayg1 gene. The Ayg1p converted the naphthopyrone YWA1 to T4HN with a release of the acetoacetic acid. Although Ayg1p does not show significant homology with known enzymes, a serine protease-type hydrolytic motif is present in its sequence, and serine-specific inhibitors strongly inhibited the activity. To identify its catalytic residues, site-directed Ayg1p mutants were expressed in Escherichia coli, and their enzyme activities were examined. The single substitution mutations S257A, D352A, and H380A resulted in a complete loss of enzyme activity in Ayg1p. These results indicated that the catalytic triad Asp352-His380-Ser257 constituted the active-site of Ayg1p. From a Dixon plot analysis, 2-acetyl-1,3,6,8-tetrahydroxynaphthalene was found to be a strong mixed-type inhibitor, suggesting the involvement of an acyl-enzyme intermediate. These studies support the mechanism in which the Ser257 at the active site functions as a nucleophile to attack the YWA1 side-chain 1'-carbonyl and cleave the carbon-carbon bond between the naphthalene ring and the side chain. Acetoacetic acid is subsequently released from the Ser257-O-acetoacetylated Ayg1p by hydrolysis. An enzyme with activity similar to Ayg1p in melanin biosynthesis has not been reported in any other organism.

Yamamura, H., S. Ugawa, et al. (2004). "Capsazepine Is a Novel Activator of the {delta} Subunit of the Human Epithelial Na+ Channel." J. Biol. Chem. **279**(43): 44483-44489.

http://www.jbc.org/cgi/content/abstract/279/43/44483

The amiloride-sensitive epithelial Na+ channel (ENaC) regulates Na+ homeostasis into cells and across epithelia. So far, four homologous subunits of mammalian ENaC have been isolated and are denoted as {alpha}, {beta}, {gamma}, and {delta}. The chemical agents acting on ENaC are, however, largely unknown, except for amiloride and benzamil as ENaC inhibitors. In particular, there are no agonists currently known that are selective for ENaC{delta}, which is mainly expressed in the brain. Here we demonstrate that capsazepine, a competitive antagonist for transient receptor potential vanilloid subfamily 1, potentiates the activity of human ENaC{delta}{beta}{gamma} (hENaC{delta}{beta}{gamma}) heteromultimer expressed in Xenopus oocytes. The inward currents at a holding potential of -60 mV in hENaC{delta}{beta}{gamma}-expressing oocytes were markedly enhanced by the application of capsazepine ([&ge]1 {micro}M), and the capsazepine-induced current was mostly abolished by the addition of 100 {micro}M amiloride. The stimulatory effects of capsazepine on the inward current were concentration-dependent with an EC50 value of 8 {micro}M. Neither the application of other vanilloid compounds (capsaicin, resiniferatoxin, and olvanil) nor a structurally related compound (dopamine) modulated the inward current. Although hENaC{delta} homomer was also significantly activated by capsazepine, unexpectedly, capsazepine had no effect on hENaC{alpha} and caused a slight decrease on the hENaC{alpha}{beta}{gamma} current. In conclusion, capsazepine acts on ENaC{delta} and acts together with protons. Other vanilloids tested do not have any effect. These findings identify capsazepine as the first known chemical

activator of ENaC{delta}.

Massimi, I., E. Park, et al. (2002). "Identification of a Novel Maturation Mechanism and Restricted Substrate Specificity for the SspB Cysteine Protease of Staphylococcus aureus." J. Biol. Chem. **277**(44): 41770-41777.

