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Molecular and Biochemical Parasitology (107)

Akopyants, N. S., S. W. Clifton, et al. (2001). "A survey of the Leishmania major Friedlin strain V1 genome by shotgun sequencing: a resource for DNA microarrays and expression profiling." Molecular and Biochemical Parasitology **113**(2): 337.

http://www.sciencedirect.com/science/article/B6T29-42R0SFR-M/2/028584d9e948600ab64c410016a6c822

Akopyants, N. S., R. S. Matlib, et al. (2004). "Expression profiling using random genomic DNA microarrays identifies differentially expressed genes associated with three major developmental stages of the protozoan parasite Leishmania major." Molecular and Biochemical Parasitology **136**(1): 71.

http://www.sciencedirect.com/science/article/B6T29-4C5G696-1/2/fa3bea71f46aef5e15b8791e6bb43ca1

To complete its life cycle, protozoan parasites of the genus Leishmania undergo at least three major developmental transitions. However, previous efforts to identify genes showing stage regulated changes in transcript abundance have yielded relatively few. Here we used expression profiling to assess changes in transcript abundance in three stages: replicating promastigotes and infective non-replicating metacyclics, which occur in the sand fly vector, and in the amastigote stage residing with macrophage phagolysosomes in mammals. Microarrays were developed containing 11,484 PCR products that included a number of known genes and 10,464 random 1 kb genomic DNA fragments. Arrays were hybridized in triplicate and genes showing two-fold or greater changes in 2/3 experiments were scored as differentially expressed. Remarkably, only about one percent of the DNAs expression varied by this criteria, in either stage comparison. Northern blot analysis confirmed the predicted change in mRNA abundance for most of these (68%). This set of genes included most of those previously identified in the literature as differentially regulated as well as a number of novel genes. Notably, Leishmania maxicircle transcripts showed strong up-regulation in metacyclic and amastigote parasites, probably associated with changes in parasite energy metabolism. However, current data suggest that expression profiling using shotgun DNA libraries significantly underestimates the extent of regulated transcripts.

Allen, T. E. and B. Ullman (1994). "Molecular characterization and overexpression of the hypoxanthine-guanine phosphoribosyltransferase gene from Trypanosoma cruzi." Molecular and Biochemical Parasitology **65**(2): 233.

http://www.sciencedirect.com/science/article/B6T29-476RNG1-

3Y/2/0c119e9f62f57a24836dc9f4207d87a2

The hypoxanthine-guanine phosphoribosyltransferase gene (HGPRT) enzyme in Trypanosoma cruzi is a rational target for the treatment of Chagas disease. To evaluate the T. cruzi HGPRT in detail, the HGPRT gene (hgprt) was cloned from a genomic library of T. cruzi DNA and sequenced. Translation of the nucleotide sequence of the hgprt revealed an open reading frame of 663 bp that encoded a 25.5-kDa polypeptide of 221 amino acids. The T. cruzi HGPRT exhibited only 24%, 25%, and 21% amino acid sequence identity to its human, Plasmodium falciparum and Schistosoma mansoni counterparts, respectively, but was 50% identical to the T. brucei HGPRT protein. Northern analysis of T. cruzi RNA revealed a 1.8-kb hgprt transcript, while Southern blots of genomic DNA suggested that hgprt was a single copy gene within the T. cruzi genome. The T. cruzi hgprt was inserted into the pBAce expression plasmid and transformed into Escherichia coli that are deficient in hypoxanthine and guanine phosphoribosylating activities. High levels of soluble, enzymatically active T. cruzi HGPRT were obtained, and this expression complemented the bacterial phosphoribosyltransferase deficiencies. The recombinant HGPRT was purified to apparent homogeneity by GTP-agarose affinity chromatography and recognized hypoxanthine, guanine, and allopurinol, but not adenine or xanthine, as substrates. The availability of the hgprt clone and large amounts of pure HGPRT protein provide a foundation for a structurebased drug design strategy for the treatment of Chagas disease.

Arnot, D. E., C. Roper, et al. (1993). "Digital codes from hypervariable tandemly repeated DNA sequences in the Plasmodium falciparum circumsporozoite gene can genetically barcode isolates." Molecular and Biochemical Parasitology **61**(1): 15.

http://www.sciencedirect.com/science/article/B6T29-476M17K-HG/2/6bef586c2992d4690a4877e2e3e386ed

DNA typing systems currently used in parasitology involve either hybridising Southern blots with repetitive sequence probes or amplifying genomic sequences using the polymerase chain reaction (PCR). Both such approaches assay allelic length variation, usually in unexpressed tandemly repeated DNA sequences. Where an appropriate target locus exists, an alternative PCR-based strategy which reveals allelic sequence variation in tandemly repeated DNA offers a more accurate and internally controlled assay. We describe such a strategy for the rapid extraction of information on tandem repeat sequence variation from hypervariable alleles, and apply it to the Plasmodium falciparum CS gene. The extreme variability of such DNA 'barcodes' can be used to identify parasite stocks and lineages. This system is also potentially useful for population genetic and epidemiological studies since it offers the possibility of following the spread of distinctively marked parasite genotypes in samples taken from infected individuals.

Aslund, L., L. Carlsson, et al. (1994). "A gene family encoding heterogeneous histone H1 proteins in Trypanosoma cruzi." Molecular and Biochemical Parasitology **65**(2): 317.

http://www.sciencedirect.com/science/article/B6T29-476RNG1-46/2/3e56ed35c5c972cd50e0c63b09cf571d

A gene family encoding a set of histone H1 proteins in Trypanosoma cruzi is described. The sequence of 3 genomic and 4 cDNA clones revealed the presence of several motifs characteristic of histone H1 although heterogeneity at the polypeptide level was evident. The clones encode histone H1 proteins of an unusually small size (74-97 amino acids) which lack the globular domain found in histone H1 of higher eukaryotes. All histone H1 mRNAs from T. cruzi are polyadenylated although no typical polyadenylation signal was found. Furthermore the genes

encoding the histone H1 proteins in T. cruzi are found in a tandem array containing 15-20 gene copies per haploid genome. This tandem array is located on a large chromosome of 2.2 Mb.

Barrett, M. P., A. MacLeod, et al. (1997). "A single locus minisatellite sequence which distinguishes between Trypanosoma brucei isolates." Molecular and Biochemical Parasitology **86**(1): 95.

http://www.sciencedirect.com/science/article/B6T29-4F1BR83-1/2/4e1e14931a8cf504c09e59831f0c9f3c

Becuwe, P., S. Gratepanche, et al. (1996). "Characterization of iron-dependent endogenous superoxide dismutase of Plasmodium falciparum." Molecular and Biochemical Parasitology **76**(1-2): 125.

http://www.sciencedirect.com/science/article/B6T29-4287HV2-D/2/abc7d9ea5c19b3a5250b8e7fded1f8bd

Two main superoxide dismutase activities at isoelectric points (pI) 6.2 and 6.8 and two minor at pI 5.6 and 6.4 were found in crude extracts of Plasmodium falciparum. These activities were cyanide-resistant and hydrogen peroxide-sensitive and represented 20-30% of the total SOD activity found in the crude extract. A fragment of 424 bp, amplified from genomic DNA from P. falciparum, was cloned and sequenced. The deduced amino acid sequence identified this fragment as a coding region of an SOD gene. A cDNA corresponding to SOD was then isolated from a P. falciparum cDNA library and sequenced. The deduced amino acid sequence of SOD (197 aa) was compared with 32 known Fe- or Mn-SODs by the 'DARWIN' system. This analysis showed that theparasitic enzyme was related to typical Fe-SODs. The SOD subunit was purified and the N-terminal sequence, determined up to 29 residues, corresponded to that of cDNA isolated. The iron-dependent SOD activity found in Plasmodium falciparum represents the first level of the antioxidant defence system of the parasite. It is also the first SOD characterized in the parasitic Apicomplexa phylum whose sequence can be compared to equivalent iron-dependent enzymes known in other protozoa and bacteria.

Benjamin, P. A., I. T. Ling, et al. (1999). "Antigenic and sequence diversity at the C-terminus of the merozoite surface protein-1 from rodent malaria isolates, and the binding of protective monoclonal antibodies." Molecular and Biochemical Parasitology **104**(2): 147.

http://www.sciencedirect.com/science/article/B6T29-3XWJR7T-1/2/c80e16309316373230381e4c31bea211

Merozoite surface protein-1 (MSP-1) is a major candidate in the development of a vaccine against malaria. Immunisation with a recombinant fusion protein containing the two Plasmodium yoelii MSP-1 C-terminal epidermal growth factor-like domains (MSP-119) can protect mice against homologous but not heterologous challenge, and therefore, antigenic differences resulting from sequence diversity in MSP-119 may be crucial in determining the potential of this protein as a vaccine. Representative sequence variants from a number of distinct P. yoelii isolates were expressed in Escherichia coli and the resulting recombinant proteins were screened for binding to a panel of monoclonal antibodies (Mabs) capable of suppressing a P. yoelii YM challenge infection in passive immunisation experiments. The sequence polymorphisms affected the binding of the antibodies to the recombinant proteins. None of the Mabs recognised MSP-119 of P. yoelii yoelii 2CL or 33X or P. yoelii nigeriensis N67. The epitopes recognised by the Mabs were further distinguished by their reactivity with the other fusion proteins. The extent of sequence

variation in MSP-119 among the isolates was extensive, with differences detected at 35 out of the 96 positions compared. Using the 3-dimensional structure of the Plasmodium falciparum MSP-119 as a model, the locations of the amino acid substitutions that may affect Mab binding were identified. The DNA sequence of MSP-119 from two Plasmodium vinckei isolates was also cloned and the deduced amino acid sequence compared with that in other species.

Bhatia, A., N. S. Daifalla, et al. (1999). "Cloning, characterization and serological evaluation of K9 and K26: two related hydrophilic antigens of Leishmania chagasi." Molecular and Biochemical Parasitology **102**(2): 249.

http://www.sciencedirect.com/science/article/B6T29-3X70V6T-4/2/95b01e7b95d9f0525e7b925094241ea3

We report here the molecular cloning and characterization of two related hydrophilic antigens of Leishmania chagasi. These two antigens have predicted molecular weights of ~9 and 26 kDa and detect antibodies in sera of patients with kala-azar (k). Thus, to maintain consistency with nomenclature of the previously described 39kDa diagnostic antigen of L. chagasi (k39 [1]), these antigens are being referred to as k9 and k26. A significant difference between k9 and k26 is the presence of 11 copies of a 14 amino acid repeat in the open reading frame of k26. The region flanking the repeats of k26 shares a 69% identity with the open reading frame of k9. The recombinant proteins encoded by both antigens are very hydrophilic and show aberrant migration on SDS PAGE. Results of Southern blot analysis reveal that k9 and k26 are conserved to varying degrees among various Leishmania species. Interestingly, the repeat region of k26 is specific to L. chagasi and L. donovani while the flanking region is conserved among several other species. Transcript levels of k26 are significantly upregulated in the amastigote stage of the parasite. Our results show that recombinant K26 is specific in detecting antibodies in infection sera from visceral leishmaniasis (VL) patients. Thus rK26 may complement rK39 in a more accurate diagnosis of VL in the old and the new world.

Bickle, Q. and R. L. Coppel (1992). "A fourth family of the Plasmodium falciparum S-antigen." Molecular and Biochemical Parasitology **56**(1): 141.

http://www.sciencedirect.com/science/article/B6T29-47726BD-3F/2/49311e1a9fc88a25b14d364e58775c90

The S-antigen of Plasmodium falciparum is a highly diverse heat stable protein that is located in the parasitophorous vacuole of the mature asexual intraerythrocytic parasite. The gene for S-antigen exists within the parasite population as multiple alleles at a single locus. Its sequence contains a large central block of tandemly arranged peptides that are identical or very similar in one allele but differ widely in sequence, repeat length and number among different alleles, and consequently antigenic specificity. Thus, antibodies directed against the repeat region can be used to define the serotype of an S-antigen. Flanking this repeat block are 2 short regions of non-repetitive sequence which have been described as occurring in three different forms, each of which is used to define a single S-antigen family. We present the S-antigen sequence for the isolate 3D7 which defines not only a novel serotype but also a novel S-antigen family. The central repeat block is composed of 57 copies of an 8-residue peptide with consensus sequence ED(E/K)VSNG(R/G). Comparison of the four S-antigen families reveals that they differ considerably from each other with variation being most pronounced in the carboxy terminal-flanking region. This pattern of sequence variation differs considerably from that found for MSA-1 and MSA-2, the only other diverse proteins of P. falciparum for which sequence information is available.

Black, C. G., L. Wang, et al. (2003). "Apical location of a novel EGF-like domain-containing protein of Plasmodium falciparum." Molecular and Biochemical Parasitology **127**(1): 59.

http://www.sciencedirect.com/science/article/B6T29-47K22Y4-1/2/60015e76df260caa8d74c19d5403fd22

Using bioinformatics analyses of the unfinished malaria genome sequence, we have identified a novel protein of Plasmodium falciparum that contains two epidermal growth factor (EGF)-like domains near the C-terminus of the protein. The sequence contains a single open reading frame of 1572 bp with the potential to encode a protein of 524 residues containing hydrophobic regions at the extreme N- and C-termini which appear to represent signal peptide and glycosylphosphatidylinositol (GPI)-attachment sites, respectively. RT-PCR analysis has confirmed that the novel gene is transcribed in asexual stages of P. falciparum. Antibodies to the EGF-like domains of the novel protein are highly specific and do not cross-react with the EGF-like domains of MSP1, MSP4, MSP5 or MSP8 expressed as GST fusion proteins. Antisera to the C-terminal fragments react with two bands of 80 and 36 kDa in P. falciparum parasite lysates whereas antisera to the most N-terminal fusion protein only recognises the 80 kDa band, suggesting that the novel protein may undergo processing in a similar way to MSP1 and MSP8, but with fewer cleavage events. Immunoblot analysis of stage-specific parasite samples reveals that the protein is present in trophozoites, schizonts and in isolated merozoites. The protein partitions in the detergent-enriched phase after Triton X-114 fractionation and is localised to the surfaces of trophozoites, schizonts and free merozoites in an apical distribution. Based on the accepted nomenclature in the field we now designate this protein MSP10. We have shown that the MSP10 fusion proteins are in a conformation that can be recognised by human immune sera and that there is very limited sequence diversity in an ~l kb region of MSP10, encompassing the two EGF-like domains. A sequence similar to MSP10 can be identified in the available P. yoelii genomic sequence, offering the possibility of ascertaining whether this novel protein can induce host protective responses in an in vivo model.

Bridge, M. A., Q. Zhou, et al. (1998). "Cloning and characterization of the kinetoplastid membrane protein-11 gene locus of Trypanosoma brucei." Molecular and Biochemical Parasitology **91**(2): 359.

http://www.sciencedirect.com/science/article/B6T29-3SHB4XY-G/2/8eadbdefa0bb1d0945ae106d8307ff1e

Bringaud, F., D. R. Robinson, et al. (2000). "Characterization and disruption of a new Trypanosoma brucei repetitive flagellum protein, using double-stranded RNA inhibition." Molecular and Biochemical Parasitology **111**(2): 283.

http://www.sciencedirect.com/science/article/B6T29-423JB0G-5/2/7a1d75370a47743cbe86fd5206252843

In Trypanosoma brucei, we have cloned a gene ~5 kb downstream of the glucose transporter gene cluster, containing a variable number of 102 bp repeats. This gene encodes a protein with no homologues in the data bases. Antibodies raised against the 34 amino acids repeated motif recognized proteins ranging from 145 to 270 kDa, depending on strains, in both bloodstream and procyclic forms of T. brucei. A correlation was established between the apparent molecular mass of the detected proteins and the number of 34 amino acid repeats which varies from 3 to 40. We have called this protein the flagellum transition zone component (FTZC) due to its localization to

the proximal region of the axoneme, within the transition zone. FTZC is the only reported example of a trypanosomal protein present in the transition zone. To determine the role of FTZC we developed a new strategy of gene inactivation based on conditional expression of double-stranded RNA. In the presence of tetracycline, expression of the double-stranded RNA, we observed a complete disappearance of FTZC in the EATRO 1125 and EATRO 427 strains of T. brucei. Molecular ablation of FTZC does not generate any obvious phenotype such as, lethality, modification of growth rate or cellular shape, in the growth conditions used.

Bringaud, F., C. Vedrenne, et al. (1998). "Conserved organization of genes in trypanosomatids." Molecular and Biochemical Parasitology **94**(2): 249.

http://www.sciencedirect.com/science/article/B6T29-3TGVK85-9/2/91d841b54ce58cd5de6f91dffba04ede

Trypanosomatids are unicellular protozoan parasites which constitute some of the most primitive eukaryotes. Leishmania spp, Trypanosoma cruzi and members of the Trypanosoma brucei group, which cause human diseases, are the most studied representatives of this large family. Here we report a comparative analysis of a large genomic region containing glucose transporter genes in three Salivarian trypanosomes (T. brucei, T. congolense and T. vivax), T. cruzi and Leishmania donovani. In T. brucei, the 8 kb (upstream) and 14 kb (downstream) regions flanking the glucose transporter genes cluster contain two and six new genes, respectively, six of them encoding proteins homologous to known eukaryotic proteins (phosphatidylinositol 3 kinase, ribosomal protein S12, DNAJ and three small G-proteins--Rab1, YPT6 and ARL3). This gene organization is identical in T. brucei, T. congolense and T. vivax suggesting that Salivarian trypanosomes have a high level of conservation in gene organization. In T. cruzi and Leishmania, the overall organization of this cluster is conserved, with insertion of additional genes when compared with T. brucei. Phylogenetic reconstitution based on glucose transporters is in accord with the monophyly of the genus Trypanosoma and the early separation of T. vivax within Salivarian trypanosomes. On the basis of gene organization, biochemical characteristics of isoforms and phylogeny, we discuss the genesis of the glucose transporter multigene family in Salivarian trypanosomes.

