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Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis, and today I'm excited to share my conversation with Dr. Nadav Ahituv. Nadav is a professor and the director of the Institute for Human Genetics at the University of California San Francisco. For nearly 20 years, he's been at the forefront of a diverse portfolio of genetic research. And from possible gene therapy cancer treatments to the genome of bats, we get to learn all about his amazing work today. Thanks so much for tuning in. I hope you enjoy our conversation.

Nadav Ahituv, PhD 00:50
So I'm a professor in the Department of Bioengineering and Therapeutic Sciences, and also the Director for the Institute for Human Genetics at UCSF. And our work basically spans the gene regulation. How genes are turned on and off, and we do a multitude of things on that, in terms of trying to understand how mutations in them lead to disease, that lead to differences in morphology and evolution, and also develop technologies to test these sequences, and finally, use them also for therapeutics.

Steve Lewis 01:21
So starting at the beginning of your career, why don't you tell us a bit about what drew you to this area and how you got ultimately to gene regulation?

Nadav Ahituv, PhD 01:30
Sure. So when I was 13, I was diagnosed with scoliosis, and basically, I had a curved spine. And when I was 16, I actually had spinal fusion surgery. Both my little sisters also had scoliosis, so that really got me into genetics, understanding a complex genetic disease, as scoliosis is, and going all the way to therapy. And so I did my undergrad in biology, and then onto grad school basically on genetics and genetic disease, in particular. At the time, I was focused on hearing loss and deafness. And during my PhD, I cloned two genes that lead to hearing loss. And then when I was looking for a postdoc, the genome came out at the time, and I was fascinated that we only know 2% of the genome, which is the coding sequences, but we really don't understand the other 98% which has a lot of regulatory elements, basically switches that tell the genes to turn on and off at different time points, locations and amount. And so I joined a lab in Berkeley that was focused, Eddie Rubin's lab, that was focused on characterizing gene regulatory elements. And then, following my postdoc, I set out to basically combine my two sorts of backgrounds, say, human genetic disease and gene regulation, and started my own lab at UCSF where we're trying to find, as I mentioned before, mutations in these gene regulatory elements, and that the human disease. And sort of going full circle, our first grant was actually on, on limb malformation, and that allowed me to go to these meetings of birth defects and there I met Carol Weiss, who works a lot on scoliosis. And through these meetings, we actually put in a grant together and currently one of the projects in our lab is actually working on trying to characterize scoliosis. So I'm sort of finally working on my own disease, so to speak.

Steve Lewis 03:34
What made you transition from hearing loss studies to focusing on scoliosis?

Nadav Ahituv, PhD 03:39
Yeah, so following my postdoc, the hearing loss was a great sort of, you know, it was exciting times for that field, in general, for the genetics field, because we could, you know, with sequencing becoming a bit more accessible and the genome becoming accessible, we could find a lot of mutations that lead to various diseases. And as I mentioned, we, I was really interested in complex diseases. Scoliosis is a complex one. And really trying to understand the genome, not only on the coding, but on the non-coding, where I think not much was done, and sort of felt like there's a lot to find there. And so that's where I went to do my postdoc. And then starting my lab. I mean, as I mentioned, we work a little bit actually, on scoliosis. Most of the lab actually works much more on characterizing regulatory elements for neurological disorders. And we also work on evolution and characterizing regulatory elements, there focusing on human evolution, differences between humans, Neanderthals, Denisovans, and also on bats is another sort of organism that I've been fascinated since I was a kid. And so we actually do a lot of bat research on bat wing development and on bat diets. And then finally, about half the lab now works on using regulatory elements for therapeutic purposes. You can imagine, if you have a switch, if you have a disease, for example, where it is caused by low gene dosage, you have much less of the RNA and then a protein, one can sort of modulate a switch, or pump up the volume, and increase it to try to fix that disease. And we've been doing a lot of work on that and also the opposite, where you have maybe something too high of a gene dosage, you can again put down the volume and modulate that. So we work on a variety of different things, and in terms of regulatory elements. And I think it's really exciting times to be working on those with one sequencing technologies, CRISPR and three, also all the AI availability that we can better understand codes of these regulatory elements.