http://www.jbc.org/cgi/content/abstract/277/44/41770

The SspB cysteine protease of Staphylococcus aureus is expressed in an operon, flanked by the sspA serine protease, and sspC, encoding a 12.9-kDa protein of unknown function. SspB was expressed as a 40-kDa prepropeptide pSspB, which did not undergo autocatalytic maturation. Activity of pSspB was reduced compared with 22-kDa mature SspB, but it was equivalent to mature SspB after incubation with SspA, which specifically removed the pSspB N-terminal propeptide. SspC abrogated the activity of pSspB when incubated in a 1:1 complex but had no effect on SspA or papain. Activity of the pSspB{middle dot}SspC complex was restored when incubated with SspA, and SspC was cleaved by SspA but not pSspB. Thus, SspC maintains pSspB as an inert zymogen, and SspA is required for removal of the propeptide and inactivation of SspC. Like the papain protease family, SspB cleaved substrates with a hydrophobic amino acid at P2 but had a strong preference for arginine at P1. It did not cleave casein, serum albumin, IgG, or IgA, but it promoted detachment of cultured keratinocytes and cleaved fibronectin and fibrinogen at sites recognized by urokinase plasminogen activator and plasmin, respectively. It also processed high molecular weight kininogen in a manner resembling plasma kallikrein. Thus, SspB exhibits a novel maturation mechanism and mimics the specificity of plasma serine proteases.

Pinkoski, M. J., N. M. Droin, et al. (2002). "Tumor Necrosis Factor alpha Up-regulates Non-lymphoid Fas-ligand following Superantigen-induced Peripheral Lymphocyte Activation." J. Biol. Chem. **277**(44): 42380-42385.

http://www.jbc.org/cgi/content/abstract/277/44/42380

Members of the tumor necrosis factor (TNF) and TNF receptor families play important roles in inducing apoptosis and mediating the inflammatory response. Activated T lymphocytes can trigger the expression of Fas-ligand on non-lymphoid tissue, such as intestinal epithelial cells (IEC), and this, in turn, can induce apoptosis in the T cells. Here, we examine the role of TNF[alpha] in this feedback regulation. Injection of TNF[alpha] into mice caused a rapid up-regulation of Fas-ligand mRNA in IEC. TNF[alpha]-induced activation of the Fas-ligand promoter in IEC requires NF-[kappa]B as this was blocked by an I-[kappa]B[alpha]M super-repressor and by mutation of an NF-[kappa]B site in the Fas-ligand promoter. Activation of T cells by antigen induced Fas-ligand expression in IEC in vivo in wild type, but not in TNF[alpha][-]/[-] or TNFR1[-]/[-] mice. These results define a novel pathway wherein TNF[alpha], produced by activated T cells in the intestine, induce Fas-ligand expression in IEC. This is the first observation that one member of the TNF superfamily mediates the regulation of another family member and represents a potential feedback mechanism controlling lymphocyte infiltration and inflammation in the small intestine.

Rafiee, P., Y. Shi, et al. (2003). "Cellular Redistribution of Inducible Hsp70 Protein in the Human and Rabbit Heart in Response to the Stress of Chronic Hypoxia: ROLE OF PROTEIN KINASES." J. Biol. Chem. **278**(44): 43636-43644.

http://www.jbc.org/cgi/content/abstract/278/44/43636

Many infants who undergo cardiac surgery have a congenital cyanotic defect where the heart is chronically perfused with hypoxemic blood. Infant hearts adapt to chronic hypoxemia by activation of intracellular protein kinase signal transduction pathways. However, the involvement of heat shock protein 70 in adaptation to chronic hypoxemia and its role in protein kinase signaling pathways is unknown. We determined expression of message and subcellular protein distribution for inducible (Hsp70i) and constitutive heat shock protein 70 (Hsc70) in chronically hypoxic and normoxic infant human and rabbit hearts and their relationship to protein kinases. In chronically hypoxic human and rabbit hearts message levels for Hsp70i were elevated 4- to 5-fold compared with normoxic hearts, Hsp70i protein was redistributed from the particulate to the cytosolic fraction. In normoxic infants Hsp70i protein was distributed almost equally between the cytosolic and particulate fractions. Hsc70 message and subcellular distribution of Hsc70 protein were unaffected by chronic hypoxia. We then determined if protein kinases influence Hsp70i protein subcellular distribution. In rabbit hearts SB203580 and chelerythrine reduced Hsp70i message levels, whereas SB203580, chelerythrine, and curcumin reversed the subcellular redistribution of Hsp70i protein caused by chronic hypoxia, with no effect in normoxic hearts, indicating regulation of Hsp70i message and subcellular distribution of Hsp70i protein in chronically hypoxic rabbit hearts is influenced by protein kinase C and mitogen-activated protein kinases, specifically p38 MAPK and JNK. We conclude the Hsp70 signal transduction pathway plays an important role in adaptation of infant human and rabbit hearts to chronic hypoxemia.