Cheng, Q., N. Cloonan, et al. (1998). "stevor and rif are Plasmodium falciparum multicopy gene families which potentially encode variant antigens." Molecular and Biochemical Parasitology **97**(1-2): 161.

http://www.sciencedirect.com/science/article/B6T29-3VB3DPK-G/2/208f0ce7754b4ac4dc1a371d60bca5dc

Several multicopy gene families have been described in Plasmodium falciparum, including the var genes that code for the variant surface antigen PfEMP1, the stevor family of subtelomeric open reading frames and the rif interspersed repetitive elements. This report documents the chromosomal location of stevor genes, their transcription and characteristics of the deduced protein. On 14 chromosomes, 34 stevor copies were identified from the Dd2 parasite line. Most are in subtelomeric regions within 50 kb of the telomere. stevor genes are located close to var genes and rif. All stevor genes sequenced had two exons: a short exon 1 encoding a start codon and a transmembrane domain; exon 2 encoding for the remainder of the ~30 kDa protein and including two more transmembrane segments. A similar structure was found for copies of rif and its predicted protein. In both STEVOR and RIF proteins, a highly polymorphic region is predicted to be a loop on the outer side of the membrane. We propose that stevor and rif are members of a larger superfamily. The number of copies of stevor and rif, their location close to the var genes, their extreme polymorphism and the predicted structure of the proteins suggest that stevor and rif code for variant surface antigens.

de Koning, H. P., A. MacLeod, et al. (2000). "Further evidence for a link between melarsoprol resistance and P2 transporter function in African trypanosomes." Molecular and Biochemical Parasitology **106**(1): 181.

http://www.sciencedirect.com/science/article/B6T29-3YHG1V3-M/2/a30bddc43ac54afcc578bb9e31d37601

Del Valle, A., B. F. Jones, et al. (2003). "Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult Ancylostoma caninum." Molecular and Biochemical Parasitology **129**(2): 167.

http://www.sciencedirect.com/science/article/B6T29-48S2S16-1/2/76e493b64afa696aa040e6ae2395a302

Hookworms, bloodfeeding intestinal nematodes, are a leading cause of iron deficiency anemia in the developing world. These parasites have evolved potent mechanisms of interfering with mammalian hemostasis, presumably for the purpose of facilitating bloodfeeding. Adult Ancylostoma caninum worm extracts contain an activity that inhibits platelet aggregation and adhesion by blocking the function of two cell surface integrin receptors, Glycoprotein IIb/IIIa and GPIa/IIa. Using rpHPLC, the hookworm platelet inhibitor activities have been purified from protein extracts of A. caninum. Because the two inhibitory activities co-purified through multiple chromatographic steps, have similar molecular masses and share identical N-terminal as well as internal amino acid sequence homology, it is likely that they represent a single gene product. A cDNA corresponding to the purified hookworm platelet inhibitor (HPI) protein has been cloned from adult A. caninum RNA, and the translated amino acid sequence shows significant homology to Neutrophil Inhibitory Factor and Ancylostoma Secreted Proteins, suggesting that these related hookworm proteins represent a novel class of integrin receptor antagonists. Polyclonal antibodies raised against the recombinant HPI protein recognize corresponding native proteins in A. caninum extracts and excretory/secretory products, and immunohistochemistry data have identified the cephalic glands as the major source of the inhibitor within the adult hookworm. These data suggest that HPI is secreted by the adult stage of the parasite at the site of intestinal attachment. As such, it may represent a viable target for a vaccine-based strategy aimed at interfering with hookworm-induced gastrointestinal hemorrhage and iron deficiency anemia.

Dissanayake, S., X. Min, et al. (1991). "Detection of amplified Wuchereria bancrofti DNA in mosquitoes with a nonradioactive probe." Molecular and Biochemical Parasitology **45**(1): 49.

http://www.sciencedirect.com/science/article/B6T29-476N04N-9B/2/2a39a79e4f4ee70cc684d0397454a485

A technique to identify Wuchereria bancrofti larvae in mosquito vectors with an enzyme-labeled DNA probe is described. To overcome the low sensitivity of nonradioactive detection methods, analyte DNA was amplified by polymerase chain reaction (PCR). Oligonucleotide primers were used to amplify W. bancrofti-specific DNA fragments of 380 and 650 bp, respectively. Parasite DNA in mosquito extracts was isolated free of inhibitors of the PCR by hybridization to a biotinylated DNA fragment (IWb 67), which hybridizes to DNA from most filarial species, followed by absorption of the resulting DNA hybrids onto avidin-coated acrylic beads. PCR-amplified DNA was detected with a biotin-labeled W. bancrofti-specific repeat DNA (IWb 35) coupled to avidin-alkaline phosphatase and the chemiluminescent substrate, AMPPD(TM). The DNA equivalent of less than one larva can be detected by this method in mosquito extracts. The sensitivity of

detection was comparable to that of radioactive probes and the assay is suitable for field application in endemic countries.

Durand, R., S. Jafari, et al. (2001). "Analysis of pfcrt point mutations and chloroquine susceptibility in isolates of Plasmodium falciparum." Molecular and Biochemical Parasitology **114**(1): 95.

http://www.sciencedirect.com/science/article/B6T29-43JXFX5-B/2/b1485f5e39d96b0eb5e922ae2a3b7c0c

Recent transfection based studies demonstrated that cg2, a candidate gene for chloroquine resistance in Plasmodium falciparum, was not the resistance determinant. A further analysis of the initial 36 kb locus comprising the cg2 gene led to the discovery of another gene, pfcrt, which was absolutely associated with chloroquine resistance in forty parasite lines [Fidock DA, Nomura T, Talley AT, Su XZ, Cooper R, Dzekunov SM, Ferdig MT, Ursos LMB, Sidhu ABS, Naude B, Deitsch KW, Su XZ, Wootton JC, Roepe PD, Wellems TE. Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell 2000;6:861-71]. The aim of this study was to evaluate, in 146 unselected clinical isolates obtained mostly from non-immune travellers returning from various endemic countries to France in years 1995-1999, the association between in vitro chloroquine resistance and the sequence of a part of the pfcrt gene. For comparison, the determination of the cg2 kappa and the pfmdr1 codon 86 genotypes were also performed on the same isolates. As determined by an isotopic semi-microtest, 70 isolates were susceptible to chloroquine (50% inhibitory concentrationpfcrt gene spanning codons 72-76, followed by sequencing showed three distinct genotypes: one type associated with susceptible isolates, one type associated mostly with resistant isolates and one type found in a resistant isolate originating from South America. Three different zones could be defined according to the status of codon 76. For 50% inhibitory concentration valuesn=47), all isolates but one had K76 (wild type). For 50% inhibitory concentration values located between 40 and 60 nM, isolates had either K76 (n=5) or K76T (mutant type) (n=6). For 50% inhibitory concentration values>60 nM (n=88), all isolates had K76T. A lack of a strong association between the pfmdr1 N86Y mutation and in vitro chloroquine resistance was observed. Cg2 genotypes were less strongly linked than pfcrt genotypes with in vitro chloroquine susceptibility in isolates located below 40 and above 60 nM. Further studies are needed to determine the reliability of the pfcrt gene as a genetic marker for chloroquine resistance.

Durrand, V., A. Berry, et al. (2004). "Variations in the sequence and expression of the Plasmodium falciparum chloroquine resistance transporter (Pfcrt) and their relationship to chloroquine resistance in vitro." Molecular and Biochemical Parasitology **136**(2): 273.

http://www.sciencedirect.com/science/article/B6T29-4CG0MCM-1/2/44e9c88287e095ceebc80634e81bc2d4

Chloroquine has been widely used for malaria treatment and prophylaxis for several decades, but its usefulness has now declined with the emergence of chloroquine resistance. Recent studies showed that the K76T mutation in the PfCRT protein, initially associated to chloroquine-resistant parasites, is sometimes also present in susceptible parasites, suggesting that other factors control the expression of the resistance phenotype. Here, we sought new mutations in the Pfcrt gene and used real-time PCR to investigate variations in the expression level of this gene with respect to the in vitro response to chloroquine. About 40 Cambodian isolates of Plasmodium falciparum were selected on the basis of their response to chloroquine in vitro. The Pfcrt gene was characterised by amplifying and sequencing the full-length cDNA. Twelve point mutations--M74I, N75D/E, K76T, A144F, L148I, I194T, A220S, Q271E, N326S, T333S, I356T and R371I--

were detected. Mutations identified at positions 144, 148, 194 and 333 had never been described before. These mutations define six distinct haplotypes, distributed heterogeneously throughout Cambodia. Only the mutations at positions 74-76, 220 and 271 were significantly associated with the in vitro response to chloroquine. Three major haplotypes--MNK/A/Q, IDT/S/E and IET/S/E--accounted for all the isolates examined. The MNK/A/Q haplotype corresponded to susceptible isolates whereas parasites with the IDT/S/E haplotype displayed an intermediate response to chloroquine and those with the IET/S/E haplotype displayed the highest IC50 values. Phylogenic analysis suggested that the IDT and IET haplotypes (positions 74-76) arose independently from the wild-type MNK sequence. We found that the expression level of Pfcrt, evaluated by real-time PCR, had no effect on the response of the parasite to the drug in vitro. Similarly, in a CQ-resistant strain short-term cultured in the presence of CQ, no change was observed in the level of transcripts. These results are discussed in light of recent finding suggesting the possible involvement of other transporters in CQ-resistance.

El-Sayed, N. M. A. and J. E. Donelson (1997). "A survey of the Trypanosoma brucei rhodesiense genome using shotgun sequencing." Molecular and Biochemical Parasitology **84**(2): 167.

http://www.sciencedirect.com/science/article/B6T29-3S6D230-2/2/c330b9c78c8e48725e523759c1a0d267

A comparison of the efficiency of sequencing random genomic DNA fragments versus random cDNAs for the discovery of new genes in African trypanosomes was undertaken. Trypanosome DNA was sheared to a 1.5-2.5 kb size distribution, cloned into a plasmid and the sequences at both ends of 183 cloned fragments determined. Sequences of both kinetoplast and nuclear DNA were identified. New coding regions were discovered for a variety of proteins, including cell division proteins, an RNA-binding protein and a homologue of the Leishmania surface protease GP63. In some cases, each end of a fragment was found to contain a different gene, demonstrating the proximity of those genes and suggesting that the density of genes in the African trypanosome genome is quite high. Repetitive sequence elements found included telomeric hexamer repeats, 76 bp repeats associated with VSG gene expression sites, 177 bp satellite repeats in minichromosomes and the Ingi transposon-like elements. In contrast to cDNA sequencing, no ribosomal protein genes were detected. For the sake of comparison, the sequences of 190 expressed sequence tags (ESTs) were also determined, and a similar number of new trypanosomal homologues were found including homologues of another putative surface protein and a human leucine-rich repeat-containing protein. We conclude from this analysis and our previous work that sequencing random DNA fragments in African trypanosomes is as efficient for gene discovery as is sequencing random cDNA clones.

Ellenberger, D. L., N. J. Pieniazek, et al. (1992). "Cloning and characterization of the Wuchereria bancrofti S15 ribosomal protein." Molecular and Biochemical Parasitology **52**(1): 131.

http://www.sciencedirect.com/science/article/B6T29-476MP60-20/2/0ea4d0646e8e54122cf7ff7a3bc37e82

Fodinger, M., S. Ortner, et al. (1993). "Pathogenic Entamoeba histolytica: cDNA cloning of a histone H3 with a divergent primary structure." Molecular and Biochemical Parasitology **59**(2): 315.

http://www.sciencedirect.com/science/article/B6T29-476RND2-3G/2/1cd89e122840f8990ba27b222d0d4c50

Entamoeba histolytica has an unusual nuclear structure characterized by a low degree of chromatin condensation and the absence of stainable metaphase chromosomes. Although nucleosome-like particles were observed, no information about histones was available so far. In this paper we describe a cDNA clone with significant homology to H3 histones that was isolated from a library of pathogenic E. histolytica.The complete cDNA encodes a 15-kDa polypeptide, which like the histone sequence from Volvox carteri is shorter by one residue than the human homologue. The amino acid sequence has only 69% identity with human H3.3 histone and 67% identity with the human H3.1 histone. This is the highest degree of sequence divergence observed for any eukaryote H3 histone sequence. Our results indicate that this divergence may contribute to the unusual chromatin structure of E. histolytica.

Foley, M., L. C. Ranford-Cartwright, et al. (1992). "Rapid and simple method for isolating malaria DNA from fingerprick samples of blood." Molecular and Biochemical Parasitology **53**(1-2): 241.

http://www.sciencedirect.com/science/article/B6T29-47725WD-X/2/cf072d684091e499dbdff8322dc05bd0

Francis, S. E., I. Y. Gluzman, et al. (1996). "Characterization of native falcipain, an enzyme involved in Plasmodium falciparum hemoglobin degradation." Molecular and Biochemical Parasitology **83**(2): 189.

http://www.sciencedirect.com/science/article/B6T29-3W324NM-7/2/cc2be53b640f837b8ff9b35d8da87ada

In Plasmodium falciparum, a cysteine protease known as falcipain has been implicated in the essential metabolic process of hemoglobin degradation. Parallel lines of investigation, using native or recombinant enzyme, have led to differing conclusions about the specificity and role of this protease. We have now determined that (1) Native falcipain does not cleave hemoglobin unless this substrate has first been denatured by reducing agents, acid-acetone treatment or plasmepsin action. (2) Reducing agents such as glutathione cannot denature hemoglobin in the presence of catalase, which is accumulated in the digestive vacuole. (3) The purified native enzyme has kinetics similar to those obtained, with trophozoite extract, but substantially different from those of recombinant enzyme. (4) Although there are numerous cysteine protease genes in the P. falciparum genome, the falcipain gene is the only one whose transcript can be detected in the early intraerythrocytic parasites. We conclude that falcipain likely works by degrading hemoglobin fragments after initial aspartic protease attack has denatured the substrate. We propose that falcipain inhibitors block the initial steps of degradation indirectly by promoting vacuolar accumulation of osmotically active hemoglobin peptides.

Galinski, M. R., C. Corredor-Medina, et al. (1999). "Plasmodium vivax merozoite surface protein-3 contains coiled-coil motifs in an alanine-rich central domain." Molecular and Biochemical Parasitology **101**(1-2): 131.

http://www.sciencedirect.com/science/article/B6T29-47YYBD3-D/2/5979110abccd429f1330665614318c78

Plasmodium merozoites are covered with a palisade layer of proteins that are arranged as organized bundles or appear as protruding spikes by electron microscopy. Here we present a third Plasmodium vivax merozoite surface protein, PvMSP-3, which is associated with but not

anchored in the merozoite membrane. Serum from a P. vivax immune squirrel monkey was used to screen a [lambda]gt11 P. vivax genomic DNA (gDNA) library. Plaque-selected antibodies from clone no. 6.1, and rabbit antisera against its encoded protein, produced a pattern in immunofluorescence assays (IFAs) that is consistent with a localization at the surface of mature schizonts and free merozoites. Specific antisera also agglutinated merozoites and recognized a protein of 150000 Da by SDS-PAGE. The complete msp-3 gene and flanking sequences were cloned from a P. vivax [lambda] Dash II gDNA library and also partly characterized by RACE (rapid amplification of cDNA ends). The immediate upstream sequence contains non-coding repeats and a putative protein-encoding open reading frame (ORF), which are also present on the msp-3 5'RACE gene product. Pvmsp-3 encodes a protein with a calculated mass of 89573 Da, which has a potential signal peptide and a major central alanine-rich domain (31%) that exhibits largely [alpha]-helical secondary structure and is flanked by charged regions. The protein does not have a putative transmembrane domain or a consensus sequence for a glycosylphosphatidylinositol (GPI) anchor modification. However, the alanine-rich domain has heptad repeats that are predicted to form coiled-coil tertiary structures, which mediate protein-protein interactions. PvMSP-3 is structurally related to P. falciparum MSP-3 and the 140000 Da MSP of P. knowlesi. Characterization of PvMSP-3, thus, also begins to define a new interspecies family of evolutionarily related Plasmodium merozoite proteins.

