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Interview by Ute Deichmann with Ellen Rothenberg

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Ellen Rothenberg joined the Division of Biology of the California Institute of Technology in 1982, since 1994 she is full professor. She is one of the leading molecular immmunologists, focusing on gene regulatory mechanisms for T-cell development from stem cells.

From biochemistry to molecular immunology

UD: You are one of the leading molecular immunologists today, and one of the few women in the field. What did you study, why did you choose molecular biology, and why immunology as the main fields of your research?

ER: When I was a kid, I wanted to be a physicist. I was extremely interested in physics and I thought it was fantastic. But this is just when I was young. I started to get a little bit more of a historical sense and realized that the discoveries in physics that I envied people for were in days earlier in the 20th century and not necessarily still waiting for me at the end of the 20th century. But that was really a little bit childish. By the time I was in high school I had a wonderful biology course with two teachers, a senior male teacher and a lively young female teaching assistant. The course was very biochemically oriented for a high school class at that time - this was maybe 1966 - and they were beginning to have an idea about, interestingly not yet DNA and RNA, gene expression and analysis, but some palpable sense of how protein structure contributes to function. It was extremely exciting to think that molecular structure could confer living properties on something. And so I got fascinated with biochemistry, and by the time I came to Harvard as an undergraduate, I thought that I wanted to be a biochemist, a structural biochemist. Not in the way that people are crystallizing everything now, but I was just really interested in the deductive pathway towards protein structure and function and using different kinds of perturbations like modifications of certain amino acid side chains to map the residues that have to contact each other.

I didn't actually know what kind of biology I wanted, though, because I was so ignorant still at that point. And what made a big difference to me was that I was given a marvelous undergraduate tutor at Harvard. They had this program where you would be a student taking classes, but you would also have someone who met with you one-on-one, a little bit like the English Oxford system. And I got a magnificent stroke of luck; I got Boris Magasanik as my tutor. Boris Magasanik was a Hungarian Jew who had come to America in 1938; one of the last chances to get here.

UD: From Vienna?

ER: From Vienna. But he was originally Hungarian and had come here via Vienna. He had become a very close friend of Salvador Luria - they were both faculty at MIT. But Boris also had a joint appointment with Harvard, and that was how I got connected with him. He is a magnificent person, because he has this idea about decoding of complex gene regulatory networks, all the way to the way they propagate into the complex regulation of the activities of the proteins that are encoded. [NB Boris Magasanik died just a few months days? after this interview, on December 25, 2013]

UD: But gene regulatory networks were not yet known then.

ER: That's right, they weren't really. But he was working on glutamine metabolism in bacteria – basically nitrogen metabolism. And it turned out that there are many levels of regulation for the glutamine metabolic enzymes and glutamine synthetases, both at the level of post-translational regulation of the proteins that change their enzymatic activity and at the level of transcriptional regulation – which depends on whether there's enough glutamine in the cell. And so early on, he was doing Jacob/Monod related work. He was part of that circle, I think, of intellectuals. But he applied it to a system in which transcription wasn't the end of the regulation. He took enormous pleasure in how the higher-order regulation of nitrogen metabolism in general included layers of transcriptional regulation, included layers of metabolite transporter gene regulation, and included levels of metabolite-based enzyme modification regulation. He loved the logic of this and he loved the idea of a control system. Not just a simple mechanism, but a whole system.

Now, he never talked in those ways about his work. But he asked questions. He was part of this fantastic tradition of asking questions. And I think under his influence I got more and more interested in gene regulation as something that

wasn't solved yet – something that was still in the future. But he led me in one strange direction, which was to encourage me to apply for an M.D./Ph.D. program at Harvard and MIT that they had just set up.

I was still very unformed. This is all a way of telling you how slowly I came to the idea of what I was interested in, in Biology. I loved the challenge of logic, but I still was in love with biochemistry. But I also found schoolwork easy, and I thought, "oh, wouldn't it be nice to have an extra degree." So going to medical school was a huge event in my life, because I finally discovered what it means, really, to hate what you're doing. It was amazing. I mean, within three weeks I was fantasizing about suicide, because it was so anti-intellectual. It was all about authoritarianism and about rote memorization. And I realized that there are some things I hate so much, even in something nominally close to my work, that I won't do them. So now suddenly I have to actually find what I do like. And I was taking a couple of courses along with this medical program. One was immunology and one was virology. They were both fascinating. I don't think that I really took the immunology course the right way, but I fell in love with the virology course, and that drew me into David Baltimore's lab. And so I decided that I would switch to becoming an MIT graduate student so that I could work on viral genetics. And what I loved, again -

UD: You went to MIT in order to embark on viral genetics?