Sancho, R., N. Marquez, et al. (2004). "Imperatorin Inhibits HIV-1 Replication through an Sp1-dependent Pathway." J. Biol. Chem. **279**(36): 37349-37359.

http://www.jbc.org/cgi/content/abstract/279/36/37349

Coumarins and structurally related compounds have been recently shown to present anti-human immunodeficiency virus, type 1 (HIV-1) activity. Among them, the dietary furanocoumarin imperatorin is present in citrus fruits, in culinary herbs, and in some medicinal plants. In this study we report that imperatorin inhibits either vesicular stomatitis virus-pseudotyped or gp160-enveloped recombinant HIV-1 infection in several T cell lines and in HeLa cells. These recombinant viruses express luciferase as a marker of viral replication. Imperatorin did not inhibit the reverse transcription nor the integration steps in the viral cell cycle. Using several 5' long terminal repeat-HIV-1 constructs where critical response elements were either deleted or mutated, we found that the transcription factor Sp1 is critical for the inhibitory activity of imperatorin induced by both phorbol 12-myristate 13-acetate and HIV-1 Tat. Moreover in transient transfections imperatorin specifically inhibited phorbol 12-myristate 13-acetate-induced transcriptional activity of the Gal4-Sp1 fusion protein. Since Sp1 is also implicated in cell cycle progression we further studied the effect of imperatorin on cyclin D1 gene transcription and protein expression and in HeLa cell cycle progression. We found that imperatorin strongly inhibited cyclin D1 expression and arrested the cells at the G1 phase of the cell cycle. These results highlight the potential of Sp1 transcription factor as a target for natural anti-HIV-1 compounds such as furanocoumarins that might have a potential therapeutic role in the management of AIDS.

Ngo, T. T., M. K. Bennett, et al. (2002). "A Role for Cyclic AMP Response Element-binding Protein (CREB) but Not the Highly Similar ATF-2 Protein in Sterol Regulation of the Promoter for 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase." J. Biol. Chem. **277**(37): 33901-33905.

http://www.jbc.org/cgi/content/abstract/277/37/33901

Sterol regulatory element-binding proteins (SREBPs) activate promoters for key genes of metabolism to keep pace with the cellular demand for lipids. In each SREBP-regulated promoter, at least one ubiquitous co-regulatory factor that binds to a neighboring recognition site is also required for efficient gene induction. Some of these putative co-regulatory proteins are members of transcription factor families that all bind to the same DNA sequence elements in vitro and are often expressed in the same cells. These two observations have made it difficult to assign specific and redundant functions to the unique members of a specific gene family. We have used the chromatin immunoprecipitation (ChIP) technique coupled with a transient complementation assay in Drosophila SL2 cells to directly compare the ability of two members of the CREB/ATF family to function as co-regulatory proteins for SREBP-dependent activation of the HMG-CoA reductase promoter. Results from both of these experimental systems demonstrate that CREB is an efficient SREBP co-regulator but ATF-2 is not.