Galinski, M. R., P. Ingravallo, et al. (2001). "Plasmodium vivax merozoite surface proteins-3[beta] and-3[gamma] share structural similarities with P. vivax merozoite surface protein-3[alpha] and define a new gene family." Molecular and Biochemical Parasitology **115**(1): 41.

http://www.sciencedirect.com/science/article/B6T29-433NRFC-4/2/f5314e56543e3f35419a9f26b8ef3c5a

The genes encoding two merozoite surface proteins of Plasmodium vivax that are related to PvMSP3 [1] are reported. One of these genes was identified within P. vivax [lambda]gt11 clone 5.4, which was selected by immunoscreening with a Saimiri monkey antiserum. The insert DNA of this clone was used as a probe to isolate the complete gene from a P. vivax [lambda]DASH genomic (g) DNA library. Antibodies to recombinant 5.4 and subsequent fusion proteins produce a pattern of circumferential surface fluorescence by indirect immunofluorescence assays (IFA) on segmented schizonts and free intact merozoites, and recognize a 125 kDa protein via western immunoblots. The gene, however, encodes a protein with a calculated size of 75677 Da, and 3' and 5' RACE analyses were employed to confirm the size of the gene and its coding region. The second related P. vivax gene was isolated by hybridization of a fragment of an orthologous P. knowlesi gene. The encoded proteins of all three related P. vivax genes have putative signal peptides, large central domains that contain >20% alanine residues bound by charged regions, are predicted to form [alpha]-helices with heptad repeat coiled-coil structures, and do not have a hydrophobic region that could anchor them to the surface of the merozoite. Although the overall identity in amino acid alignment among the three encoded proteins is low (P. knowlesi and P. falciparum.

Garside, L. H. and W. C. Gibson (1995). "Absence of the glutamic acid/alanine-rich protein (GARP) genes in the Nannomonas species Trypanosoma simiae and T. godfreyi." Molecular and Biochemical Parasitology **74**(2): 211.

http://www.sciencedirect.com/science/article/B6T29-4002FPG-S/2/4fb776c648820e0e95e7206c04e817f0

Gonzalez, G., P. Spinelli, et al. (2000). "Molecular characterization of P-29, a metacestode-specific component of Echinococcus granulosus which is immunologically related to, but distinct from, antigen 5." Molecular and Biochemical Parasitology **105**(2): 177.

http://www.sciencedirect.com/science/article/B6T29-3YDGG2B-1/2/cb8a8366578da0fec94742c3ebe542bc

In this work the characterization of P-29, a novel 29 kDa antigen from Echinococcus granulosus is reported. E. granulosus was identified while looking for parasite antigens distinct from those present in hydatid cyst fluid. A monoclonal antibody (mAb 47H.PS) prepared against protoscolex components revealed that P-29 is localized to the tegument and rostellum of protoscoleces, and to the germinal layer of the cyst, but it is absent in hydatid cyst fluid or adult worm extracts. Several internal fragments of P-29 showed sequence identity to the amino acid sequence encoded by Eg6, a partial gene sequence reported to code for an epitope of antigen 5 (Ag5), one of the major diagnostic antigens of the parasite. We confirmed that Eg6 encodes a sub-fragment of P-29 by mapping the epitope of mAb 47H.PS, and isolating the full length P-29 cDNA. Since Eg6 had been postulated to encode a fragment of Ag5, we specifically studied the relationship of P-29 and Ag5 by: (i) examining the cross-reactivity displayed by different mAbs; (ii) comparison of their peptide finger prints; and (iii) a comparative study of their diagnostic value. Our results prove unequivocally that P-29 and Ag5 are immunologically related, but different proteins, raising several questions on the current knowledge of Ag5.

Graham Clark, C. and O. J. Pung (1994). "Host specificity of ribosomal DNA variation in sylvatic Trypanosoma cruzi from North America." Molecular and Biochemical Parasitology **66**(1): 175.

http://www.sciencedirect.com/science/article/B6T29-476RNB7-2Y/2/8f71c5bbd68fa51173949bfd9814475d

Greenhalgh, C. J., A. Loukas, et al. (1999). "The organization of a galectin gene from Teladorsagia circumcincta." Molecular and Biochemical Parasitology **101**(1-2): 199.

http://www.sciencedirect.com/science/article/B6T29-47YYBD3-K/2/f4b1c28e8aa69f45e7d34a6c085d4b53

Galectins are a family of soluble [beta]-galactoside-binding lectins that are conserved amongst a broad range of organisms. We have previously isolated cDNA clones coding for galectins from the sheep gastrointestinal nematode parasites Teladorsagia circumcincta, Haemonchus contortus and Trichostrongylus colubriformis, revealing a high level of identity between these molecules. This subsequent study reports the organization of the T. circumcincta Tci-gal-1 galectin gene. The coding region is broken into eight exons covering 6.6 kbp, with introns ranging in size from 55 base pairs (bp) to 2.8 kbp. Comparisons with recently reported galectin structures from Caenorhabditis elegans reveal strong architectural similarity between galectins from the parasitic and free-living nematodes, but this structure is not conserved in mammalian galectins.

Hancock, K., S. Pattabhi, et al. (2004). "Characterization and cloning of GP50, a Taenia solium antigen diagnostic for cysticercosis." Molecular and Biochemical Parasitology **133**(1): 115.

http://www.sciencedirect.com/science/article/B6T29-49YH604-1/2/f74bedd93ea3f376a3d64d8a5e2b8710

GP50, a Taenia solium protein diagnostic for cysticercosis has been cloned, sequenced, and characterized. GP50 is one diagnostic component of the lentil lectin purified glycoprotein (LLGP) antigens that have been used for antibody-based diagnosis of cysticercosis in a Western blot assay for nearly 15 years. GP50 is a glycosylated and GPI-anchored membrane protein. The native protein migrates at 50 kDa, but the predicted molecular weight of the mature protein is 28.9. Antigenically active recombinant GP50 has been expressed in a baculovirus expression system. The antigenic activity of both the native and recombinant proteins is dependent upon the correct formation of disulfide bonds. GP50, purified from cysticerci, has two homologs expressed in the adult worm, TSES33 and TSES38. Both are diagnostic for taeniasis. In spite of the amino acid similarities between GP50 and the TSES proteins, each appears to be a stage-specific antigen. A preliminary evaluation of recombinant GP50 in a Western blot assay showed 100% specificity for cysticercosis and 90% sensitivity for cysticercosis positive serum samples reactive with the GP50 component of LLGP.

Harnett, W., K. M. Houston, et al. (1999). "Molecular cloning and demonstration of an aminopeptidase activity in a filarial nematode glycoprotein." Molecular and Biochemical Parasitology **104**(1): 11.

http://www.sciencedirect.com/science/article/B6T29-3XT0BP8-2/2/f6e364c5fcdc5cd600f9add2af93029c

ES-62 is an abundant phosphorylcholine-containing secreted glycoprotein of the filarial nematode Acanthocheilonema viteae. Using an antiserum directed against the parasite molecule, 3 cDNAs of size, ~1.5-1.6 kbp were isolated from an A. viteae expression library. Sequence analysis in combination with N-terminal amino acid sequencing of purified ES-62 revealed that each clone contained a full-length cDNA for ES-62 corresponding to 474 amino acid residues but differed in their 5' and 3' untranslated regions. Characterisation of the 5' end of ES-62 mRNA using 5' rapid amplification of cDNA ends showed that it coded for a signal sequence. Several tryptic peptides were independently sequenced using quadruple-time-of-flight mass spectrometry and used to confirm the cDNA sequence. The mature protein was found to contain three potential N-linked glycosylation sites. Comparison of the derived amino acid sequence of ES-62 with the SwissProt database identified a sequence (between amino acid residues approximately 250 and 350 of mature ES-62) with significant similarity to several bacterial/fungal aminopeptidases. Incubation of ES-62 with leucine-7-amino-4-methylcoumarin as substrate confirmed that ES-62 possessed aminopeptidase activity.

Harrop, S. A., N. Sawangjaroen, et al. (1995). "Characterization and localization of cathepsin B proteinases expressed by adult Ancylostoma caninum hookworms." Molecular and Biochemical Parasitology **71**(2): 163.

http://www.sciencedirect.com/science/article/B6T29-40078MY-2/2/5abaed59056e97a1aec32f847dbaf71d

The hookworm Ancylostoma caninum induces human eosinophilic enteritis (EE), probably via allergic responses to its secretions. Cysteine and metallo-proteinases may be the components of these secretions that elicit hypersensitivity reactions. In order to characterize genes encoding cysteine proteinases (CP) secreted by A. caninum, an adult hookworm cDNA library was constructed and screened with a cloned fragment of a hookworm CP gene. This fragment was obtained using consensus oligonucleotide, CP-gene-specific primers in the polymerase chain reaction. cDNAs encoding two CPs were obtained from the library and sequenced. The first gene, AcCP-1, encoded a cathepsin B-like zymogen CP of 343 amino acids (aa), predicted to be processed in vivo into a mature CP of 255 aa. Closest nucleotide identities were to Haemonchus contortus cysteine protease (61%) and to human cathepsin B (60%). The second gene, AcCP-2,

encoded a mature CP of 254 aa, that showed 86% identity to AcCP-1, and 58% and 47% identity to bovine cathepsin B and human cathepsin B, respectively. Rabbit antisera raised against recombinant AcCP-1 reacted with esophageal, amphidial and excretory glands in formalin-fixed, paraffin embedded sections of both male and female adult hookworms, and with an antigen of approx. 40 kDa in Western blot analysis of excretory/secretory products from adult hookworms. Together, these immuno-hybridization results strongly suggest that the CP encoded by the AcCP-1 gene is secreted by hookworms. These are the first reported CP genes from hookworms. Proteinases encoded by these genes may be responsible for the CP activity that we have shown previously to be secreted by adult A. caninum.

Hermsen, C. C., D. S. C. Telgt, et al. (2001). "Detection of Plasmodium falciparum malaria parasites in vivo by real-time quantitative PCR." Molecular and Biochemical Parasitology **118**(2): 247.

http://www.sciencedirect.com/science/article/B6T29-443K24H-2/2/bb27e4e1808589f46acc6b8cd058950d

Hoekstra, R., M. Otsen, et al. (1999). "Characterisation of a polymorphic Tc1-like transposable element of the parasitic nematode Haemonchus contortus." Molecular and Biochemical Parasitology **102**(1): 157.

http://www.sciencedirect.com/science/article/B6T29-3X3BNCR-G/2/c85ca031f7adfb5e940f97604493bdf8

Hctc1, a member of the Tc1-family of transposable elements was isolated from the parasitic nematode Haemonchus contortus. Hctc1 is 1590 bp long, is flanked by 55 bp inverted repeats and carries a single open reading frame of a 340 amino acid transposase-like protein. Hctc1 is similar to Tc1 of Caenorhabditis elegans and elements Tcb1 and Tcb2 of Caenorhabditis briggsae in the inverted terminal repeats, the open reading frame, as well as the target insertion sequence. Furthermore, the copy number of Hctc1 is comparable with the Tc1 copy number in low copy strains of C. elegans. The sequence of Hctc1 is highly variable in H. contortus due to deletions, insertions and point mutations, with at least five distinct length variants of Hctc1. Most of the Hctc1 variation was within rather than between H. contortus populations. The high level of sequence variation is probably due to variation generally found for members of the Tc1-family, as well as a high background level of genetic variation of H. contortus.

Hoekstra, R., M. Otsen, et al. (2000). "Transposon associated markers for the parasitic nematode Haemonchus contortus." Molecular and Biochemical Parasitology **105**(1): 127.

http://www.sciencedirect.com/science/article/B6T29-3XYG4H5-D/2/47e07b0b204477663ff88c7fcff7014d

We have previously characterized a Tc1-like transposable element Hctc1, from the parasitic nematode Haemonchus contortus. Here we describe the genetic variation of Hctc1 insertion sites in H. contortus populations differing in geographical origin, resistance to chemotherapeutics and level of inbreeding. Clear differences between populations were observed on Southern blots with a Hctc1-specific probe. Sequencing the 5'- or 3'-flanks of individual Hctc1 integration sites allowed the design of PCR reactions between a Hctc1-specific primer and the flanking regions. This revealed a considerable variation of integration sites of Hctc1 both within and between populations, although several integrations were shared by populations of different geographical

origin. For four of the eight markers allele frequencies were shifted during selection for resistance to chemotherapeutics and/or inbreeding. For two positions both the 5' and 3' regions flanking Hctc1 were isolated and PCR showed that for these two positions the variation of transposon associated markers between populations were indeed caused by variation in integration of Hctc1. For these two positions co-dominant markers were developed. These results indicate that Hctc1 insertions may serve as genetic markers for H. contortus.

Hoekstra, R., A. Visser, et al. (1997). "Characterization of an acetylcholine receptor gene of haemonchus contortus in relation to levamisole resistance." Molecular and Biochemical Parasitology **84**(2): 179.

http://www.sciencedirect.com/science/article/B6T29-3S6D230-3/2/2b074169fe9e621c39a05057d57b1f86

The anthelmintic drug levamisole is thought to bind to nicotinic acetylcholine receptors of nematodes. It is possible that resistance to this drug is associated with either a change in binding characteristics or a reduction in the number of nicotinic acetylcholine receptors. Therefore, the molecular mechanism of levamisole resistance in the parasitic nematode Haemonchus contortus was studied by isolating and characterising cDNA clones encoding a putative ligand binding nicotinic acetylcholine receptor subunit, HCA1, of two susceptible and one levamisole resistant population. Hca1 is related to unc-38, a nicotinic acetylcholine receptor subunit gene associated with levamisole resistance in Caenorhabditis elegans. Although extensive sequence analyses of hca1 sequences revealed poly-morphism at amino acid level, no association with levamisole resistance could be detected. Restriction fragment length polymorphism analyses confirmed that, although polymorphism was detected, no selection of a specific allele of hca1 has taken place during selection for levamisole resistance in various levamisole resistant populations.

Hong, S.-J., J.-Y. Lee, et al. (2001). "Molecular cloning and characterization of a mu-class glutathione S-transferase from Clonorchis sinensis." Molecular and Biochemical Parasitology **115**(1): 69.

http://www.sciencedirect.com/science/article/B6T29-433NRFC-7/2/1b104eb3e917867dde6cdf1ee6fb2c30

In biliary passages, Clonorchis sinensis causes epithelial hyperplasia and is assumed to promote carcinogenesis. Glutathione S-transferase (GST) is an antioxidant enzyme involved in phase II defense in trematodes. A clone (pcsGSTM1) encoding a GST was identified by screening a C. sinensis cDNA library with a PCR-synthesized cDNA probe. The predicted amino acid sequence encoded by pcsGSTM1 cDNA had a high degree of sequence identity and folding topology similar to the mu-class GSTs. The estimated molecular mass of the protein, 26 kDa, was consistent with an expression by pcsGSTM1 cDNA. The bacterially expressed recombinant csGSTM1 protein possessed an enzymatic GST activity and conjugated GSH to reactive carbonyls of lipid peroxidation. The recombinant csGSTM1 protein did not share antigenic epitope(s) with GSTs of Fasciola hepatica, Paragonimus westermaniand Schistosoma japonicum. The csGSTM1 was identified to a mu-class GST in C. sinensis.

Hope, M., A. MacLeod, et al. (1999). "Analysis of ploidy (in megabase chromosomes) in Trypanosoma brucei after genetic exchange." Molecular and Biochemical Parasitology **104**(1): 1.

http://www.sciencedirect.com/science/article/B6T29-3XT0BP8-

1/2/a0bf75560f2251154b7e9d31396a68f5

The megabase chromosomes of Trypanosoma brucei are normally diploid, but the extent to which this ploidy is maintained when parasites undergo genetic exchange is not known. To investigate this issue, a panel of 30 recombinant clones resulting from the co-transmission through tsetse flies of three different parental T. brucei lines in all pair-wise combinations (STIB 247, STIB 386 and TREU 927/4) were examined. These clones are products of 28 different mating events; four of them result from self-fertilisation and the others are F1 hybrids. DNA contents of the three parental lines were determined by flow cytometry and shown to differ only slightly with DNA content increasing in the order 927/4<247<386. Flow cytometry of the recombinant clones indicated DNA contents were similar to the parents in 28 clones and raised approximately 1.5 times the parental values in only two. The two F1 hybrid progeny with raised DNA contents were shown by marker analysis to be trisomic for seven independent loci indicating that they were probably triploid whereas progeny with DNA contents similar to parental values inherited a single allele from each parent for four independent loci indicating that they were diploid.

Joshua, G. W. P. and C. Y. Hsieh (1995). "Stage-specifically expressed genes of Angiostrongylus cantonensis: identification by differential display." Molecular and Biochemical Parasitology **71**(2): 285.

http://www.sciencedirect.com/science/article/B6T29-40078MY-M/2/35a1218f51488569b335eaaef3644344

Kaneko, O., T. Tsuboi, et al. (2001). "The high molecular mass rhoptry protein, RhopH1, is encoded by members of the clag multigene family in Plasmodium falciparum and Plasmodium yoelii." Molecular and Biochemical Parasitology **118**(2): 223.

http://www.sciencedirect.com/science/article/B6T29-4465W14-4/2/25beab1a27c9ad5110b72f741bd68df8

Malarial merozoite rhoptries contain a high molecular mass protein complex called RhopH. RhopH is composed of three polypeptides, RhopH1, RhopH2, and RhopH3, encoded by distinct genes. Using monoclonal antibody-purified protein complex from both Plasmodium falciparum and Plasmodium yoelii, peptides were obtained by digestion of RhopH1 and their sequence determined either by mass spectrometry or Edman degradation. In both species the genes encoding RhopH1 were identified as members of the cytoadherence linked asexual gene (clag) family. In P. falciparum the family members on chromosome 3 were identified as encoding RhopH1. In P. yoelii two related genes were identified and sequenced. One of the genes, pyrhoph1a, was positively identified as encoding RhopH1 by the peptide analysis and the other gene, pyrhoph1a-p, was at least transcribed. Genes in the clag family present in both parasite species have a number of conserved features. The size and location of the P. yoelii protein complex in the rhoptries was confirmed. The first clag gene identified on chromosome 9 was implicated in cytoadherence, the binding of infected erythrocytes to host endothelial cells; this study shows that other members of the family encode merozoite rhoptry proteins, proteins that may be involved in merozoite-erythrocyte interactions. We propose that the family should be renamed as rhoph1/clag.