Steve Lewis 05:43
Building off of that, how much have you integrated the in-silico approach toward biomarker identification and then ultimately toward your gene regulation modulation approach in your lab?

Nadav Ahituv, PhD 05:56
Yeah, quite a lot. I mean, for first, predicting gene regulatory elements now, and we've, together with Jay Shendure and the University of Washington, almost 15 years ago, we developed this technology called massively parallel reporter assays, where you can test hundreds of thousands of sequences for regulatory activity. So now that we have this sort of technology, we could take all the sequences that we characterize and use machine learning and try and see basically, can we predict other regulatory elements? Can we predict their tissue specificity? Can we predict the effect of variance? And I should mention that it's not only we, it's many other labs and people have been using these data sets and doing this, and that has enabled really a great leap in terms of our ability, one to identify gene regulatory elements, identify if they're cell type or tissue specific, and also trying to understand variants. And I think we'll keep on doing that. In terms of diagnostics, and yeah, we've also been using a lot of sequencing analysis and AI also to try to use biomarkers in regulatory elements as part of that for in particular, for cell-free DNA for cancer, to try to see if you could potentially identify cancers in a quicker, earlier time point. And that's really crucial, for example, for cancer, right. Because the earlier you detect, the better you are. And so that's another sort of small part of the lab that we've tried to take advantage of both sorts of machine learning and the sequencing for this.

Steve Lewis 07:28
Over the course of your career, did it go from focus on one gene or one sequence, even in the intergenic DNA, to the multigenic analysis? Did you always know that it was going to be a feasibility to kind of study the complex conditions across different gene impacts that ultimately define these conditions?

Nadav Ahituv, PhD 07:52
Yeah. I mean, when I started my postdoc working on gene regulatory elements, the way things were done at the time is we had only three genomes. So we had the human, the mouse and the fugu fish. And so what we did in the lab that I joined to do my postdoc specialized in is doing comparative genomics, where you can compare, for example, the human genome to the mouse genome to the fish genome and look at things that are conserved through evolution. And if they're, of course, all the coding sequences, you see them conserved, but you can also find a lot of non-coding sequences that are conserved, and that would suggest that that sequence could be a regulatory element. And then over the years a lot of new technologies came out that could allow us to characterize these regulatory elements in a genome wide manner, along with sequencing technologies that improved and cut down in cost. And so now you could easily find in a genome wide manner tons of regulatory elements using technologies like chip seq or ATAC seq or CUT&RUN, CUT&Tag and so forth. And that really revolutionized our ability to find things genome wide. And what I should note is all these technologies are descriptive. If you find something that gets bound, for example, by a transcription factor, whereas the certain histone mark, with these technologies, it doesn't necessarily mean that they're functional regulatory elements. And then, sort of the missing step was trying to do these assays, functional assays, that actually test is that sequence a functional regulatory element. And that, as I mentioned before, along with Jay Shendure's lab, we're able to develop these massively parallel reporter assays that can test hundreds of thousands of sequences for function. And those actually tell you are these sequences functional regulatory elements.

Steve Lewis 09:39
So building off of the massively parallel reporter assays. Tell me about that?

Nadav Ahituv, PhD 09:45
Yeah, so when I started my lab, and basically the way you would test for sequence, if it's a regulatory element, is you put it in front of a reporter gene like green fluorescent protein or lacZ or luciferase. And if it's a regulatory element that will turn on the protein and make RNA and then make a protein that you could basically see. But the issue with that is you can only test one sequence at a time, and so it's really not high throughput. And so the trick that Jay came up with was basically sticking in a 15 base pair bar code along with that protein. So now, if the sequence is a regulatory element, it will make RNA of, let's say that green fluorescent protein, but also make RNA of that barcode. And so now you can clone hundreds of thousands of sequences with hundreds of thoudands of barcodes, and then your readout, instead of looking at a GFP or so forth, is just taking RNA from the cell and reading that RNA barcode. And so if it's a regulatory element, you should see RNA of the barcode. If it's not, you won't see RNA of that barcode. And that is basically allowed, and over the years, what we're working on is