Valcourt, U., J. Gouttenoire, et al. (2002). "Functions of Transforming Growth Factor-beta Family Type I Receptors and Smad Proteins in the Hypertrophic Maturation and Osteoblastic Differentiation of Chondrocytes." J. Biol. Chem. **277**(37): 33545-33558.

http://www.jbc.org/cgi/content/abstract/277/37/33545

We investigated the effects of bone morphogenetic protein (BMP)-2, a member of the transforming growth factor-[beta] superfamily, on the regulation of the chondrocyte phenotype, and we identified signaling molecules involved in this regulation. BMP-2 triggers three concomitant responses in mouse primary chondrocytes and chondrocytic MC615 cells. First, BMP-2 stimulates expression or synthesis of type II collagen. Second, BMP-2 induces expression of molecular markers characteristic of pre- and hypertrophic chondrocytes, such as Indian hedgehog, parathyroid hormone/parathyroid hormone-related peptide receptor, type X collagen, and alkaline phosphatase. Third, BMP-2 induces osteocalcin expression, a specific trait of osteoblasts. Constitutively active forms of transforming growth factor-[beta] family type I receptors and Smad proteins were overexpressed to address their role in this process. Activin receptor-like kinase (ALK)-1, ALK-2, ALK-3, and ALK-6 were able to reproduce the hypertrophic maturation of chondrocytes induced by BMP-2. In addition, ALK-2 mimicked further the osteoblastic differentiation of chondrocytes induced by BMP-2. In the presence of BMP-2, Smad1, Smad5, and Smad8 potentiated the hypertrophic maturation of chondrocytes, but failed to induce osteocalcin expression. Smad6 and Smad7 impaired chondrocytic expression and osteoblastic differentiation induced by BMP-2. Thus, our results indicate that Smad-mediated pathways are essential for the regulation of the different steps of chondrocyte and osteoblast differentiation and suggest that additional Smad-independent pathways might be activated by ALK-2.

Joosten, M., M. Blazquez-Domingo, et al. (2004). "Translational Control of Putative Protooncogene Nm23-M2 by Cytokines via Phosphoinositide 3-Kinase Signaling." J. Biol. Chem. **279**(37): 38169-38176.

http://www.jbc.org/cgi/content/abstract/279/37/38169

The expansion and differentiation of hematopoietic progenitors is regulated by cytokine and growth factor signaling. To examine how signal transduction controls the gene expression program required for progenitor expansion, we screened ATLAS filters with polysome-associated mRNA derived from erythroid progenitors stimulated with erythropoietin and/or stem cell factor. The putative proto-oncogene nucleoside diphosphate kinase B (ndpk-B or nm23-M2) was identified as an erythropoietin and stem cell factor target gene. Factor-induced expression of nm23-M2 was regulated specifically at the level of polysome association by a phosphoinositide 3-kinase-dependent mechanism. Identification of the transcription initiation site revealed that nm23-

M2 mRNA starts with a terminal oligopyrimidine sequence, which is known to render mRNA translation dependent on mitogenic factors. Recently, the nm23-M2 locus was identified as a common leukemia retrovirus integration site, suggesting that it plays a role in leukemia development. The expression of Nm23 from a retroviral vector in the absence of its 5'-untranslated region caused constitutive polysome association of nm23-M2. Polysome-association and protein expression of endogenous nm23-M2 declined during differentiation of erythroid progenitors, suggesting a role for Nm23-M2 in progenitor expansion. Taken together, nm23-m2 exemplifies that cytokine-dependent control of translation initiation is an important mechanism of gene expression regulation.

He, H., F. Soncin, et al. (2003). "Elevated Expression of Heat Shock Factor (HSF) 2A Stimulates HSF1-induced Transcription during Stress." J. Biol. Chem. **278**(37): 35465-35475.