Kapp, K., J. Knobloch, et al. (2004). "The Schistosoma mansoni Src kinase TK3 is expressed in the

gonads and likely involved in cytoskeletal organization." Molecular and Biochemical Parasitology **138**(2): 171.

http://www.sciencedirect.com/science/article/B6T29-4D98DD1-2/2/61e949c6482a6d3e5c237c6d3da8f5b1

Cytoplasmic protein tyrosine kinases of the Src family play a pivotal role in the regulation of cellular processes including proliferation and differentiation. Among other functions, Src kinases are involved in regulating the cell architecture. In an approach to identify protein tyrosine kinases from the medically important parasite Schistosoma mansoni, we isolated the TK3 gene by degenerate primer PCR and cDNA library screening. Sequencing of the complete cDNA and data-base analyses indicated that TK3 is a Src family kinase. Its predicted size of 71 kDa was confirmed by Western blot analysis. Southern blot analysis showed that TK3 is a single-copy gene, and Northern blot and RT-PCR experiments indicated its expression in both sexes and throughout development. Localization studies by in situ hybridization and immunolocalization revealed that TK3 is predominantly expressed in the reproductive organs such as the testes of the male and the ovary as well as the vitellarium of the female. Its enzymatic activity was confirmed by functional analyses. In transient transfection experiments with HEK293 cells, TK3 phosphorylated the well-known Src-kinase substrate p130 Cas, an intracellular scaffolding protein. Yeast two-hybrid screenings in a heterologous invertebrate system identified dAbi, vinculin and tubulin as binding partners, representing molecules that fulfill functions in the cell architecture of many organisms. These findings suggest that TK3 may play a role in signal transduction pathways organizing the cytoskeleton in the gonads of schistosomes.

Katzer, F., S. McKellar, et al. (1998). "Phylogenetic analysis of Theileria and Babesia equi in relation to the establishment of parasite populations within novel host species and the development of diagnostic tests." Molecular and Biochemical Parasitology **95**(1): 33.

http://www.sciencedirect.com/science/article/B6T29-3TNYXRX-4/2/8ab6deed7f83df0ab3ae1805c33481f9

The divergence of parasites is important for maintenance within an established host and spread to novel host species. In this paper we have carried out phylogenetic analyses of Theileria parasites isolated from different host species. This was performed with small subunit ribosomal RNA sequences available in the data bases and a novel sequence amplified from Theileria lestoquardi DNA. Similar phylogenetic studies were carried out with sequences representing the major merozoite/piroplasm surface antigen (mMPSA) from the data base, and novel sequences representing 2 mMPSA alleles from T. lestoquardi, a full length sequence of a Theileria taurotragi mMPSA gene and partial sequences of two new allelic variants of the Babesia equi mMPSA gene homologue. The analysis indicated that the pathogenic sheep parasite T. lestoquardi has most probably evolved from a common ancestor of T. annulata. Interestingly, the level of mMPSA sequence diversity found for T. lestoquardi was surprisingly low, while diversity between the B. equi sequences was higher than that found within any of the classical Theileria species. The possible implications of these results for the establishment of Theileria parasites within novel species are discussed. Extensive cross-reactivity of a range of antisera was found when tested against recombinant mMPSA polypeptides from different Theileria (including B. equi) species. The cross-reactivity between mMPSA polypeptides and sequence diversity are relevant for the development of species specific diagnostic tests.

Kedzierski, L., C. G. Black, et al. (2000). "Characterisation of the merozoite surface protein 4/5 gene of Plasmodium berghei and Plasmodium yoelii." Molecular and Biochemical Parasitology **105**(1): 137.

http://www.sciencedirect.com/science/article/B6T29-3XYG4H5-F/2/84ae053949eb1b3e6832a1be1511a7b3

The genes encoding merozoite surface protein 4/5 (MSP4/5) from Plasmodium berghei and Plasmodium yoelii have been cloned and completely sequenced. Comparisons of the predicted protein sequences with those of Plasmodium chabaudi MSP4/5 and Plasmodium falciparum MSP4 and MSP5 show general structural similarities. All predicted proteins contain hydrophobic signal sequences, potential GPI attachment sequences and a single epidermal growth factor (EGF)-like domain at the C-terminus. The amino acid sequence of the EGF-like motif is highly conserved in rodent malaria species and also shows a considerable degree of similarity with the EGF-like domains found in the P. falciparum proteins. Both the P. yoelii and P. berghei genes show evidence of both spliced and unspliced mRNA at steady state. This phenomenon is similar to that seen for the P. chabaudi MSP4/5 gene, and is believed to be involved in regulation of protein expression. We describe here the construction of clones expressing full length recombinant protein. Antibodies directed against recombinant MSP4/5 proteins recognise a single polypeptide on parasite material and show crossreactivity between MSP4/5 from different murine malaria species, but do not crossreact with either MSP4 or MSP5 from P. falciparum. The various antisera show reactivity against reduction sensitive epitopes as well as reduction insensitive epitopes.

Kyes, S., Z. Christodoulou, et al. (2002). "Stage-specific merozoite surface protein 2 antisense transcripts in Plasmodium falciparum." Molecular and Biochemical Parasitology **123**(1): 79.

http://www.sciencedirect.com/science/article/B6T29-46955JJ-1/2/26ee563a4e1b6c70c56f794b52501ffa

Kyes, S., H. Taylor, et al. (1997). "Genomic representation of var gene sequences in Plasmodium falciparum field isolates from different geographic regions." Molecular and Biochemical Parasitology **87**(2): 235.

http://www.sciencedirect.com/science/article/B6T29-3V92333-F/2/ba5fb3987d4e2252108e6ab3b7faf6c0

Lin, D. T., N. D. Goldman, et al. (1996). "Stage-specific expression of a Plasmodium falciparum protein related to the eukaryotic mitogen-activated protein kinases." Molecular and Biochemical Parasitology **78**(1-2): 67.

http://www.sciencedirect.com/science/article/B6T29-4287HY4-1J/2/d91963b583bc7c62fc7bc711b302b170

We have identified a putative protein kinase gene from both Plasmodium falciparum cDNA and genomic DNA libraries. The nucleotide sequence contains an open-reading frame of 2646 bp, which codes for a predicted protein of 882 amino acid residues. Comparison of the predicted amino acid sequence with those in GenBank suggests that this gene codes for a protein similiar to the mitogen-activated protein (MAP) kinase of other organisms. This MAP kinase-related protein, named PfMRP, contains the TDY dual phosphorylation site upstream of the highly conserved VATRWYRAPE sequence in subdomain VIII. PfMRP contains an unusually large and highly charged domain within its carboxyl-terminal segment, which includes two repetitive sequences of either a tetrapeptide or octapeptide motif. PfMRP gene is located on chromosome

14. Northern blot analysis of total RNA reveals the presence of a single mRNA transcript ~4.2 kb in length, which is predominantly expressed in gametocytes and gametes/zygotes.

Lin, Y., T. L. Lin, et al. (2002). "Variation in primary sequence and tandem repeat copy number among i-antigens of Ichthyophthirius multifiliis." Molecular and Biochemical Parasitology **120**(1): 93.

http://www.sciencedirect.com/science/article/B6T29-44KWT4D-1/2/a5ce50eb08167dff86d034c153e9a7a7

The immobilization antigens (i-antigens) of Ichthyophthirius multifiliis are potential vaccine candidates for the prevention of 'white spot' disease in freshwater fish. These antigens vary with respect to antigenicity and molecular mass, and at least five i-antigen serotypes have been identified among parasite isolates thus far. In previous studies, the gene and corresponding cDNA encoding a ~48 kDa i-antigen from parasite isolate G1 (serotype A), had been cloned and sequenced. We now report on the isolation of two new genes, designated IAG52A[G5] and IAG52B[G5], encoding ~52/55 kDa i-antigens from a parasite isolate representing a different serotype, namely, D. Based on their deduced sequences, the ~52/55 kDa gene products have the same structural features as the 48 kDa protein including hydrophobic N- and C-termini, periodic cysteine residues with the potential for metal binding, and tandemly repetitive amino acid sequence domains that span their length. Nevertheless, the products of these genes vary in their tandem repeat copy number, and share only ~50% homology overall. When expressed in heterologous systems, the products of the newly described genes react strongly with monospecific polyclonal antisera against the i-antigens of serotype D and are clearly i-antigens. It would nevertheless appear that mRNA transcripts from the two genes are present at widely different levels within parasites themselves. Analysis at the protein level using 2-D SDS-PAGE would further suggest that multiple i-antigens are expressed within the same serotype at any given time.

Ling, I. T., O. Kaneko, et al. (2003). "Characterisation of the rhoph2 gene of Plasmodium falciparum and Plasmodium yoelii." Molecular and Biochemical Parasitology **127**(1): 47.

http://www.sciencedirect.com/science/article/B6T29-47GHDVR-1/2/9ad9fdd5845cedd774103a7ba28ea4e3

The high molecular mass protein complex (RhopH) in the rhoptries of the malaria parasite consists of three distinct polypeptides with estimated sizes in Plasmodium falciparum of 155 kDa (PfRhopH1), 140 kDa (PfRhopH2) and 110 kDa (PfRhopH3). Using a number of reagents, including a new mAb 4E10 that is specific for the PfRhopH complex, it was shown that the RhopH complex is synthesised during schizogony and transferred intact to the ring stage in newly invaded erythrocytes. The genes encoding RhopH1 and RhopH3 have already been identified and characterised in both P. falciparum and Plasmodium yoelii. In this report, we describe the identification of the gene for RhopH2 in both these parasite species. Peptide sequences were obtained from purified RhopH2 proteins and used to generate oligonucleotide primers and search malaria sequence databases. In a parallel approach, mAb 4E10 was used to identify a clone coding for RhopH2 from a P. falciparum cDNA library. The sequences of both P. falciparum and P. yoelii genes for RhopH2 were completed and compared. They both contain nine introns and there is a high degree of similarity between the deduced amino acid sequences of the two proteins. The P. falciparum gene is a single copy gene located on chromosome 9, and is transcribed in schizonts.

Loukas, A., P. Hunt, et al. (1999). "Cloning and expression of an aquaporin-like gene from a parasitic nematode." Molecular and Biochemical Parasitology **99**(2): 287.

http://www.sciencedirect.com/science/article/B6T29-3W7XCB3-G/2/a73fe0b8c559f68808753b05916ffb17

Loukas, A., P. M. Selzer, et al. (1998). "Characterisation of Tc-cpl-1, a cathepsin L-like cysteine protease from Toxocara canis infective larvae." Molecular and Biochemical Parasitology **92**(2): 275.

http://www.sciencedirect.com/science/article/B6T29-3SY3HCY-S/2/78281b97f554cbe6d4f30218b91b8a04

Cysteine proteases play vital biological roles in both intracellular and extracellular environments. A cysteine protease migrating at 30 kDa was identified in somatic extracts of Toxocara canis larvae (TEX), by its binding to the biotinylated inhibitor Phe-Ala-CH2F. TEX proteases readily cleaved the cathepsin L- and B-specific peptide susbstrate Z-Phe-Arg-AMC and to a lesser extent, the cathepsin B-specific peptide Z-Arg-Arg-AMC. Excretory/secretory (TES) products of T. canis larvae did not cleave either substrate. Partial sequence encoding the 5' end of a cysteine protease cDNA from infective T. canis larvae was then obtained from an expressed sequence tag (EST) project. The entire cDNA (termed Tc-cpl-1) was subsequently sequenced and found to encode a preproenzyme similar to cathepsin L-like proteases (identities between 36 and 69%), the closest homologues being two predicted proteins from Caenorhabditis elegans cosmids, a cathepsin L-like enzyme from Brugia pahangi and a range of parasite and plant papain-like proteases. Sequence alignment with homologues of known secondary structure indicated several charged residues in the S1 and S2 subsites involved in determining substrate specificity. Some of these are shared with human cathepsin B, including Glu 205 (papain numbering), known to permit cleavage of Arg-Arg peptide bonds. The recombinant protease (rTc-CPL-1) was expressed in bacteria for immunisation of mice and the subsequent antiserum shown to specifically react with the 30 kDa native protease in TEX. Sera from mice infected with the parasite also contained antibodies to rTc-CPL-1 as did sera from nine patients with proven toxocariasis; control sera did not. Larger scale studies are underway to investigate the efficacy of rTc-CPL-1 as a diagnostic antigen for human toxocariasis, the current test for which relies on whole excretory/secretory antigens of cultured parasites.

Lovett, J. L., D. K. Howe, et al. (2000). "Molecular characterization of a thrombospondin-related anonymous protein homologue in Neospora caninum." Molecular and Biochemical Parasitology **107**(1): 33.

http://www.sciencedirect.com/science/article/B6T29-3YS98DW-2/2/0538ef5a34be2b58099bb9e26255cb17

Thrombospondin-related anonymous protein (TRAP) family members participate in attachment and invasion of host cells by apicomplexan parasites. A TRAP homologue in Neospora caninum strain Nc-1 (NcMIC2) was cloned, sequenced and found to be 61% identical (75% similar) at the amino acid level to Toxoplasma gondii MIC2 (TgMIC2). Similar to TgMIC2, the predicted amino acid sequence of NcMIC2 contains one integrin-like domain (I or A domain), five thrombospondin (TSP) repeats, a putative transmembrane spanning region and intracellular C-terminus, and was localized to micronemes by cryo-immunoelectron microscopy. The secretion of NcMIC2 was temperature dependent and was induced at or above 25[deg]C. The secreted form of NcMIC2 released into the medium was found to be proteolytically processed such that it lacked the C-terminal domain. Secretion of NcMIC2 was regulated by calcium, since several agents which

raise intracellular calcium levels were shown to promote NcMIC2 secretion and chelation of [Ca2+]i abrogated release. As a member of the growing family of apicomplexan TRAP proteins, NcMIC2 may play an important role in attachment and invasion by N. caninum into host cells.

MacLeod, A., C. M. R. Turner, et al. (1999). "A high level of mixed Trypanosoma brucei infections in tsetse flies detected by three hypervariable minisatellites." Molecular and Biochemical Parasitology **102**(2): 237.

http://www.sciencedirect.com/science/article/B6T29-3X70V6T-3/2/bf6a663bd1c732235fb943a901ab396a

The issue of whether genetic exchange occurs at a significant frequency in natural populations of Trypanosoma brucei is controversial and one of the arguments against a high frequency has been the apparent lack of host infections with mixtures of trypanosome genotypes. Three minisatellite markers (MS42, CRAM, 292) within the coding regions of three genes have been identified and PCR based methods developed for detecting variation at these loci using crude lysates of infected blood as templates. Initial PCR analysis, using primers flanking the repeats, of DNA from two cloned stocks of the parasite has shown that two DNA fragments of different size were amplified from each stock. Analysis of the inheritance of these fragments into the F1 progeny of crosses demonstrated that the different size fragments were alleles that segregated in a Mendelian manner. The alleles at each of the three loci segregated independently consistent with their localisation on three different chromosomes. Analysis of a series of cloned isolates from tsetse flies showed that these loci were highly variable giving heterozygosities of 94% and the identification of 12 distinct alleles in a sample of 17 cloned isolates. In order to determine whether isolates are heterogeneous in terms of trypanosome genotype, the allelic variation at these three loci was examined in uncloned samples from tsetse flies isolated in Kiboko, Kenya and Lugala, Uganda. A significant proportion of the isolates (36% in Lugala and 47% in Kiboko) contained more than two alleles at one or more of the loci thus demonstrating that a high proportion of tsetse flies were infected with more than one genotype of trypanosomes. This was established, unequivocally, for two isolates by generating a series of cloned trypanosome lines from each and determining the genotype of each clone; one isolate (927) contained seven different genotypes with a high proportion of the possible combinations of alleles at each locus. These results indicate the possibility of frequent genetic exchange in the field, they imply that a significant proportion of mammalian hosts must contain mixtures of different trypanosome genotypes and they demonstrate the advantages of using minisatellite markers for the analysis of the population structure of T. brucei.

Mann, V. H., T. Huang, et al. (1994). "Sequence variation in the circumsporozoite protein gene of Plasmodium vivax appears to be regionally biased." Molecular and Biochemical Parasitology **68**(1): 45.

http://www.sciencedirect.com/science/article/B6T29-476TY3B-6/2/25f5f05fe0f0f445f323489d506cde52

We have sequenced the circumsporozoite protein gene from 16 isolates from China, the Philippines, Papua New Guinea and the Solomon Islands. We found very limited polymorphisms in the non-repetitive regions of the circumsporozoite gene from these isolates. All samples from China contained a 36-base insert 3' to the repeats previously seen only in a North Korean isolate. Limited variation was found in the repeat regions, which allowed these and previously sequenced isolates to be classified into groups based on repeat structure. These groupings also correlate with the geographical origin of the isolates.