really improving this technology, and not just we, but many other labs make it much more high throughput, to make it work in more complex cells. Like making it work with lentivirus, where you get the ability to infect neurons, but also integrate into the genome and get a readout from within the genome. And a variety of different methods have been developed beautifully by many other labs, also to improve this technology and make it much more high throughput. That technology basically, I think, is sort of the missing piece that we have. We had all these descriptive technologies, and now we have this functional one. And I think the ultimate sort of technology as far as a complement now is the use of high throughput CRISPR screens, also where you can modulate. You can, for example, delete in a high throughput manner, thousands of regulatory elements and look at the expression of them. The single cell technology, for example, you can use what's called CRISPR activation, where basically the scissors of the CRISPR are mutated, but it can bind, so use it as a delivery truck to bind to a specific location. So you can have it bind to a regulatory element and see if it increases the activity of a gene or CRISPR inactivation, where you can bind and see if it decreases. And so through these high throughput screens, and we can do these assays now that we try to find regulatory elements, but in genome, in their endogenous location where they are instead of be massively parallel, where they take them out of context. I think both have advantages and disadvantages, but combining both now, which we've been doing also has been a lot of great ways to really characterize and drill down on regulatory elements.

Steve Lewis 12:46
Do you think that there is an opportunity for improvement in some of these areas? What do you see on the horizon?

Nadav Ahituv, PhD 12:55
I think the high throughput CRISPR screens, they're currently limited in the numbers, and I think we got to improve those. I think other ways, and other starting, but I think could be improved is taking advantage of CRISPR prime editing, where you're not basically deleting or doing CRISPRa or -i, but putting very specific mutations into a sequence. And so you can imagine doing, for example, saturation mutagenesis is taking a regulatory element, you know that functions, and doing all possible mutations in it, and seeing the effect that it has on the target gene that it regulates. And so I think a lot of that will come up over the years. And through that, we can really learn the effect of variants in regulatory elements. We could also, would be a dream if we could, for example, sequence a person, get all the variants that this person has, and then in a cell, we could model all these variants, basically insert all these variants and see how it affects certain phenotypes in a high throughput manner. And I think we need a lot of that in the future to then later be able to develop personal gene therapies for the person based on the functional output that we get for all their variants.

Steve Lewis 14:09
We're excited to be in season three of Speaking of Mol Bio, and we know that we have you, our loyal listeners, to thank for the growing success of our podcast series. As a thank you, we're offering a free portable wireless speaker so you can listen to the podcast or your music anywhere. I have one at my desk, and I love how easily it connects to my phone. It's nice when I want to break from my headphones or want to share what I'm listening to with others. I hope you'll visit thermofisher.com/molbiopodcast to request yours today. Please note this item is only available in some

regions and only while supplies last. Again, visit thermofisher.com/molbiopodcast to request yours. And now back to our interview.

Steve Lewis 14:57
Before we dive deeper into the science and the molecular biology aspect of your work, I want to circle back to what you mentioned about the comparative animal physiology, and you mentioned bats. I'm very curious how you integrated the evolution of bats and why? What is the why behind that?

Nadav Ahituv, PhD 15:18
Yeah, so ever since I was a kid, I was fascinated by bats. I think they're the most amazing animals that we have. For me, they're like superheroes, where, you know, you have one, they can fly, right. And two, not all bats, but some bats have echolocation, where, you know, it's quite amazing. They live all over the world. And some hibernate, some don't. And then for us, we've been fascinated primarily with two things and working on in the lab also on them. One is wing development, how bats develop wing and until a certain stage, and the wing of the bat, and sort of the limb of the bats looks normal, and then suddenly the forelimb starts becoming what's a wing during development. And so we've been fortunate there to work with an Nicola Illing, who's in the University of Cape Town, who can actually collect bat embryos in the wild. And so we've done quite a lot of genomics on them, both forelimb and hindlimb and the great paired comparison and to find regulatory elements that are, and we think, important for wing development. And as a follow up to that, we've swapped a few of these sequences in the mouse, taking out the mouse sequence, putting in the bat sequence, and seeing what we get. And we don't get flying mice, sadly, in the lab, but we do get very subtle phenotypes like a bit longer limb, or a bit more skin, or hind limbs that look like a bat and so. Definitely, really interesting. I think it also shows that evolution is not sort of a one and done, but really a variety of different changes, some minute that need you to get to that point. And then the second thing that we've worked a lot on bats is diet. So I think bats are amazing in terms of their diets, and they eat, and the first ancestor was insect eating bat. But then a lot of changes have happened to make bats be able to eat fruit or nectar or fish or blood and so forth. And we've been focusing in particular on the fruit bats. And that happened twice in evolution, both in the old-world bats and the new world bats, they just eat so much fruit which has tons of sugar and don't get diabetes. And so we've been focusing on trying to understand how they can eat so much sugar without getting diabetes, and have done a lot of genomics on them. And have also been fortunate to go to Belize and in the past few years, and to this bat-a-thon organized by Nancy Simmons and Natural History Museum in New York, where there's 50 bat researchers that do field work unlike us that, you know, sit in the computer or in the lab all day and be able to collect bats for this project. And that's been an amazing trip that, you know, my favorite trip to go to every year.