http://www.jbc.org/cgi/content/abstract/278/37/35465

Heat shock factor 2 (HSF2) belongs to a family of structurally related transcription factors, which share the property of binding to heat shock elements in the promoters of hsp molecular chaperone genes. However, unlike HSF1, which is essential for hsp gene transcription, the cellular functions of HSF2 are not well known. Here we show that human HSF2, although an ineffective activator of the hsp70 promoter in vitro and in vivo in the absence of stress, participates in the activation of the hsp70 promoter by heat shock. HSF2 was not, however, activated by heat shock in cells deficient in functional HSF1, suggesting a requirement for HSF1 in HSF2-mediated transcriptional enhancement. In addition, HSF2 regulation involves differential activity of two isoforms, HSF2A and HSF2B, which arise from alternative splicing of a common hsf2 gene. Under basal conditions, both HSF2 isoforms are ineffective in activating the hsp70 transcription. However, heat shock differentially activates HSF2A in vivo. This phenomenon appears to be physiologically significant, as human myeloprogenitor cells differentiating along the erythroid lineage express HSF2A de novo and undergo a large increase in capacity to activate the hsp70 promoter. Our experiments further show that HSF1 is physically associated with HSF2 in the cell and that such binding is enhanced by heat shock. Our data suggest a mechanism involving the formation of heterocomplexes between HSF1 and HSF2 with enhanced activity to activate the hsp70 promoter when compared with HSF1 or HSF2 homotrimers.

McDermott, N. B., D. F. Gordon, et al. (2002). "Isolation and Functional Analysis of the Mouse RXRgamma 1 Gene Promoter in Anterior Pituitary Cells." J. Biol. Chem. **277**(39): 36839-36844.

http://www.jbc.org/cgi/content/abstract/277/39/36839

The retinoid X receptor (RXR) isoform RXR[gamma] has limited tissue expression, including brain, skeletal muscle, and anterior pituitary gland. Within the anterior pituitary gland, RXR[gamma] expression is limited primarily to the thyrotropes. In this report, we have isolated ~3 kb of 5'-flanking DNA of the mouse RXR[gamma]1 gene. We have identified the major transcription start site in the thyrotrope-derived TtT-97 cells. Transient transfection studies show that a 1.4-kb promoter fragment has full promoter activity in TtT-97 cells. This promoter has much less activity in thyrotrope-derived [alpha]TSH cells, pituitary-derived GH3 somatomammotropes, and non-pituitary CV-1 cells. None of these cell lines has detectable RXR[gamma]1 mRNA. A previous report has identified a non-consensus direct repeat (DR-1) element in the RXR[gamma]2 gene promoter region that mediates stimulation of promoter activity by 9-cis-retinoic acid (9-cis-RA). Inspection of the RXR[gamma]1 promoter region revealed a non-consensus DR-1 element at [-]232 bp from the transcription start site. Interestingly, RXR[gamma]1 promoter activity was suppressed 50% by 9-cis-RA in the TtT-97 thyrotropes. Further experiments in non-pituitary cells showed that suppression of RXR[gamma]1 promoter

activity was RXR-dependent. Mutagenesis of the DR-1 element abrogated suppression of promoter activity by 9-cis-RA, suggesting that this negative regulation requires both RXR and this specific DR-1 element. In summary, we have isolated the mouse RXR[gamma]1 gene promoter region and identified the major start site in thyrotropes. Promoter activity is uniquely suppressed by 9-cis-RA through a DR-1 element. Isolation and characterization of the mouse RXR[gamma]1 promoter region provides a tool for further investigation focusing on thyrotrope-specific gene expression as well as negative regulation of genes by retinoic acid.

Tabuchi, A., H. Sakaya, et al. (2002). "Involvement of an Upstream Stimulatory Factor as Well as cAMP-responsive Element-binding Protein in the Activation of Brain-derived Neurotrophic Factor Gene Promoter I." J. Biol. Chem. **277**(39): 35920-35931.