Martin, S. A. M., F. J. Thompson, et al. (1995). "The construction of spliced leader cDNA libraries from the filarial nematode Brugia pahangi." Molecular and Biochemical Parasitology **70**(1-2): 241.

http://www.sciencedirect.com/science/article/B6T29-4007985-12/2/ed21685061287f1b6465fa51c7c9e4ab

Mathieu-Daude, F., J. Stevens, et al. (1995). "Genetic diversity and population structure of Trypanosoma brucei: clonality versus sexuality." Molecular and Biochemical Parasitology **72**(1-2): 89.

http://www.sciencedirect.com/science/article/B6T29-4007FV1-9/2/77f896d911106ed9ceb52068dae0aa85

Genomic fingerprinting by arbitrarily primed PCR was used to analyze the genetic variability among 59 Trypanosoma brucei stocks representing the three T. brucei subspecies isolated from various hosts and different countries in Africa. 14 oligonucleotide primers revealed 355 polymorphic binary characters which were used for phenetic and phylogenetic analysis and to perform recombination tests exploring the linkage disequilibrium in the sample. These was good concordance between arbitrarily primed PCR polymorphisms and isoenzyme data previously collected for many of the same strains [1]. However, the arbitrarily primed PCR typing was more discerning than multilocus enzyme electrophoresis typing. Phenetic and phylogenetic analysis using arbitrarily primed PCR markers did not confirm T. brucei brucei and T. brucei rhodesiense as separate subspecies, but T. brucei gambiense group I was monophyletic, confirming this group as suitable for the subspecies status. With this exception, there were no clear lineages among the sample, other than clustering of East African stocks and clustering of West African stocks. Some features of the phylogenetic analysis suggested that the population structure was not strictly clonal though recombination tests showed linkage disequilibrium, even in the absence of repeated genotypes. While genotypes appear stable enough for tracking in applied studies, sexuality will impact at the evolutionary time scale, and may be more frequent under some ecological conditions. The arbitrarily primed PCR approach should be an effective and simple approach to follow epidemics and to quantify the role of sexuality in T. brucei populations.

Milner, J. D. and S. L. Hajduk (1999). "Expression and localization of serum resistance associated protein in Trypanosoma brucei rhodesiense." Molecular and Biochemical Parasitology **104**(2): 271.

http://www.sciencedirect.com/science/article/B6T29-3XWJR7T-C/2/e30fb82ade83f45a390ca59f684a590c

The trypanosome lytic factor (TLF) is a primate specific innate defense mechanism that restricts the host range of African trypanosomes. Trypanosoma brucei rhodesiense, the causative agent of the acute form of human sleeping sickness, is resistant to the cytolytic action of TLF. By differential display PCR we have identified a gene in T. b. rhodesiense that is preferentially expressed in cell lines resistant to TLF. The protein sequence predicted from the gene shows homology to the trypanosome variable surface glycoprotein (VSG) gene family and in particular, to the previously reported human serum resistance associated gene (SRA). The amount of SRA mRNA is over 1000-fold higher in TLF resistant cells relative to TLF sensitive trypanosomes. Treatment of TLF sensitive trypanosomes with increasing concentrations of TLF in mice results in the selection of parasites that have reverted back to the TLF resistant phenotype. These trypanosomes also showed high levels of SRA mRNA. Antibodies against recombinant SRA react with a 59 kDa protein on western blots of total cell protein from TLF resistant trypanosomes but

not TLF sensitive cells. Indirect immunofluorescence revealed that SRA is a cell surface protein present only in TLF resistant trypanosomes. These results suggest that TLF resistance in human sleeping sickness trypanosomes is a consequence of the selective, high level expression of a cell surface molecule(s). In addition, these studies support the role of TLF as a major factor in human serum mediated killing of susceptible trypanosomes.

Molitor, I. M., S. Knobel, et al. (2004). "Translation initiation factor eIF-5A from Plasmodium falciparum." Molecular and Biochemical Parasitology **137**(1): 65.

http://www.sciencedirect.com/science/article/B6T29-4CF73K0-1/2/590376726fba900b9cd81901666c7180

Eukaryotic translation initiation factor (eIF-5A) is a highly conserved and essential protein that contains the unique amino acid hypusine. The first step in the post-translational biosynthesis of hypusine, the transfer of an aminobutyl moiety from the polyamine substrate spermidine to the [epsiv]-amino group of a specific lysine residue in the eIF-5A precursor, is catalyzed by the enzyme deoxyhypusine synthase. A cDNA encoding a protein homologous to eIF-5A was isolated by plaque hybridization from a cDNA library of Plasmodium falciparum. The cloned cDNA contains an open reading frame encoding a protein of 161 amino acids, which shares a high sequence identity with other eukaryotic eIF-5A sequences. A phylogenetic tree constructed with eIF-5A from P. falciparum and 16 other eIF-5A sequences of eukaryotic and archaeal origin reveals that plasmodial eIF-5A together with other apicomplexan eIF-5A show a higher degree of homology to plant proteins than to animal and fungal sequences. The plasmodial eIF-5A gene was expressed as a six-histidine tagged fusion protein in Escherichia coli. Radioactive incorporation studies with [1,8-3H] spermidine indicated that this protein can serve as a substrate for human deoxyhypusine synthase. Results of quantitative real-time PCR studies with synchronized erythrocytic stages of P. falciparum revealed no significant induction or downregulation but only some variation in the expression level of plasmodial eIF-5A in ring, trophozoite and schizont stage.

Moormann, A. M., P. A. Hossler, et al. (1999). "Deferoxamine effects on Plasmodium falciparum gene expression." Molecular and Biochemical Parasitology **98**(2): 279.

http://www.sciencedirect.com/science/article/B6T29-3VS7MM5-C/2/201dfe21c598e143723762bf34e25e04

Mowatt, M. R., A. Aggarwal, et al. (1991). "Carboxy-terminal sequence conservation among variant-specific surface proteins of Giardia lamblia." Molecular and Biochemical Parasitology **49**(2): 215.

http://www.sciencedirect.com/science/article/B6T29-476W89M-4/2/dbd7e0b76d145052a3c2c13321d98a2f

Antigenic variation in the parasitic protozoan Giardia lamblia was studied by characterizing the expression and genomic organization of a variant-specific surface protein (VSP) gene. Transcripts from this gene, vsp1267, were abundant in the cloned variant WB/1267, but undetectable in the parental clone from which WB/1267 was derived or in variant progeny of WB/1267. Two identical copies of vsp1267 exist in the WB/1267 genome, separated by 3 kb and arranged as convergent transcription units. Primer extension sequencing and S1 nuclease protection analysis suggested that the 5' untranslated region (UTR) of VSP1267 mRNA consists

of a single nucleotide (nt). Primer extension sequencing mapped the site of VSP1267 transcript polyadenlyation 25 nt beyond the termination codon. vsp1267 contained no introns and predicted a cysteine-rich polypeptide with features common to other VSPs. Comparison of vsp1267 with another VSP gene sequence revealed striking conservation, both at the nucleotide and amino acid levels, at the 3' ends of the genes. An oligonucleotide derived from this region detected size-variant VSP transcripts in 4 of 5 G. lamblia clones analyzed, suggesting the general utility of this probe in studying VSP genes and their expression.

Nakaar, V., D. Bermudes, et al. (1998). "Upstream elements required for expression of nucleoside triphosphate hydrolase genes of Toxoplasma gondii." Molecular and Biochemical Parasitology **92**(2): 229.

http://www.sciencedirect.com/science/article/B6T29-3SY3HCY-M/2/1141bf6fbb8b38ca438cce58c3f0a85c

Nucleoside triphosphate hydrolase is an abundant protein secreted by the obligate protozoan parasite Toxoplasma gondii. The protein has apyrase activity, degrading ATP to the di- and mono-phosphate forms. Because T. gondii is incapable of de novo synthesis of purines, it is postulated that NTPase may be used by the parasite to salvage purines from the host cell for survival and replication. To elucidate the molecular mechanisms of NTP gene expression, we isolated from the virulent RH strain of T. gondii the putative promoter region of three tandemly repeated NTP genes (NTP1, 2, 3). Using deletion constructs linked to the chloramphenicol acetyl transferase (CAT) reporter gene, we defined an active promoter within the first 220 bp. Sequence analysis of this region reveals the lack of a TATA box, but the promoter region is associated with a sequence which resembles an initiator element (Inr) in the NTP1 and NTP3 genes. This sequence which is similar to other Inrs known to regulate the expression of a wide variety of RNA polymerase II genes, is required for NTP expression. The NTP3 promoter contains sufficient information for developmentally regulated expression of CAT activity when the actively replicating stage tachyzoite differentiates into the dormant bradyzoite form.

Ortner, S., M. Binder, et al. (1997). "Molecular and biochemical characterization of phosphoglucomutases from Entamoeba histolytica and Entamoeba dispar." Molecular and Biochemical Parasitology **90**(1): 121.

http://www.sciencedirect.com/science/article/B6T29-3RWWJ1V-C/2/43f283496701309e14430788bc04ffa4

Entamoeba histolytica and Entamoeba dispar have only recently been defined as two separate species. E. histolytica, the pathogenic species, is the microorganism causing invasive intestinal amoebiasis and/or liver abscess, while the morphologically similar E. dispar is nonpathogenic and noninvasive. The gold standard for the distinction of the two species has been the isoenzyme electrophoresis of phosphoglucomutases (EC 5.4.2.2) and hexokinases (EC 2.7.1.1), but there had also been a controversy about the possibility of a conversion of isoenzyme patterns. In this study, we cloned the phosphoglucomutase (PGM) cDNAs from the pathogenic and the nonpathogenic species. The deduced amino acid sequences were only 2.4% different. The cDNAs were expressed in Escherichia coli under the control of a T7 RNA polymerase promoter. The recombinant polypeptides displayed strong phosphoglucomutase activity, each of the recombinant enzymes comigrated with its natural counterpart from E. histolytica and E. dispar in the starch gel electrophoresis. Our results give a biochemical interpretation of the PGM isoenzyme pattern and support the clear distinction between the two species.

Otsen, M., M. E. Plas, et al. (2000). "Microsatellite diversity of isolates of the parasitic nematode Haemonchus contortus." Molecular and Biochemical Parasitology **110**(1): 69.

http://www.sciencedirect.com/science/article/B6T29-4164TTH-7/2/28652f557b908b4a21419451c660424a

The alarming development of anthelmintic resistance in important gastrointestinal nematode parasites of man and live-stock is caused by selection for specific genotypes. In order to provide genetic tools to study the nematode populations and the consequences of anthelmintic treatment, we isolated and sequenced 59 microsatellites of the sheep and goat parasite Haemonchus contortus. These microsatellites consist typically of 2-10 tandems CA/GT repeats that are interrupted by sequences of 1-10 bp. A predominant cause of the imperfect structure of the microsatellites appeared mutations of G/C bp in the tandem repeat. About 44% of the microsatellites were associated with the HcREP1 direct repeat, and it was demonstrated that a generic HcREP1 primer could be used to amplify HcREP1-associated microsatellites. Thirty microsatellites could be typed by polymerase chain reaction (PCR) of which 27 were polymorphic. A number of these markers were used to detect genetic contamination of an experimental inbred population. The microsatellites may also contribute to the genetic mapping of drug resistance genes.

Pachebat, J. A., I. T. Ling, et al. (2001). "The 22 kDa component of the protein complex on the surface of Plasmodium falciparum merozoites is derived from a larger precursor, merozoite surface protein 7." Molecular and Biochemical Parasitology **117**(1): 83.

http://www.sciencedirect.com/science/article/B6T29-43X1FVP-8/2/e57d830ca0be51b96de6661618bd5070

The gene coding for merozoite surface protein 7 has been identified and sequenced in three lines of Plasmodium falciparum. The gene encodes a 351 amino acid polypeptide that is the precursor of a 22-kDa protein (MSP722) on the merozoite surface and non-covalently associated with merozoite surface protein 1 (MSP1) complex shed from the surface at erythrocyte invasion. A second 19-kDa component of the complex (MSP719) was shown to be derived from MSP722 and the complete primary structure of this polypeptide was confirmed by mass spectrometry. The protein sequence contains several predicted helical and two beta elements, but has no similarity with sequences outside the Plasmodium databases. Four sites of sequence variation were identified in MSP7, all within the MSP722 region. The MSP7 gene is expressed in mature schizonts, at the same time as other merozoite surface protein genes. It is proposed that MSP722 is the result of cleavage by a protease that may also cleave MSP1 and MSP6. A related gene was identified and cloned from the rodent malaria parasite, Plasmodium yoelii YM; at the amino acid level this sequence was 23% identical and 50% similar to that of P. falciparum MSP7.

Pandrea, I., D. Mittleider, et al. (2005). "Phylogenetic relationships of methionine aminopeptidase 2 among Encephalitozoon species and genotypes of microsporidia." Molecular and Biochemical Parasitology **140**(2): 141.

http://www.sciencedirect.com/science/article/B6T29-4FB3V7W-1/2/450f1ce04512a31c66c40d172cde56ca

This report describes the characterization and phylogenetic analysis of the deduced amino acid

sequences of methionine aminopeptidase 2 (MetAP-2) enzymes from microsporidian species and genotypes of the genus Encephalitozoon. Fragments of DNA encoding 318 to 335 amino acid residues of the MetAP-2 genes were isolated from genomic DNA prepared from cultured spores of Encephalitozoon hellem, Encephalitozoon intestinalis, and Encephalitozoon cuniculi genotypes I-III. Sequence comparisons of the deduced amino acid residues indicated that the microsporidian sequences are MetAP-2-like rather than MetAP-1-like. Alignments demonstrated that the new Encephalitozoon sequences included sequences and structures conserved in eukaryotic MetAP-2s, including the five conserved, active site residues, Asp, Asp, His, Glu, and His, considered to be critical for catalysis and for coordinating the cation (e.g., cobalt) co-factor, and included residues known to interact with the antibiotic, fumagillin. The primary structure of the Encephalitozoon MetAP-2s, however, showed some dissimilarity with human and yeast MetAP-2s, including the absence of the NH2-terminal polylysine tract. Phylogenetic comparison of these Encephalitozoon MetAP-2s with orthologues from related species and from other informative taxa confirmed that the MetAP-2s of these Encephalitozoon species and strains are closely related to each other and cluster with MetAP-2s.

Park, J. B., S. J. Son, et al. (2005). "Molecular and electrophysiological characterization of nucleotide-sensitive chloride current-inducing protein of Fasciola hepatica." Molecular and Biochemical Parasitology **140**(2): 197.

http://www.sciencedirect.com/science/article/B6T29-4FCG1NG-1/2/64a0d752f0ee0a987f0984b0fcb514cb

Nucleotide-sensitive chloride current regulating proteins (ICln's) of the chloride channels have been characterized from man and animals. An ICln of Fasciola hepatica (ICln-Fh) consisting of 231 amino acids revealed high similarities to both consensus domain of ICln's and two acidic residue-abundant patches in its C-terminus. Native ICln-Fh protein was confirmed present in F. hepatica soluble extract by immunoblotting. The recombinant ICln-Fh protein expressed in collagenase-defolliculated Xenopus oocytes induced fast rising and outward rectifying Cl- currents (ICln-Fh). The recombinant ICln-Fh protein, however, did not trigger cell swelling-induced Cl- currents (ICl-swell). The ICln-Fh currents were significantly reduced by substituting external Cl- with gluconic acid and by externally adding cAMP. Collectively, these results suggest that ICln-Fh protein is an inducer of Cl- currents in F. hepatica.

Parmley, S. F., S. Yang, et al. (1994). "Molecular characterization of a 65-kilodalton Toxoplasma gondii antigen expressed abundantly in the matrix of tissue cysts." Molecular and Biochemical Parasitology **66**(2): 283.

http://www.sciencedirect.com/science/article/B6T29-476F3GF-B/2/0226918225372d724b40ddc204a5fd93

We describe the cloning and characterization of a novel antigen expressed in the bradyzoite stage of Toxoplasma gondii. A cDNA library was constructed in bacteriophage [lambda]gt11 Sfi-Not using messenger RNA molecules isolated from cysts of the ME49 strain of T. gondii. The recombinant phage library was subjected to screening with polyclonal antibodies against bradyzoite antigens. This screening identified a recombinant antigen that was recognized strongly by polyclonal antibodies against bradyzoite antigens as well as by sera from mice chronically infected with T. gondii. The native antigen is a protein of 65 kDa that localized to the matrix of the cyst and the cyst wall surrounding the bradyzoites. The antigen was found to be expressed abundantly in cysts but could not be detected in tachyzoites or within the parasitophorous vacuole of tachyzoite infected host cells. Genomic and cDNA sequence of the gene revealed an open reading frame encoding 452 amino acids interrupted by 2 introns: a 503-bp intron located in the 5'

untranslated region preceding the protein coding sequence and a 110-bp intron located 95 bp downstream of the first ATG.