Steve Lewis 18:06
That's super interesting. I did not know about the evolutionary divergence and diversity that you were describing, but that's incredibly interesting. I would be remiss if I didn't tug on the science fiction thread of the flying mice. But I'll take it a little bit of a different direction, because you mentioned echolocation. There are other animals that do that. Do you think you'll find regulatory components at some point in the future related to that?

Nadav Ahituv, PhD 18:34

Yeah, so there's beautiful work done in the past by lab in Utah, where characterized basically sequences that changed a lot in evolution for certain traits, and one of them was echolocation. And they found actually sequences like that, both in bats and in dolphin and others. So I think there, there is some work and trying to characterize these regions. There's beautiful work by quite a few labs also on trying to understand, sort of the neuroscience of the echolocation in bats and how they use it and how they take advantage of it, and particularly the insect bats are the ones that use echolocation to basically find their prey. And there's beautiful work and videos also that you could check on the web on how they do that with echolocation.

Steve Lewis 19:18
One more high-level question, because I am curious your lens on it, because I think you probably saw this inflection point, and like you said, you came up with a technology that's really interesting and applied in a lot of different laboratories around the world. Did the concept of modulation, just at a high level, modulation, compared to a binary switch. Was that a challenging topic to reach over the course of your career, because now it's sort of accepted, right? You have modulation, but I have to imagine earlier, probably my guess would be the 80s or 90s. It was possibly thought as more of a binary approach to regulation?

Nadav Ahituv, PhD 20:11
I think we just didn't know as much. We didn't have the genome wide technologies that we had there. So there, there are, there were in the 80s and 90s also definitely a regulatory element, enhancers, promoters that people characterize. They're just more on a one-by-one basis than, you know, and people would take them and mutate them and do these, you know, luciferase assays that I mentioned, one-by-one. So I think the advantage of the genome wide approach is really getting and, you know, hundreds of thousands and millions of these sequences, sort of annotated in the genome. And that's also thanks to a lot of big projects, consortiums where many groups of people came together to basically characterize these in a more genome-wide manner. And so I think having that now, and with the era of sort of AI now, in combination, is really leading to our better understanding of them. And so it's nice to see how the technology sort of constantly keeps improving and increasing, sort of, if you look at our ability to predict these regulatory element, you know, it's constantly now going up, in particular now due to AI, that's helping a lot in that.

Steve Lewis 21:22
Aside from sequencing, what other molecular technologies are using on a regular basis?

Nadav Ahituv, PhD 21:28
For these massively parallel reporter assays I mentioned, we do tons of cloning, and we use oligo synthesis, which is also seen DNA synthesis, has also seen a beautiful revolution. So, for example, we can order, you know, 600,000 oligos the size of 300 base pairs, or even 500 base pairs, and clone all of these libraries, right. It's easy now to make these big viral libraries and then inject or infect whatever we want with them. So that's been a lot of fun. Same for, you know, CRISPR libraries, and CRISPR technology has been great to make these large-scale libraries and be able to do that.

Steve Lewis 22:04

Speaking a bit more about kind of cloning and de novo gene synthesis, I have to imagine, for your area, when you're talking about transcription and modulation, is, has that really accelerated the pace of discovery?