http://www.jbc.org/cgi/content/abstract/277/39/35920

The use of different brain-derived neurotrophic factor (BDNF) gene promoters results in the differential production of 5'-alternative transcripts, suggesting versatile functions of BDNF in neurons. Among four BDNF promoters I, II, III, and IV (BDNF-PI, -PII, -PIII, and -PIV), BDNF-PI was markedly activated, as well as BDNF-PIII, by Ca2+ signals evoked via neuronal activity. However, little is known about the mechanisms for the transcriptional activation of BDNF-PI. Using rat cortical neurons in culture, we assigned the promoter sequences responsible for the Ca2+ signal-mediated activation of BDNF-PI and found that the Ca2+-responsive elements were located in two separate (distal and proximal) regions and that the DNA sequences in the proximal region containing cAMP-responsive element (CRE), which is overlapped by the upstream stimulatory factor (USF)-binding element, were largely responsible for the activation of BDNF-PI. CRE-binding protein (CREB) family transcription factors and USF1/USF2 bind to this overlapping site, depending upon their preferred sequences which also control the magnitude of the activation. Overexpression of dominant negative CREB or USF reduced the BDNF-PI activation. These findings support that not only CREB but also USF1/USF2 contributes to Ca2+ signal-mediated activation of BDNF-PI through the recognition of an overlapping CRE and USF-binding element.

Bream, J. H., D. L. Hodge, et al. (2004). "A Distal Region in the Interferon-{gamma} Gene Is a Site of Epigenetic Remodeling and Transcriptional Regulation by Interleukin-2." J. Biol. Chem. **279**(39): 41249-41257.

http://www.jbc.org/cgi/content/abstract/279/39/41249

Interferon-{gamma} (IFN-{gamma}) is a multifunctional cytokine that defines the development of Th1 cells and is critical for host defense against intracellular pathogens. IL-2 is another key immunoregulatory cytokine that is involved in T helper differentiation and is known to induce IFN-{gamma} expression in natural killer (NK) and T cells. Despite concerted efforts to identify the one or more transcriptional control mechanisms by which IL-2 induces IFN-{gamma} mRNA expression, no such genomic regulatory regions have been described. We have identified a DNase I hypersensitivity site [~]3.5-4.0 kb upstream of the transcriptional start site. Using chromatin immunoprecipitation assays we found constitutive histone H3 acetylation in this distal region in primary human NK cells, which is enhanced by IL-2 treatment. This distal region is also preferentially acetylated on histones H3 and H4 in primary Th1 cells as compared with Th2 cells. Within this distal region we found a Stat5-like motif, and in vitro DNA binding assays as well as in vivo chromosomal immunoprecipitation assays showed IL-2-induced binding of both Stat5a and Stat5b to this distal element in the IFNG gene. We examined the function of this Stat5-binding motif by transfecting human peripheral blood mononuclear cells with -3.6 kb of IFNG-luciferase constructs and found that phorbol 12-myristate 13-acetate/ionomycin-induced transcription was

augmented by IL-2 treatment. The effect of IL-2 was lost when the Stat5 motif was disrupted. These data led us to conclude that this distal region serves as both a target of chromatin remodeling in the IFNG locus as well as an IL-2-induced transcriptional enhancer that binds Stat5 proteins.

Morinobu, A., Y. Kanno, et al. (2004). "Discrete Roles for Histone Acetylation in Human T Helper 1 Cell-specific Gene Expression." J. Biol. Chem. **279**(39): 40640-40646.

http://www.jbc.org/cgi/content/abstract/279/39/40640

To better understand the control of T helper (TH) 1-expressed genes, we compared and contrasted acetylation and expression for three key genes, IFNG, TBET, and IL18RAP and found them to be distinctly regulated. The TBET and the IFNG genes, but not the IL18RAP gene, showed preferential acetylation of histones H3 and H4 during TH1 differentiation. Analysis of acetylation of specific histone residues revealed that H3(Lys-9), H4(Lys-8), and H4(Lys-12) were preferentially modified in TH1 cells, suggesting a possible contribution of acetylation of these residues for induction of these genes. On the other hand, the acetylation of IL18RAP gene occurred both in TH1 and TH2 cells the similar kinetics and on the same with residues, demonstrating that selective histone acetylation was not universally the case for all TH1-expressed genes. Histone H3 acetylation of IFNG and TBET genes occurred with different kinetics, however, and was distinctively regulated by cytokines. Interleukin (IL)-12 and IL-18 enhanced the histone acetylation of the IFNG gene. By contrast, histone acetylation of the TBET gene was markedly suppressed by IL-4, whereas IL-12 and IL-18 had only modest effects suggesting that histone acetylation during TH1 differentiation is a process that is regulated by various factors at multiple levels. By treating Th2 cells with a histone deacetylase inhibitor, we restored histone acetylation of the IFNG and TBET genes, but it did not fully restore their expression in TH2 cells, again suggesting that histone acetylation explains one but not all the aspects of TH1-specific gene expression.