Peterson, G. C., A. E. Souza, et al. (1993). "Characterization of a Trypanosoma brucei nuclear gene encoding a protein homologous to a subunit of bovine NADH:ubiquinone oxidoreductase (complex I)." Molecular and Biochemical Parasitology **58**(1): 63.

http://www.sciencedirect.com/science/article/B6T29-476M0YJ-DH/2/d038d1f0f0359158cba6cbbff60efd88

A Trypanosoma brucei gene has been identified that encodes a protein predicted to be a component of the trypanosome homologue of mitochondrial NADH:ubiquinone oxidoreductase (complex I). High homology was found to a 20-kDa component of the iron-sulfur protein fraction of bovine mitochondrial NADH:ubiquinone oxidoreductase and the products of the ndhK locus of Paramecium tetraurelia mitochondria and the NQ06 locus of Paracoccus denitrificans. The homology extends to several other proteins predicted to function as part of electron transport systems, including the psbG/ndhK gene products of chloroplast and cyanobacterial genomes which are thought to be subunits of a NADH-plastoquinone oxidoreductase involved in chlororespiration. The T. brucei ndhK counterpart is nuclearly encoded. An extended amino terminus of the T. brucei ndhK with structural similarity to mitochondrial presequences indicates that its transfer into mitochondria is likely. Stumpy and slender bloodforms and procyclic forms all possess similar levels of ndhK transcripts despite previous reports of stage-regulated expression of complex I-like activity.

Prapunwattana, P., W. Sirawaraporn, et al. (1996). "Chemical synthesis of the Plasmodium falciparum dihydrofolate reductase-thymidylate synthase gene." Molecular and Biochemical Parasitology **83**(1): 93.

http://www.sciencedirect.com/science/article/B6T29-3W31576-9/2/1e641ff29c06d0051fe709e18d7e4bb1

Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a well-known target for pyrimethamine and cycloguanil. The low amounts of enzyme obtainable from parasites or the currently available heterologous expression systems have thus far hindered studies of this enzyme. The 1912-base pair P. falciparum DHFR-TS gene was designed based on E. coli codon preference with unique restriction sites evenly placed throughout the coding sequence. The gene was designed and synthesized as three separated domains: the DHFR domain, the junctional sequence, and the TS domain. Each of these domains contained numerous unique restriction sites to facilitate mutagenesis. The three domains were assembled into a complete DHFR-TS gene which contained 30 unique restriction sites in the coding sequence. The bifunctional DHFR-TS was expressed from the synthetic gene as soluble enzyme in E. coli about 10-fold more efficiently than from the wild-type sequence. The DHFR-TS from the syntheic gene had kinetic properties similar to those of the wild-type enzyme and represents a convenient source of protein for further study. The unique restriction sites in the coding sequence permits easy mutagenesis of the gene which should facilitate further understanding of the molecular basis of antifolate resistance in malaria.

Price, R., G. Robinson, et al. (1997). "Assessment of pfmdr 1 gene copy number by tandem competitive polymerase chain reaction." Molecular and Biochemical Parasitology **85**(2): 161.

http://www.sciencedirect.com/science/article/B6T29-453CBPG-3/2/9aacd3868b68539293646c52bd024bfb

The pfmdr 1 gene encodes a Plasmodium falciparum homologue of the human P-glycoprotein expressed on the surface of the parasite food vacuole. Variation in copy number and specific codon mutations of pfmdr 1 have been implicated in the development of parasite resistance to antimalarial drugs. We describe here the technique of Tandem-Competitive Polymerase Chain Reaction (TC-PCR), which allows accurate measurement of pfmdr 1 copy number in parasite DNA obtained directly from small quantities (100 [mu]l) of red blood cells. We reliably quantified pfmdr 1 in previously well characterised strains of Plasmodium falciparum with differing pfmdr 1 gene copy numbers using starting amounts of between 3000 and 40 000 gene copies. We then used TC-PCR to determine pfmdr 1 gene copy number in field specimens of venous blood taken from 10 patients with malaria contracted along the Thai--Burmese border. In this region of high grade parasite resistance to mefloquine greater than 70% of samples had a copy number greater than 1 of pfmdr 1 determined with a repeatability coefficient of 0.58.

Priest, J. W. and S. L. Hajduk (1994). "Developmental regulation of Trypanosoma brucei cytochrome c reductase during bloodstream to procyclic differentiation." Molecular and Biochemical Parasitology **65**(2): 291.

http://www.sciencedirect.com/science/article/B6T29-476RNG1-44/2/f0adae08148d94df5f337552f3b4e409

The bloodstream forms of the protozoan parasite Trypanosoma brucei lack spectrally detectable cytochromes and satisfy energy requirements mainly by glycolysis. When infected blood is ingested by the tse-tse fly vector, the bloodstream form cells differentiate to procyclic forms that have fully functional mitochondria. Procyclic cells have cyanide-sensitive, cytochrome-mediated electron transport and the full complement of TCA cycle enzymes. The developmental regulation of the cytochrome c reductase complex was examined at the RNA and protein levels. RNase T1 protection studies and Northern blot analyses demonstrated that bloodstream and procyclic form cells constitutively expressed the genes for two nuclear encoded cytochrome c reductase subunits, cytochrome c1 and subunit 4. Polyadenylated transcripts of both genes were present in bloodstream form cells at up to 20% of the procyclic cell levels. These levels were significantly up-regulated sometime after the onset of differentiation to the procyclic form. Despite the presence of subunit mRNAs in bloodstream form cells, subunit proteins were not detected until the cells had been allowed to differentiate in vitro for 6 h. Procyclic cell levels of subunit proteins and holocytochromes were reached by 48 h. Our results suggest that cytochrome c reductase is developmentally regulated at multiple levels, some involving post-transcriptional mechanisms.

Priest, J. W., J. P. Kwon, et al. (2000). "Cloning of the immunodominant 17-kDa antigen from Cryptosporidium parvum." Molecular and Biochemical Parasitology **106**(2): 261.

http://www.sciencedirect.com/science/article/B6T29-3YN92NP-6/2/f26006b1fcab2bfaafb2ae0d7c57a308

Infection with Cryptosporidium parvum causes a self-limiting diarrheal illness in immunocompetent humans and is associated with the development of a serum IgG antibody response dominated by the 27-kDa and 17-kDa parasite surface antigens. Antibodies against the 27-kDa and 17-kDa antigens may serve as useful markers for past infection in population-based studies of the risk factors associated with Cryptosporidium infection. A recombinant form of the 17-kDa antigen would be useful both in epidemiologic studies and in studies of the role of the

humoral response in immunity. We have partially purified and sequenced the immunodominant 17-kDa surface antigen from sporozoites, and we have cloned a 975 bp open reading frame from C. parvum that includes all of the 17-kDa antigen peptide sequences. We show immunologic identity between a recombinant form of the protein and the native 17-kDa antigen. We conclude that the carboxy-terminal fragment of the cloned protein is the authentic 17-kDa antigen.

Putaporntip, C., S. Jongwutiwes, et al. (2000). "Intragenic recombination in the 3' portion of the merozoite surface protein 1 gene of Plasmodium vivax." Molecular and Biochemical Parasitology **109**(2): 111.

http://www.sciencedirect.com/science/article/B6T29-4118BBC-3/2/ceb2788d743483f7c13987b4884bfc92

To date, little has been known about the extent of sequence variation in the C-terminal part of the Plasmodium vivax merozoite surface protein 1 (PvMSP1) which has been considered to be a potential vaccine candidate. Here, we examined the variation in the region encompassing interspecies conserved blocks (ICBs) 8 and 10 of PvMSP1 by DNA sequencing of 14 Thai isolates and three Brazilian isolates. Eighteen different alleles were detected. Three new sequence types had been identified in polymorphic region between ICB8 and CB9: one was possibly a result of intragenic recombination between the Belem and Salvador I alleles and the others displayed unique repeats. A striking variation was observed in a stretch of 38 codons in polymorphic block between conserved block CB9 and ICB10, resulting in eight different sequence types, probably generated by interallelic recombination at a single or multiple sites. There is no apparent linkage between these two polymorphic sites. On the other hand, a single or stretches of nucleotide substitutions are dimorphic like in Plasmodium falciparum MSP1 (PfMSP1) in the remaining parts, creating microheterogeneity of sequences. The C-terminal 19 kDa-encoding region was extremely conserved with a single dimorphic exchange at a known position. Thus, this study provides evidence of intragenic recombination occurring in the 3' portion of PvMSP1 and suggests that the 3' portion of PvMSP1 is more diverse than that in PfMSP1.

Qari, S. H., Y.-P. Shi, et al. (1998). "Predicted and observed alleles of Plasmodium falciparum merozoite surface protein-1 (MSP-1), a potential malaria vaccine antigen." Molecular and Biochemical Parasitology **92**(2): 241.

http://www.sciencedirect.com/science/article/B6T29-3SY3HCY-N/2/742796e1f1a8bb1ce4cab958f2f34861

The 19-kDa antigenic domain of Plasmodium falciparum merozoite surface protein (MSP)-1 is a potential malaria vaccine candidate. Based on the amino acid substitution, four known alleles, E-TSR (PNG-MAD20 type), E-KNG (Uganda-PA type), Q-KNG (Wellcome type), and Q-TSR (Indo type) of this domain have been identified. Using single or double crossover recombinational events, we predicted the existence of additional alleles of this antigen. The presence of the predicted alleles was determined in parasite isolates from western Kenya, by undertaking a cross-sectional and a longitudinal study. Of the ten predicted alleles, we have revealed the presence of three new alleles: E-KSG-L (Kenya-1 type); E-KSR-L (Kenya-2 type); and E-KNG-F (Kenya-3 type). The results of this study suggest that it may be possible to predict the complexity of the genetic makeup of natural parasite populations.

Qin, C., G. Jones, et al. (1991). "Identification of a common Plasmodium epitope (CPE) recognised by a

pan-specific inhibitory monoclonal antibody." Molecular and Biochemical Parasitology **49**(1): 73.

http://www.sciencedirect.com/science/article/B6T29-47GMFFS-10/2/e35c256f422f002d51b245cf76e51a82

A Plasmodium falciparum genomic expression library was screened with a monoclonal antibody produced from mice infected with Plasmodium yoelii. Eleven unique clones were isolated all of which contained the sequence NKND, IKND or KKND. This sequence was confirmed as the epitope of M26-32 by testing a series of overlapping peptides and the allowable substitutions determined by testing the binding of M26-32 to peptides containing all possible single amino acid replacements of NKND. Potential epitopes of M26-32 occur in many plasmodial proteins and this is consistent with the large number of proteins recognised in these parasites by Western blotting. Since this monoclonal antibody shows marked in vitro inhibition of P. falciparum growth, these data suggest that an anti-malarial vaccine may be produced by targeting such common plasmodial epitopes without necessarily identifying the corresponding antigens.

Que, X., S. G. Svard, et al. (1996). "Developmentally regulated transcripts and evidence of differential mRNA processing in Giardia lamblia." Molecular and Biochemical Parasitology **81**(1): 101.

http://www.sciencedirect.com/science/article/B6T29-3W25F2X-P/2/de7b20220acae58be4085328b5c20abe

Although encystation and excystation are crucial to transmission of Giardia lamblia, little is known about the regulation of these very distinct differentiation processes. Fingerprinting of giardial mRNA populations throughout the time course of differentiation demonstrated complex patterns in mRNA differential display. Certain transcripts appeared or increased, while others decreased or disappeared at specific times, in response to physiologic stimuli that mimic key stages in parasite descent through the host gastrointestinal tract. This approach has allowed the direct identification of critical stages in differentiation, as well as isolation of genes which may be crucial to the development of G. lamblia. One stage-specific single copy gene (ENC6) whose transcript is greatly upregulated during encystation was analyzed further. Partial sequence analysis revealed no correspondence with known genes. 3'-rapid amplification of cDNA ends (3'-RACE) analysis of ENC6transcripts at various times of encystation revealed two polyadenylation sites. The more proximal site, 10 nucleotides past the single classic AGTAAA sequence, was utilized only during encystation and its transcript increased ~ 16-fold during the first 24 h of encystation. In contrast, a slightly divergent polyadenylation site 288 nucleotides downstream from the open reading frame (ORF) was used during both vegetative growth and encystation, although its transcript was present at low levels. These studies are the first evidence of differential mRNA processing in G. lamblia and suggest a potential role of the 3'-untranslated region (3'-UTR) in modulating gene expression during differentiation of this primitive eukaryote.

Ranford-Cartwright, L. C., P. Balfe, et al. (1991). "Genetic hybrids of Plasmodium falciparum identified by amplification of genomic DNA from single oocysts." Molecular and Biochemical Parasitology **49**(2): 239.

http://www.sciencedirect.com/science/article/B6T29-476W89M-6/2/461a49cb541a6cbf5901bfb4c9263da5

Individual oocysts from Plasmodium falciparum-infected Anopheles gambiae and Anopheles stephensi mosquitoes have been examined by the PCR technique, after their removal from the midgut. The DNA obtained from these oocysts has been amplified using oligonucleotide primers

specific for part of the merozoite surface antigen MSA-1 gene. This technique distinguishes oocysts which are the products of self-fertilisation events from those which are the products of cross-fertilisation between different parasite clones.

Ranie, J., V. P. Kumar, et al. (1993). "Cloning of the triosephosphate isomerase gene of Plasmodium falciparum and expression in Escherichia coli." Molecular and Biochemical Parasitology **61**(2): 159.

http://www.sciencedirect.com/science/article/B6T29-476M13N-FT/2/0d80e961e30f3526a75887904218282e

A major supply of energy in the rapidly multiplying intraerythrocytic Plasmodium falciparum is from the glycolytic pathway. We have isolated the cDNA and genomic clones of the glycolytic enzyme, triosephosphate isomerase (TPI) by polymerase chain reaction (PCR). Degenerate oligonucleotides obtained by reverse translation of conserved polypeptide sequences derived from TPIs of other organisms, were used to prime PCR on P. falciparum DNA. The P. falciparum TPI gene is interrupted by a single intron which divides the coding region into two exons. The coding region encodes a protein of 248 amino acids which is of the same size as TPIs from other organisms and shares 42--45% homology with other known eukaryotic TPIs. On comparison with human TPI the catalytic domain was found to be highly conserved, while significant variations occurred at the other regions in the protein sequence. The P. falciparum TPI gene was cloned into the expression vector pTrc99A and hyperexpressed as an unfused protein in Escherichia coli. The 28-kDa protein was shown to be catalytically active.

Rao, K. V. N., M. Eswaran, et al. (2000). "The Wuchereria bancrofti orthologue of Brugia malayi SXP1 and the diagnosis of bancroftian filariasis." Molecular and Biochemical Parasitology **107**(1): 71.

http://www.sciencedirect.com/science/article/B6T29-3YS98DW-5/2/bfad1cc10d2ce2c8105d06449fa4a470

The gene encoding the Wuchereria bancrofti orthologue of the Brugia malayi-derived diagnostic antigen SXP1 was identified from a W. bancrofti L3 cDNA library and characterized. The Wb-sxp-1 cDNA encoded a basic protein with a calculated molecular mass of 20.8 kDa. Wb-SXP-1 was 85% identical to the SXP1 protein described from B. malayi (Bm-SXP-1). The Wb-SXP-1 sequence also showed significant identity with proteins described from B. pahangi, Onchocerca volvulus, Acanthochilonema vitea, Ascaris suum, Loa loa, Litomosoides sigmodontis and Caenorhabditis elegans. The presence of a number of invariant and conserved residues in all of these nematode-derived molecules suggests that Wb-SXP-1 is a member of a new protein family. A recombinant form of Wb-SXP-1 was produced and it was determined that the anti-Wb-SXP-1 antibody response in patients with W. bancrofti infections was restricted to the IgG4 subclass. An anti-Wb-SXP-1 IgG4 ELISA was developed and this assay was found to be 100% sensitive for patients with patent W. bancrofti infection. Sera from individuals experiencing chronic pathology, endemic normals or patients with non-filarial nematode infections had no detectable IgG4 against Wb-SXP-1. While patients with patent Onchocerca volvulus infections were uniformly negative in the Wb-SXP-1 assay, 40% of sera from patent Loa loa infections were positive. When Bm-SXP-1 was used as the antigen under identical conditions, the assay was 88% specific for patent W. bancrofti infections and the antigen was recognized by antibodies from both O. volvulus and L. loa infections. The results strongly suggested that, for certain diagnostic filarial antigens, the use of same-species molecules can enhance the specificity of diagnostic tests.