Nadav Ahituv, PhD 22:20
Yeah. I mean, what we've been doing, for example, with these massively parallel reporter assays, as I mentioned, is better understanding the regulatory code. And so we've looked at it as a language problem, and we sort of attack it in two different ways. One is, if you know sequences or that work right, if you know sort of a set of words, you can take them and basically change them until and see what happens. And so we've taken, for example, 20 regulatory elements that we know are regulatory elements, and did saturation mutagenesis on them, where we sort of mutate every possible base from, let's say it's an A to a C to a T or a G, and then test them to see the effect. And you know, with that, we can test thousands of different mutations and sort of try to understand the code. What makes that sequence work as a regulatory element, and what doesn't. You can look at it also in the opposite way. You can take a neutral sequence, a sequence that you know is not a regulatory element and then start putting in a transcription factor binding sites, basically sequences where transcription factors will bind and turn it on to make a regulatory element. And there we can play a lot with the grammar. We can put them in different numbers or different distances or different orientations, and then, you know, we could test, as I mentioned, hundreds of thousands of these, and then see what works what doesn't. And through that, we can learn about the grammar and like, do we need more of these transcription factor binding sites? Do we need certain combinations to get this to work, and so forth? And so, having the ability to synthesize basically DNA in a high throughput manner, which now we could do and get hundreds of thousands of oligos, and then having the ability to make these libraries and test them in cell culture, and has been, you know, amazing for us in order to do that.

Steve Lewis 24:09
There's a lot of therapeutic benefits to being able to work on those transcription factor binding sites and the combinations.

Nadav Ahituv, PhD 24:19
Yeah, for example, for the saturation mutagenesis, we took the telomerase promoter, the TERT promoter, which mutations in that promoter in some cancers like glioblastoma or melanoma, are one of the most common mutations ever in cancer. And for example, about 80 plus percent of patients with glioblastoma will have a mutation in that promoter. And they these are in particular, two specific mutations that either one of them make the telomerase promoter active suddenly, and then it starts working, and making telomerase basically work, and that allows for a cancer progression. So we took that promoter did all possible mutations. And of course, the top two activating, or the top two that I mentioned that are in cancer, but there's many other activating mutations, and so that helps now with patients, where you find, you sort of have a catalog of all the possible mutations, and you can see if that new mutation that's not the one that was previously found, is causing that. And then the second thing is, we see a lot of mutations that actually reduce activity of the telomerase promoter. And we could, based on where they are and what they do, we could try to understand what might be binding to the telomerase promoter, and if we can basically use an antagonist for it, we could reduce the activity of the promoter. And you can imagine using that, and that as a therapy, basically, for telomerase. And so

really understanding how these switches work would be a great way. And then you can even take it further, where you could, for example, you mentioned prime editing, you could potentially mutate that regulatory element to make it work less or more to rescue diseases that are involved with gene dosage, for example. So if you have a disease, for example, that the mutation just leads to less of that protein being made, you can mutate specifically with prime editing the promoter that will make the promoter work just much harder, much stronger, or pump up the volume, and then that could compensate for the sort of lower amount of the protein being made.

Steve Lewis 26:27
And of course, at a high level you are describing, ultimately, maybe gene therapy as an approach toward cancer treatment. Is that correct?