Perrier, E., R. Perrier, et al. (2004). "Ca2+ Controls Functional Expression of the Cardiac K+ Transient Outward Current via the Calcineurin Pathway." J. Biol. Chem. **279**(39): 40634-40639.

http://www.jbc.org/cgi/content/abstract/279/39/40634

The transient outward K+ current (Ito) modulates transmembrane Ca2+ influx into cardiomyocytes, which, in turn, might act on Ito. Here, we investigated whether Ca2+ modifies functional expression of Ito. Whole-cell Ito were recorded using the patch clamp technique in single right ventricular myocytes isolated from adult rats and incubated for 24 h at 37 {degrees}C in a serum-free medium containing various Ca2+ concentrations ([Ca2+]o). Increasing the [Ca2+]o from 0.5 to 1.0 and 2.5 mM produced a gradual decrease in Ito density without change in current kinetics. Quantitativereverse transcriptase-PCR showed that a decrease of the Kv4.2 mRNA could account for this decrease. In the acetoxymethyl ester form of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM)-loaded myocytes (a permeant Ca2+ chelator), Ito density increased significantly when cells were exposed for 24 h to either 1 or 2.5 mM [Ca2+]o. Moreover, 24-h exposure to the Ca2+ channel agonist, Bay K8644, in 1 mM [Ca2+]o induced a decrease in Ito density, whereas the Ca2+ channel antagonist, nifedipine, blunted Ito decrease in 2.5 mM [Ca2+]o. The decrease of Ito in 2.5 mM [Ca2+]o was also prevented by co-incubation with either the calmodulin inhibitor W7 or the calcineurin inhibitors FK506 or cyclosporin A. Furthermore, in myocytes incubated for 24 h with 2.5 mM [Ca2+]o, calcineurin activity was significantly increased compared with 1 mM [Ca2+]o. Our data suggest that modulation of [Ca2+]i via L-type Ca2+ channels, which appears to involve the Ca2+/calmodulin-regulated protein phosphatase calcineurin, down-regulates the functional

expression of Ito. This effect might be involved in many physiological and pathological modulations of Ito channel expression in cardiac cells, as well other cell types.

Pelicano, H., L. Feng, et al. (2003). "Inhibition of Mitochondrial Respiration: A NOVEL STRATEGY TO ENHANCE DRUG-INDUCED APOPTOSIS IN HUMAN LEUKEMIA CELLS BY A REACTIVE OXYGEN SPECIES-MEDIATED MECHANISM." J. Biol. Chem. **278**(39): 37832-37839.

http://www.jbc.org/cgi/content/abstract/278/39/37832

Cancer cells are under intrinsic increased oxidative stress and vulnerable to free radical-induced apoptosis. Here, we report a strategy to hinder mitochondrial electron transport and increase superoxide [IMG]f1.gif" BORDER="0"> radical generation in human leukemia cells as a novel mechanism to enhance apoptosis induced by anticancer agents. This strategy was first tested in a proof-of-principle study using rotenone, a specific inhibitor of mitochondrial electron transport complex I. Partial inhibition of mitochondrial respiration enhances electron leakage from the transport chain, leading to an increase in [IMG]f1.gif" BORDER="0"> generation and sensitization of the leukemia cells to anticancer agents whose action involve free radical generation. Using leukemia cells with genetic alterations in mitochondrial DNA and biochemical approaches, we further demonstrated that As2O3, a clinically active anti-leukemia agent, inhibits mitochondrial respiratory function, increases free radical generation, and enhances the activity of another [IMG]f2.gif" BORDER="0"> agent against cultured leukemia cells and primary leukemia cells isolated from patients. Our study shows that interfering mitochondrial respiration is a novel mechanism by which As2O3 increases generation of free radicals. This novel mechanism of action provides a biochemical basis for developing new drug combination strategies using As2O3 to enhance the activity of anticancer agents by promoting generation of free radicals.