Reinitz, D. M., B. D. Aizenstein, et al. (1992). "Variable and conserved structural elements of trypanosome variant surface glycoproteins." Molecular and Biochemical Parasitology **51**(1): 119.

http://www.sciencedirect.com/science/article/B6T29-476TYTC-35/2/383d00da090ae030eb4f3ba712e2fa1a

The characterization of B cell epitopes on the trypanosome variant surface glycoprotein (VSG) rests on elucidation of variant specific amino acid sequences that may be exposed or buried as a result of the natural conformation of these molecules in the surface coat. Despite the fact that different VSGs have heterogeneous primary sequences and unique antigenic characteristics, recent high resolution X-ray crystallographic analyses of VSGs have revealed a conserved 3-dimensional structure common to these surface proteins [19]. We took advantage of this conserved structural conformation to help predict which variant subregions of VSG molecules may contain exposed or buried variant specific B cell epitopes. Using Staden data tables, we aligned the deduced amino acid sequence of Trypanosoma brucei rhodesiense LouTat 1 VSG, a molecule that has been characterized immunologically in this laboratory, with 12 other complete VSG sequences including the T. b. brucei MiTat 1.2 VSG that has been characterized in crystallographic studies. Results of this analysis predict that there are eight defined clusters of variant amino acids which may contribute to exposed B cell epitopes, and ten defined clusters of variant amino acids which may contribute to buried B cell epitopes, on all VSG molecules. Interestingly, this analysis also revealed a VSG consensus sequence in which certain conserved motifs are present in all VSGs. The shared elements of VSG sequences corresponded to known secondary structures present in MiTat 1.2, and included groups of conserved amino acids responsible for turns in subregions of the protein, for structural positioning of the variable residues on the exposed surface, and for the dimerization of VSG monomers. Overall, these observations may aid in the targeting and mapping of exposed and buried VSG specific B cell epitopes, and also may offer clues as to elements of the primary sequence that are important for the conserved 3-dimensional structure of antigenically distinct VSG molecules.

Russell, R., M. Pilar Iribar, et al. (1999). "Intra and inter-specific microsatellite variation in the Leishmania subgenus Viannia." Molecular and Biochemical Parasitology **103**(1): 71.

http://www.sciencedirect.com/science/article/B6T29-3XD3JHF-7/2/f1108f10c290ec89c7f762f1ea23f5f0

Leishmania species of the subgenus Viannia are responsible for a large proportion of New World leishmaniasis. Here we report the development of a set of microsatellite markers which are able to discriminate between all species within the subgenus Viannia, including the closely related species pairs: Leishmania (V.) braziliensis and Leishmania (V.) peruviana; Leishmania (V.) panamensis and Leishmania (V.) guyanensis. Potential species hybrids were uncovered in the analysis. These markers are sufficiently polymorphic such that within-species epidemiological, population and genetic studies are theoretically possible for all species analyzed.

Ruvolo, V., R. Altszuler, et al. (1993). "The transcript encoding the circumsporozoite antigen of Plasmodium berghei utilizes heterogeneous polyadenylation sites." Molecular and Biochemical Parasitology **57**(1): 137.

http://www.sciencedirect.com/science/article/B6T29-47726M3-4W/2/98b5eab3ba8726a4b3dbf6faace19590

We have employed polymerase chain reaction-based techniques to examine the transcript

encoding the circumsporozoite (CS) antigen, the immunodominant coat protein of the infectious stage of the murine parasite Plasmodium berghei. Earlier studies suggested that the 3' terminus of the CS message might be determined by transcription termination rather than by cleavage and polyadenylation, as in most eukaryotes. Here we report that a subset of CS messages are polyadenylated. Moreover, the poly(A) tails are added at multiple sites clustered within a short region 300 bp downstream from the stop codon. Whether 3' end heterogeneity is peculiar to the CS message or a common feature of plasmodial transcripts remains to be determined.

Sallicandro, P., M. G. Paglia, et al. (2000). "Repetitive sequences upstream of the pfg27/25 gene determine polymorphism in laboratory and natural lines of Plasmodium falciparum." Molecular and Biochemical Parasitology **110**(2): 247.

http://www.sciencedirect.com/science/article/B6T29-41NTB7B-6/2/bd6d0957c7fd765640cc1b2457802133

The structure of the genomic region located upstream of the gametocyte-specific gene pfg27/25 of Plasmodium falciparum was analysed in laboratory lines and field isolates of the parasite. The gene is located in a subtelomeric region of chromosome 13 in parasite clones 3D7 and HB3. Analysis of laboratory lines and field isolates of P. falciparum indicated that polymorphism upstream of pfg27/25 is mainly due to the structure of a repetitive DNA region located at about half a kilobase from the pfg27/25 coding sequence. Different types of repetitive sequences are present in this region, whose copy number is variable in different parasite lines. In addition a GC-rich sequence element contained in this region, which is proposed to be the startpoint of pfg27/25 mRNA, presents either a direct or a reverse orientation in different parasite lines. Genomic deletions upstream of the pfg27/25 gene are also described in two laboratory lines of the parasite, which eliminate two newly identified malaria genes, orf P and orf Gap, from the genome of these parasites. One of them, orf Gap, deleted from the reference parasite clone 3D7, is abundantly expressed as mature mRNA in asexual parasites. PCR analysis on 64 field isolates of P. falciparum indicated that orf P and orf Gap sequences are present in all tested samples of naturally propagating parasites.

Santos, M. A. M., N. Garg, et al. (1997). "The identification and molecular characterization of Trypanosoma cruzi amastigote surface protein-1, a member of the trans-sialidase gene super-family." Molecular and Biochemical Parasitology **86**(1): 1.

http://www.sciencedirect.com/science/article/B6T29-3PM2698-2/2/b05840eaebead0b303c6e9bf55923784

An accumulating body of evidence suggests that T. cruzi-infected host cells are recognized and destroyed by class I major histocompatibility complex (MHC) restricted CD8+ T-cells thus contributing to immune control of the infection [1, 2, 3, 4, 5, 6]. However, to date, only a few amastigote proteins which could be the target of this response have been described and gene sequence information is available only for the amastins [7]. In order to identify amastigote proteins which could contribute to immune detection of infected host cells, a panel of monoclonal antibodies specific for amastigote proteins was produced and screened. Three mAbs (IIIC4, VIIC1 and IIID4) were identified which recognized amastigote surface proteins of 78, 26 and 53 kDa, respectively. Screening of an amastigote cDNA expression library with mAb IIIC4 resulted in the isolation of a 2.8 Kb clone, pSI2. The derived amino acid sequence indicates that the pSI2 clone encodes an amastigote surface protein belonging to the T. cruzi trans-sialidase super-family. Based on its preferential expression in the amastigote stage we have named this protein amastigote surface protein-1 (ASP-1). ASP-1 contains the third and fourth Asp block motifs, SxDxGxTW and the fibronectin type III-like domain, VTVxNVxLYNR, thus placing it in family II of

the T. cruzi trans-sialidases [8]. ASP-1 is the first trans-sialidase family member shown to be preferentially expressed in the amastigote stage of the T. cruzi life cycle. This expression of ASP-1 on parasites in infected cells and its apparent membrane attachment by a glycosylphosphatidylinositol (GPI)-anchor makes it a prime candidate to enter the class I MHC processing and presentation pathway.

Schaap, D., G. Arts, et al. (2005). "De novo ribosome biosynthesis is transcriptionally regulated in Eimeria tenella, dependent on its life cycle stage." Molecular and Biochemical Parasitology **139**(2): 239.

http://www.sciencedirect.com/science/article/B6T29-4F29FPK-2/2/b274ae316d9ec775105f30f151e366ab

Protozoan parasites go through various developmental stages during their parasitic life, which requires the expression of different genes. To identify stage specific gene products in Eimeria tenella, a differential screening was performed comparing the intracellular schizont stage with the extracellular oocyst stage. De novo transcripts of 18S-5.8S-26S rRNA transcription units and of two ribosomal proteins (RPL5 and RPL23) were specifically identified in schizonts and were undetectable in oocysts. The stage specific transcription of pre-rRNAs (prior to processing) was confirmed with Northern blot analysis. Since the E. tenella genome contains a repeated gene cluster with an estimated 140 large rRNA transcription units, they all might be similarly regulated. Specific expression of RPL5 and RPL23 in E. tenella schizonts was also confirmed by Northern blotting. Furthermore, an analysis of the E. tenella EST database with 26,705 ESTs showed that 9.5% of all merozoite ESTs and only 0.2% of the sporozoite ESTs encoded ribosomal proteins (RPs). These ESTs encoded 69 different RPs, suggesting that most and possibly all RPs are differentially transcribed in E. tenella. Analysis of EST data from other Coccidia, such as Toxoplasma gondii, indicated a similar stage dependent transcription of RP genes. We conclude that ribosome biosynthesis is transcriptionally regulated in E. tenella and other Coccidia, such that rapidly growing parasite stages utilize much of their resources to de novo biosynthesis of ribosomes, and that "dormant" oocyst stages do not synthesize new ribosomes. The 50- to 100-fold reduction in transcription of RPs together with the reduced rRNA transcription prevents that unnecessary new ribosomes are synthesized in oocysts.

Silins, G. U., R. L. Blakeley, et al. (1996). "Characterisation of genes encoding a nucleoside monophosphate kinase and a L35 ribosomal protein from Babesia bovis." Molecular and Biochemical Parasitology **76**(1-2): 231.

http://www.sciencedirect.com/science/article/B6T29-4287HV2-P/2/9520d409b782dd43359ea3929596acd8

We have sequenced a region of the Babesia bovis nuclear genome that encodes a L35 ribosomal protein homologue (bl35) and a putative nucleoside monophosphate kinase (bnmk) that is most similar to the adenylate kinase of gram-positive bacteria and the mitochondrial form of adenylate kinase in eukaryotes. BNMK appears to be unique in that it is the first eukaryotic family member to feature a putative zinc-binding domain. bnmk and bl35 are closely linked and transcribed from opposite DNA strands. Examination of the gene structures indicate that the coding regions contain small intervening sequences that obey the GT-AG rule of eukaryotic spliceosomal introns. The single intron separates the bl35 initiation codon from the remainder of the coding region and the 6-exon bnmk gene does not appear to be differentially spliced. Both genes utilise multiple polyadenylation sites and the canonical mammalian polyadenylation signal AATAAA is absent from their 3' untranslated regions. Primer extension analyses reveal that the bnmk gene utilises a cluster of transcription start points, one of which is used most frequently. The bnmk mRNA 5' end does not appear to be cis- or trans-spliced. We report here the first evidence of intronic

sequences, as well as heterogeneous 5' and 3' ends for mRNA of a member of the Babesia genus.

Siman-Tov, M. M., R. Aly, et al. (1996). "Cloning from Leishmania major of a developmentally regulated gene, c-lpk2, for the catalytic subunit of the cAMP-dependent protein kinase." Molecular and Biochemical Parasitology **77**(2): 201.

http://www.sciencedirect.com/science/article/B6T29-3W2YGCS-9/2/3a715e5727110dac1bf97411c616849b

Protein kinases are important in the regulation of cellular processes including growth and differentiation. Using the polymerase chain reaction with oligonucleotide primers derived from conserved regions of cAMP-dependent protein kinases (PKAs), three different DNA fragments were amplified from leishmanial genomic DNA. One fragment was used to isolate a stage specific gene, c-lpk2, from a Leishmania major genomic library. This gene shows high homology to other eukaryotic PKAs, and the open reading frame encodes a 332 amino acid protein with a predicted molecular mass of 38.2 kDa. When aligned with other PKAs the leishmanial enzyme has a unique eight amino acid extension at the carboxy terminus. The c-lpk2 gene is present as a single copy in L. major, L. donovani and L. amazonensis. The 5'-flanking region contains a polypyrimidine rich tract upstream from the predicted ATG start codon. The gene is highly expressed in promastigotes and barely detectable in amastigotes of L. major. Temperature increase was shown to rapidly down-regulate c-lpk2 expression. Transfer of L. amazonensis promastigotes to 35[deg]C resulted in the rapid disappearance of c-lpk2 mRNA (>70% in 1 h), while at 26[deg]C the mRNA was more stable. The strict temperature dependence of mRNA degradation rate suggests that PKA expression is regulated post-transcriptionally.

Snounou, G., S. Viriyakosol, et al. (1993). "Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections." Molecular and Biochemical Parasitology **58**(2): 283.

http://www.sciencedirect.com/science/article/B6T29-476M11V-FC/2/ea198ae431594bf7a9ddaad7ece016e4

Genus- and species-specific sequences are present within the small subunit ribosomal RNA genes of the four human malaria parasites. Oligonucleotide primer pairs specific to each species were designed for specific amplification by the Polymerase Chain Reaction (PCR), to detect each malaria species. DNA equivalent to 5 [mu]l of blood was sufficient for the detection of each of the species. Blood samples obtained from 196 patients attending a malaria clinic in Trad province (Thailand) were analyzed. Detection and identification of the parasites, solely by electrophoretic analysis of the PCR products, has proven to be more sensitive and accurate than by routine diagnostic microscopy. A high proportion of mixed species infections were brought to light by the PCR assay. Implications for medical treatment and epidemiological studies are discussed.

Snounou, G., S. Viriyakosol, et al. (1993). "High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction." Molecular and Biochemical Parasitology **61**(2): 315.

http://www.sciencedirect.com/science/article/B6T29-476M13N-GB/2/46a76321c16c919b7639d959f6959c2f

Soto, M., J. M. Requena, et al. (1993). "Isolation, characterization and analysis of the expression of the Leishmania ribosomal PO protein genes." Molecular and Biochemical Parasitology **61**(2): 265.

http://www.sciencedirect.com/science/article/B6T29-476M13N-G5/2/bf902df200a63bdbbe2b6da32d0e73a1

Two tandemly linked genes are present in the Leishmania infantum genome that code for the acidic ribosomal PO protein. The genes are identical in the coding region, although a striking lack of nucleotide sequence conservation is observed when the boundaries of the coding regions between both genes are compared. The 3' untranslated regions of the two genes are, moreover, different in size. The deduced amino acid sequence of the L. infantum PO protein (LiPO) shows a high degree of sequence conservation, including the highly charged conserved C-terminal domain, with the ribosomal PO proteins of other eukaryotic organisms. Northern blot experiments showed that two different size class transcripts are expressed in the gene cluster and that the steady state level of each of the transcripts in logarithmic phase promastigotes is markedly different. The abundance of both transcripts is down-regulated in parasite cultures on reaching stationary phase. Since it seems that the two Leishmania ribosomal PO genes are expressed in a single polycistronic transcript, it is likely that the different levels of PO mRNAs observed in cultured cells is due to a postranscriptional regulatory mechanism.

Tabares, E., D. Ferguson, et al. (2004). "Eimeria tenella sporozoites and merozoites differentially express glycosylphosphatidylinositol-anchored variant surface proteins." Molecular and Biochemical Parasitology **135**(1): 123.

http://www.sciencedirect.com/science/article/B6T29-4BVRJYJ-2/2/b4e928d2dc77e3fac725289129ddf08b

Little is known about glycosylphosphatidylinositol (GPI)-linked surface proteins in the coccidian parasite Eimeria tenella. Examination of 28,550 EST sequences from the sporozoite and second merozoite developmental stages of the parasite led to the identification of 37 potential GPI-linked variant surface proteins, termed EtSAGs. Analysis of the complete nucleotide sequences of 23 EtSAG genes separated them into two multi-gene families. All the predicted EtSAG proteins (which vary in length from 228 to 271 residues) have an N-terminal hydrophobic signal peptide, a C-terminal hydrophobic GPI signal-anchor peptide and an extracellular domain organised around six cysteine residues, the positions of which are conserved within each family. Using specific antibodies against a small number of recombinant-expressed EtSAGs, the surface localisation and GPI-anchorage of members of both families was confirmed experimentally. Expression of EtSAGs is differentially regulated between the oocyst/sporozoite and second generation merozoite stages, with only one expressed specifically in the sporozoite, a small number expressed in both stages and the majority expressed specifically in the second generation merozoite. Preliminary data support a model in which multiple variant surface antigens are co-expressed on individual parasites, rather than a model of antigenic switching. The biological role(s) of EtSAGs and the effect(s) that expression of a complex repertoire of variant surface antigens by the second generation merozoite has on host adapted immunity are unknown.

Tachibana, M., T. Tsuboi, et al. (2002). "Two types of Plasmodium ovale defined by SSU rRNA have distinct sequences for ookinete surface proteins." Molecular and Biochemical Parasitology **122**(2): 223.

http://www.sciencedirect.com/science/article/B6T29-460DN3S-1/2/d888ee546f1f7929a420079dcf9acfc9

Tachibana, M., T. Tsuboi, et al. (2001). "Presence of three distinct ookinete surface protein genes, Pos25, Pos28-1, and Pos28-2, in Plasmodium ovale." Molecular and Biochemical Parasitology **113**(2): 341.

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Taylor, H. M., S. A. Kyes, et al. (2000). "A study of var gene transcription in vitro using universal var gene primers." Molecular and Biochemical Parasitology **105**(1): 13.

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The polymorphic multigene family, var, encodes the variant antigen, Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), present on the surface of erythrocytes infected with the human malaria parasite, P. falciparum. PfEMP1 has been implicated in the pathology of malaria through its ability to bind to host endothelial receptors and uninfected erythrocytes. Understanding the relationship between host pathology, immune response and parasite variation is crucial, but requires a method of reliably detecting and differentiating all possible var genes. Several primer pairs used to date are biased and limited in their detection capacity. Here we describe a set of PCR primers that amplify the majority of var genes in the laboratory isolates 3D7 and A4, and appear to work equally well on all isolates tested. We use these universal primers to examine the relationship between var gene transcription as assessed by reverse transcriptase-PCR (RT-PCR) with that measured by Northern analysis of parasite RNA. Phenotypically selected young parasites have multiple transcripts detected by RT-PCR, but the full-length transcript appears to be homogeneous. In addition, we demonstrate that the choice of primers used for RT-PCR is crucial in data interpretation.