Nadav Ahituv, PhD 26:40
Yeah, for that, we have also used what I mentioned before, CRISPRa in the past, we were sort of the first to show for haploinsufficiency, haplo is half, insufficient is not enough. Diseases that you can basically modulate the regulatory limit to increase. And so just giving a background on haploinsufficiency, we have two copies of a gene, one from mom, one from dad. And let's say one copy is a loss of function and doesn't provide us protein, we now get 50% of the protein being made because we just have one copy. And so we reason that if we target a regulatory element, the switch of that existing copy, and sort of pump up the volume, increase it, we might be able to fix these diseases. And there's 660 diseases in genes that lead to haploinsufficiency. And so our first foray into that was just use obesity, which was an easy, sort of, phenotype where we have mice that have one copy of a gene and are extremely obese, and then if we pump up the volume and increase the existing copy to make instead of 50%, 100% we showed very nicely that you can rescue obesity in these mice, and we did it on the top two obesity genes in humans, in SIM1 and MC4R. Just to follow up on the cancer therapy, another very exciting cancer therapy that we're developing in the lab is actually a using fat cells for cancer therapy. And fat is really, in my mind, an underutilized cell type, and it has two amazing properties. One is everything is there in the clinic to play with it. You can take it out by liposuction, and you can put it back by plastic surgery, and all of those are routinely used in the clinic. The second is, and we have basically brown fat that's near our neck, and that's sort of the good fat that burns energy into heat. But we also have, of course, white fat that stores all the fat, and that's sort of the bad fat. And what's nice about fat is you could take white fat, and if you up regulate a few genes, or even one gene, you can make it more brown, and that's called beige fat. So now it starts burning energy and becoming the good fat. And so we reasoned that this could be actually a great therapy for cancer, because we could take out fat from a patient, let's say that has cancer, and change it to become, the white fat change it to become brown fat that starts burning energy, and when it burns energy, it needs a lot of glucose and a lot of other reagents, and then we put it back next to the tumor. Now the tumor, in order to grow, the cancer, in order to grow, needs a lot of reagents. It needs a lot of sugar, it needs a lot of fatty acids, but now it has cells that really out competed for reagent. They need tons of reagents to start burning energy. And so we've shown very nicely that this really nicely suppresses the tumor, and we hope to get this published soon and show that this could be a potential new sort of cancer therapy for cancer suppression.

Steve Lewis 29:46

Wow. For anybody wondering why UCSF is renowned for cancer and also gene therapy, I think we just got a lesson in a little bit of why. That's incredibly fascinating. Thank you for sharing what excites you today that maybe we'll see in 10 years?

Nadav Ahituv, PhD 30:07
Yeah, I think, as I mentioned, our ability in my field, at least our ability to find these regulatory elements is now really improved. And so I think taking advantage of them for therapy, just like I mentioned, I think hopefully that that will grow. And, you know, I hope cell therapy, as I mentioned, I think will grow. I think also, um, one thing that we're sort of missing is our ability, you know, we're going into wireless, and we're going into AI and all these things, but our ability to control and monitor, and basically change and monitor our health, needs to improve drastically, I think, and we don't have to only find the disease once we have the symptoms, we have to find it early. And I'm very excited about, you know, there's a few companies now trying to early diagnose, but I think a lot of that is expensive, not everybody can afford, and we need to really get to the ability to monitor, you know, from cell phones, if we could monitor our, you know, our own bodies in various ways. And it's getting a bit more with wearables, but I think much more of that needs to be done so we can be preemptive in terms of disease so on that I'm extremely excited about, and I think there's a lot of potential. And also using that for even controlling gene regulation, imagine if we could basically turn on a gene with our phones in our bodies and start making it do things that are beneficial for us and so and I really hope you know that is a direction. I hope you know we can get through over the years and controlling therapy.

Steve Lewis 31:42
Well, I am incredibly grateful for this opportunity to speak with you and get to know you today. It's been a wonderful conversation. We do love to finish our episodes by asking a couple of questions that hopefully will give some insight to our listeners. What has been the greatest key to your success?

Nadav Ahituv, PhD 32:04
I think for me, it's been people. I mean, I've been fortunate to have such amazing lab members in my lab. And really, you know, it's, as I said in the beginning, it's a team sport, and really working together and with them has been, you know what has made me, you know, happy to come every day to work and really get all of these things out. And I've been fortunate to have an amazing team of members throughout the years in my lab.

Steve Lewis 32:34
And what advice would you give someone who might want to follow in your footsteps?

Nadav Ahituv, PhD 32:39
Think big. Never underestimate yourself, and we also all have a sort of imposter syndrome. I still do until today, and you know, even though I've been doing this for years. So really go out and do what you want and really be, you know, passionate and do what you want to do and really follow the things you're interested in. Yeah, don't, don't be shy and don't be afraid and go for it.

Steve Lewis 33:11

That was Dr Nadav Ahituv, Professor and Director of the Institute for Human Genetics at the University of California San Francisco. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers and good science.