Gomez, M. F., A. S. Stevenson, et al. (2002). "Opposing Actions of Inositol 1,4,5-Trisphosphate and Ryanodine Receptors on Nuclear Factor of Activated T-cells Regulation in Smooth Muscle." J. Biol. Chem. **277**(40): 37756-37764.

http://www.jbc.org/cgi/content/abstract/277/40/37756

The nuclear factor of activated T-cells (NFAT), originally identified in T-cells, has since been shown to play a role in mediating Ca2+-dependent gene transcription in diverse cell types outside of the immune system. We have previously shown that nuclear accumulation of NFATc3 is induced in ileal smooth muscle by platelet-derived growth factor in a manner that depends on Ca2+ influx through L-type, voltage-dependent Ca2+ channels. Here we show that NFATc3 is also the predominant NFAT isoform expressed in cerebral artery smooth muscle and is induced to accumulate in the nucleus by UTP and other Gq/11-coupled receptor agonists. This induction is mediated by calcineurin and is dependent on sarcoplasmic reticulum Ca2+ release through inositol 1,4,5-trisphosphate receptors and extracellular Ca2+ influx through L-type, voltage-dependent Ca2+ channels. Consistent with results obtained in ileal smooth muscle, depolarization-induced Ca2+ influx fails to induce NFAT nuclear accumulation in cerebral arteries. We also provide evidence that Ca2+ release by ryanodine receptors in the form of Ca2+ sparks may exert an inhibitory influence on UTP-induced NFATc3 nuclear accumulation and further suggest that UTP may act, in part, by inhibiting Ca2+ sparks. These results are consistent with a multifactorial regulation of NFAT nuclear accumulation in smooth muscle that is likely to involve several intracellular signaling pathways, including local effects of sarcoplasmic reticulum Ca2+ release and effects attributable to global elevations in intracellular Ca2+.

Kominato, Y., Y. Hata, et al. (2002). "Alternative Promoter Identified between a Hypermethylated Upstream Region of Repetitive Elements and a CpG Island in Human ABO Histo-blood Group Genes." J. Biol. Chem. **277**(40): 37936-37948.

http://www.jbc.org/cgi/content/abstract/277/40/37936

We have studied the expression of human histo-blood group ABO genes during erythroid differentiation, using an ex vivo culture of AC133[-]CD34+ cells obtained from peripheral blood. 5'-Rapid amplification of cDNA ends analysis of RNA from those cells revealed a novel transcription start site, which appeared to mark an alternative starting exon (1a) comprising 27 bp at the 5'-end of a CpG island in ABO genes. Results from reverse transcription-PCR specific to exon 1a indicated that the cells of both erythroid and epithelial lineages utilize this exon as the transcription starting exon. Transient transfection experiments showed that the region just upstream from the transcription start site possesses promoter activity in a cell type-specific manner when placed 5' adjacent to the reporter luciferase gene. Results from bisulfite genomic sequencing and reverse transcription-PCR analysis indicated that hypermethylation of the distal promoter region correlated with the absence of transcripts containing exon 1a, whereas hypermethylation in the interspersed repeats 5' adjacent to the distal promoter was commonly observed in all of the cell lines examined. These results suggest that a functional alternative promoter is located between the hypermethylated region of repetitive elements and the CpG island in the ABO genes.