Traub-Cseko, Y. M., M. Duboise, et al. (1993). "Identification of two distinct cysteine proteinase genes of Leishmania pifanoi axenic amastigotes using the polymerase chain reaction." Molecular and Biochemical Parasitology **57**(1): 101.

http://www.sciencedirect.com/science/article/B6T29-47726M3-4S/2/c692857c5c2407b4059e285ba5838ca1

A developmentally regulated cysteine proteinase associated with an unique lysosomal organelle, the megasome, has been described for the intracellular amastigotes of the Leishmania mexicana complex; this proteinase appears to be important in the survival of the parasite. Degenerate primers encoding the active sites residues have been used to amplify cysteine proteinase cDNA sequences from axenically cultured amastigotes of Leishmania pifanoi, a member of the L. mexicana complex. Based on sequence data, two distinct genes (Lpcys1 and Lpcys2) were identified. Although both genes are preferentially transcribed in the amastigote stage, each is distinct in genomic arrangement and chromosome location, with Lpcys2 showing evidence for the presence of 8-20 tandemly arrayed copies and mRNA levels 10-fold higher than Lpcys1. Related forms of the Lpcys1 and Lpcys2 genes exist in other species of the genus Leishmania, including Leishmania braziliensis, Leishmania major and Leishmania donovani. The protein sequence of an

abundant immunoaffinity purified amastigote cysteine proteinase (A-2) is identical to that predicted for the product of Lpcys2; immunofluorescence studies show an intracellular pattern/distribution for the A-2 proteinase consistent with a putative megasomal association. The DNA sequence of a genomic copy of Lpcys2 predicts a C-terminal extension for the proteinase; comparative sequence analyses of the C-terminal extensions found for Trypanosoma cruzi and Trypanosoma brucei reveal the selective conservation of cysteine, as well as proline and glycine residues, suggesting that conservation of folding and secondary structure may be required for biological function.

Trucco, C., D. Fernandez-Reyes, et al. (2001). "The merozoite surface protein 6 gene codes for a 36 kDa protein associated with the Plasmodium falciparum merozoite surface protein-1 complex." Molecular and Biochemical Parasitology **112**(1): 91.

http://www.sciencedirect.com/science/article/B6T29-428FK3Y-B/2/f6cbccd9ea92de591a7257dbd6139044

A complex of non-covalently bound polypeptides is located on the surface of the merozoite form of the human malaria parasite Plasmodium falciparum. Four of these polypeptides are derived by proteolytic processing of the merozoite surface protein 1 (MSP-1) precursor. Two components, a 22 and a 36 kDa polypeptide are not derived from MSP-1. The N-terminal sequence of the 36 kDa polypeptide has been determined, the corresponding gene cloned, and the protein characterised. The 36 kDa protein consists of 211 amino acids and is derived from a larger precursor of 371 amino acids. The precursor merozoite surface protein 6 (MSP-6) has been designated, and the 36 kDa protein, MSP-636. Mass spectrometric analysis of peptides released from the polypeptide by tryptic digestion confirmed that the gene identified codes for MSP-636. Antibodies were produced to a recombinant protein containing the C-terminal 45 amino acid residues of MSP-636. In immunofluorescence studies these antibodies bound to antigen at the parasite surface or in the parasitophorous vacuole within schizonts, with a pattern indistinguishable from that of antibodies to MSP-1. MSP-636 was present in the MSP-1 complex immunoprecipitated from the supernatant of in vitro parasite cultures, but was also immunoprecipitated from this supernatant in a form not bound to MSP-1. Examination of the MSP-6 gene in three parasite lines detected no sequence variation. The sequence of MSP-636 is related to that of the previously described merozoite surface protein 3 (MSP-3). The MSP-636 amino acid sequence has 50% identity and 85% similarity with the C-terminal region of MSP-3. The proteins share a specific sequence pattern (ILGWEFGGG-[AV]-P) and a glutamic acid-rich region. The remainder of MSP-6 and MSP-3 are unrelated, except at the N-terminus. Both MSP-636 and MSP-3 are partially associated with the parasite surface and partially released as soluble proteins on merozoite release. MSP-636 is a hydrophilic negatively charged polypeptide, but there are two clusters of hydrophobic amino acids at the C-terminus, located in two amphipathic helical structures identified from secondary structure predictions. It was suggested that this 35 residue C-terminal region may be involved in MSP-636 binding to MSP-1 or other molecules; alternatively, based on the secondary structure and coil formation predictions, the region may form an intramolecular anti-parallel coiled-coil structure.

Wakefield, A. E., F. J. Pixley, et al. (1990). "Amplification of mitochondrial ribosomal RNA sequences from Pneumocystis carinii DNA of rat and human origin." Molecular and Biochemical Parasitology **43**(1): 69.

http://www.sciencedirect.com/science/article/B6T29-476CTTP-DY/2/b58535790979c93e8de7fb97b5d85127

Pneumocystis carinii specific DNA sequences have been cloned from the experimental rat model.

The sequence of the gene coding for the large subunit of mitochondrial ribosomal RNA has been used to construct P. carinii specific oligonucleotide primers for the polymerase chain reaction. These oligonucleotides produced amplification of specific sequences from both P. carinii infected rat and human lung samplings, but none from a range of other organisms including potential pulmonary pathogens. Comparison of the sequence of amplified products from the infected rats and humans demonstrated limited but consistent differences between P. carinii from these two hosts and allowed for the construction of a human specific internal oligonucleotide. The application of the specific oligonucleotides for DNA amplification and subsequent Southern hybridisation affords extremely sensitive and specific detection of P. carinii in human samples, which may be applicable to both epidemiological research and clinical studies.

Wan, K.-L., V. B. Carruthers, et al. (1997). "Molecular characterisation of an expressed sequence tag locus of Toxoplasma gondii encoding the micronemal protein MIC2." Molecular and Biochemical Parasitology **84**(2): 203.

http://www.sciencedirect.com/science/article/B6T29-3S6D230-5/2/6e706d963c550c5517adf4b3d8eac21f

The expressed sequence tag (EST) dataset of Toxoplasma gondii provides a wealth of information towards gene discovery. The complete cDNA and genomic sequence of EST tgc050 locus shows that it contains five copies of the conserved thrombospondin (TSP)-like motif present in a number of molecules with adhesive properties. A conserved region implicated with the adhesive characteristic of another group of proteins including several integrins, is also present in this molecule. The protein encoded by this sequence (rc50) is strongly recognised by monoclonal antibodies to MIC2. Affinity purified anti-rc50 antisera specifically reacted with a single protein of identical molecular mass as MIC2 and exclusively labeled the micronemes of T. gondii by cryo-immunoelectron microscopy. These results demonstrate that c50 encodes for MIC2, a previously characterised microneme protein of T. gondii. The extensive sequence similarity across multiple protein domains provides evidence that the protein encoded by this locus is the homologue to the Etp100 microneme protein of Eimeria tenella.

Wataya, Y., M. Arai, et al. (1993). "DNA diagnosis of falciparum malaria using a double PCR technique: a field trial in the Solomon Islands." Molecular and Biochemical Parasitology **58**(1): 165.

http://www.sciencedirect.com/science/article/B6T29-476M0YJ-DW/2/fe2e67e070816dd4727c525895e9e3ad

Waters, N. C., K. M. Kopydlowski, et al. (2002). "Functional characterization of the acyl carrier protein (PfACP) and beta-ketoacyl ACP synthase III (PfKASIII) from Plasmodium falciparum." Molecular and Biochemical Parasitology **123**(2): 85.

http://www.sciencedirect.com/science/article/B6T29-46B6WYR-1/2/0b3d45351f39a3710c8f5d5093934cea

The genome of the malaria parasite, Plasmodium falciparum, appears to contain the proteins necessary for a Type II dissociated fatty acid biosynthetic system. Here we report the functional characterization of two proteins from this system. Purified recombinant acyl carrier protein (ACP) and [beta]-ketoacyl-ACP synthase III (KASIII) from P. falciparum are soluble and active in a truncated form. Malarial ACP is activated by the addition of a 4'-phosphopantetheine prosthetic

group derived from coenzyme A, generating holo-PfACP. Holo-PfACP is an effective substrate for the transacylase activity of PfKASIII, but substitution of a key active site cysteine in PfKASIII to alanine or serine abolishes enzymatic activity. During the schizont stage of parasite development, there is a significant up-regulation of the mRNAs corresponding to these proteins, indicating an important metabolic requirement for fatty acids during this stage.

Webb, J. R., L. L. Button, et al. (1991). "Heterogeneity of the genes encoding the major surface glycoprotein of Leishmania donovani." Molecular and Biochemical Parasitology **48**(2): 173.

http://www.sciencedirect.com/science/article/B6T29-476TYYD-3M/2/fb2d1d275e3f9258ea7daa0e4de2885d

The major surface glycoprotein of Leishmania (GP63) is present on all known species of Leishmania and likely plays an integral role during the infection of macrophages in the mammalian host. To identify regions of GP63 which may be of functional significance, the nucleotide sequence of a gene encoding GP63 of Leishmania donovani was determined and compared to the sequences reported for GP63 genes of Leishmania major and Leishmania chagasi. The GP63 nucleotide and predicted protein sequence was highly conserved among the 3 species despite their diverse geographical distribution. L. donovani GP63 is encoded by a multigene family and the gene locus contains at least 7 tandemly repeated genes and at least 3 genes which are dispersed from the tandem array. In addition, polymerase chain reaction and Southern blot analyses demonstrated that there was size heterogeneity within the pro-peptide coding regions of the multiple GP63 genes of L. donovani and that such genes were expressed concurrently in the promastigote life stage.

Wickham, M. E., J. K. Thompson, et al. (2003). "Characterisation of the merozoite surface protein-2 promoter using stable and transient transfection in Plasmodium falciparum." Molecular and Biochemical Parasitology **129**(2): 147.

http://www.sciencedirect.com/science/article/B6T29-48PDW0G-1/2/3318ab8e3eaed2e5aa101c08b38f42f1

Plasmodium falciparum merozoite surface protein (MSP)-2, is a polymorphic protein whose variable regions define two allelic families, the 3D7/IC-1 and FC27/D10 families. The gene encoding MSP-2 is located on chromosome 2 immediately 3' of the gene encoding merozoite surface protein-5 (MSP-5) with a 1096 bp intergenic region that presumably contains the MSP-2 promoter. Here we present characterization of the MSP-2 promoter using transient and stable transfection of P. falciparum. The mRNA transcription initiation site was mapped to a position 256 bp upstream of the MSP-2 translation start site. The ability of the intergenic region between MSP-5 and MSP-2 to promote the expression of chloramphenicol acetyl transferase (CAT) has been tested using a series of nested deletions in transient transfection experiments. The minimal region required for CAT expression has been defined and putative regulatory elements delineated. These nested deletions were used for heterologous expression of an FC27 family MSP-2 allele in the 3D7 allelic background in transfected 3D7 lines. In each case, the transgenic P. falciparum lines generated co-express both 3D7 and FC27 allelic forms of MSP-2 at the merozoite surface. These results have identified the functional promoter for MSP-2.

Winter, G., Q. Chen, et al. (2003). "The 3D7var5.2 (varCOMMON) type var gene family is commonly expressed in non-placental Plasmodium falciparum malaria." Molecular and Biochemical

Parasitology **127**(2): 179.

http://www.sciencedirect.com/science/article/B6T29-47RS0PH-2/2/b7ea51f8b896b633d799d505490edf46

Relapse variants in chronic Plasmodium falciparum infections are antigenically distinct from the parental parasites. The variable antigen PfEMP1 expressed at the surface of the infected erythrocyte (IE) is encoded by the var gene family with [ap]60 copies per haploid genome. Placental isolates commonly express DBL[gamma] containing subtypes of var genes with homology to either 3D7var5.2 (varCOMMON) or FCR3varCSA. Here we report that varCOMMON related genes are constitutively transcribed in [ap]60% of malaria infected children in Gabon. varCOMMON is conserved in field isolates over at least 2.1 kb. In 3D7 parasites varCOMMON is present on chromosome 5 (var5.2) and constitutively transcribed in the opposite direction to most other var genes. It lacks a regulatory intron, an acidic terminal segment and ends in telomeric repeat sequences. varCOMMON encodes a large, hypothetical PfEMP1 of a structure similar to previous placenta-binding PfEMP1s but it is not present at the IE-surface. IE of a 3D7 clone (3D7S8) transcribe varCOMMON but express a PfEMP1 distinct from varCOMMON at the surface and adhere to placental tissues through varCOMMON independent novel mechanisms. Our report suggests that expression of varCOMMON type genes is not restricted to placental malaria.

Witney, A. A., D. L. Doolan, et al. (2001). "Determining liver stage parasite burden by real time quantitative PCR as a method for evaluating pre-erythrocytic malaria vaccine efficacy." Molecular and Biochemical Parasitology **118**(2): 233.

http://www.sciencedirect.com/science/article/B6T29-442RVGR-1/2/47d190df00e1bf068fbeb283e06e4e1e

The detection and quantitation of blood stage parasitaemia is typically used as a surrogate endpoint for estimating the efficacy of vaccines targeted against the hepatic stage, as well as the erythrocytic stage, of the parasite. However, this does not provide an adequate means of evaluating the efficacy of vaccines, which may be only partially effective at the liver-stage. This is a particular concern for effective evaluation of immune enhancement strategies for candidate pre-erythrocytic stage vaccines. Here, we have developed and validated a method for detecting and quantitating liver stage parasites, using the TaqMan(R) fluorescent real-time quantitative PCR system (PE Applied Biosystems). This method uses TaqMan(R) primers designed to the Plasmodium yoelii 18S rRNA gene and rodent GAPDH to amplify products from infected mouse liver cDNA. The technique is highly reproducible as demonstrated with plasmid controls and capable of efficiently quantitating liver-stage parasite burden following a range of sporozoite challenge doses in strains of mice, which differ in their susceptibility to sporozoite infection. We have further demonstrated the capacity of this technique to evaluate the efficacy of a range of pre-erythrocytic stage vaccines. Our data establish this quantitative real-time PCR assay to be a fast and reproducible way of accurately assessing liver stage parasite burden and vaccine efficacy in rodent malaria models.

Yahiaoui, B., F. Dzierszinski, et al. (1999). "Isolation and characterization of a subtractive library enriched for developmentally regulated transcripts expressed during encystation of Toxoplasma gondii." Molecular and Biochemical Parasitology **99**(2): 223.

http://www.sciencedirect.com/science/article/B6T29-3W7XCB3-6/2/07ffb25edc670a0c74d44e2dc7cf86f7

To survive within infected hosts, Toxoplasma gondii undergoes profound metabolic and morphological changes by differentiating into a cyst characterized by its resistance to the immune system and chemotherapy. The stimulus that triggers Toxoplasma encystation and the molecular mechanisms regulating the bradyzoite phenotype are still unknown. Here, we developed a differentiation method in conjunction with a selective and subtracted cDNA strategy devised to identify developmentally regulated transcripts. We isolated and analyzed 65 cDNA clones. In addition to bradyzoite specific cDNAs previously reported, we demonstrate that twelve genes are exclusively or preferentially transcribed in the encysted bradyzoite forms of T. gondii using semi-quantitative RT-PCR. Among cDNAs identified, are those encoding predicted homologues of chaperones (mitochondrial heat shock protein 60, T-complex protein 1), DNA-damage repair protein, phosphatidylinositol synthase, glucose-6-phosphate isomerase and enolase. The identification of these genes opens the way for further study of molecular mechanisms controlling gene expression during T. gondii encystation.

Zhang, W., J. Li, et al. (2003). "A gene family from Echinococcus granulosus differentially expressed in mature adult worms." Molecular and Biochemical Parasitology **126**(1): 25.

http://www.sciencedirect.com/science/article/B6T29-47PPGCF-1/2/2d2aaff2234fed53f82f5e509cabac46

Differences in mRNA expression between immature adult worms (IAW) and mature adult worms (MAW) of Echinococcus granulosus were determined using polymerase chain reaction-based differential display (DDRT-PCR). Twenty-eight putative differential cDNA fragments were isolated, cloned and sequenced. mRNAs from IAW and MAW were probed with the labelled fragments. Six cDNA fragments (coded as egM12, egM13, egM22, egM26, egM30 and egM34) were putatively determined to be specific to MAW by Northern hybridisation. The stage-specificity of egM12, egM13 and egM34 was confirmed by RT-PCR. RNAs of IAW, MAW, protoscoleces and oncospheres, probed with egM13 and egM30, showed that the mRNAs were expressed exclusively in MAW, which implied involvement in the regulation of egg development. Using the labelled fragments to screen a cDNA library of MAW, 99 clones were identified and analysed. An alignment of selected clones showed that the MAW-specific mRNAs belonged to a family. Examination of the deduced amino acid sequence of three of the corresponding cDNAs (egM4, egM9 and egM123) indicated they were cysteine-rich and contained a 24 amino acid repeat sequence, repeated four to six times. The repeat regions were predominantly alpha helical in nature with interspersed turns, forming alternating zones of positive and negative charge. The functional significance of each of the cDNAs identified is unclear as none had significant sequence similarity to genes of known function. However, polypeptides encoded by egM4 and egM123 were recognised by antibodies in a serum pool from dogs experimentally infected with E. granulosus, suggesting they could prove of value in serodiagnosis of definitive hosts.