ER: Right. I basically dropped the Harvard M.D. part of my program and just embarked on this. And what had happened was that David Baltimore's courses made the regulation, again, of the virus's lifecycle so lucid. It was so clear and the notion that you could account for these complex host-viral interactions could just be understood so beautifully, and you could ask questions that were logical questions and you could answer them.

UD: Baltimore was at MIT?

ER: Yes, Baltimore was at MIT and he was a fairly young, but very successful professor there. But it really wasn't until I was a graduate student at MIT that I started taking courses that gave me the background in molecular genetics, in gene regulation, molecular biology – really delving into these things from a regulatory system perspective. And so my interest shifted more and more in that direction. It still took a while because my PhD was about retrovirus molecular biology and biochemistry of retrovirus replication. But I had developed more and more interest in immunology and in the regulation of cells in the immune system and

developmental regulation. This was also when I was reading on my own to prepare for my candidacy exams, and that was when I encountered Eric Davidson's papers and became very fascinated with this notion that you could embrace the whole genome.

UD: So he didn't influence you; you encountered the papers because you already worked in this direction.

ER: Right. I met him many years later because I had been fascinated with these papers which seemed to be giving you a way to think about complicated eukaryotic genomes in a way which the prokaryotic systems clearly did not represent. It was clearly a new area. So that was how I got into that. But by the time I finished my PhD at MIT I had done a lot of work on the molecular biology of these retroviruses and their interaction with the cells, how these viruses modify the cells. My classmates and my colleagues were all involved in the creation of the field.

It was fantastic. My friends were the first people to clone the oncogenes that were picked up because they were recombined into the retroviral genomes. I knew all of those people when that was going on. It was incredibly exciting. I was the first person to actually synthesize, in vitro, the whole genome of a retrovirus – clone it and show that it was actually infectious. The DNA that I made in a test tube was the life of this virus. It was fantastic.

It was an extremely exciting time, and virology – this was before there was a lot of cloning of eukaryotic genes – viruses were the probes that one had for genes in mammalian cells. And so many, many things were discovered in viruses which then later turned out to be rules for mammalian cells generally. Like splicing: RNA splicing was discovered for viruses, and I did the first heteroduplex analysis of showing the mapping of the spliced transcript against this in-vitro made, full length cDNA that I had generated. It was an incredibly exciting time to be a molecular biology student. And the horizon opened up forever. There seemed to be an extraordinary number of problems. Then I just fell in love with immunology. I thought it was interesting because the cells were so interesting.

UD: You just talked about the fascinating research field of viruses and what you could do with viruses that is also interesting for the eukaryotic cell. Now you moved to immunology. How did that happen?

ER: Well, at that time the viruses were not only interesting as viruses, but they were interesting as a window into eukaryotic cell biology. It was amazingly powerful, because you could get an entire self-contained biological system in this tiny little

package, with a genome of only 10 kilobases. So you knew that all the accountability for all the things that happened with this virus had to be contained within this tiny distance, which even the primitive tools of that time could address. A lot of people like me were interested in this, not because we really wanted to cure viral diseases, but because we were really entranced by the idea of being able to get into the mechanism of the eukaryotic cell, and this was a probe that you could actually manage. Things moved extremely fast and it was very exciting. Cloning was published in ’76, and the first cloning facilities at MIT were in ’77. I had just gotten my PhD in ’77 and we were all discussing what we would like to do with this.

At the same time, it was already known that there were some really interesting cellular systems out there where even with primitive tools, before cloning, a cell would specialize in making so much of one kind of messenger RNA that you could just look at its properties in the total RNA of the cell. One of those was red blood cells and the other one was immune cells. And so people already knew that immune cells were really interesting. And I loved that fact that these cells were active – compared to red blood cells, which are like zombies, they’re basically already dead. They have no nuclei, they’re doing nothing.

UD: Except in chicken.

ER: Yes, right, but those are dead nuclei too and they are compacted and they’re not doing anything. Whereas immune cells do “everything”. They seemed like the most marvelous kind of cell to combine the properties of the mammalian cell, with all the intricacy of their regulation, together with the autonomy of bacteria. These cells make decisions as single cells, they move around the body as single cells, and they make decisions to divide or to die as single cells. And so it seemed like the absolutely ideal cell type to go between the mammalian system, which still seemed very intimidating at that time, and also the microbial world.

And as I was saying last night, MIT was very, very, very focused on microbial systems. Salvador Luria, Boris Magasanik, many other people there, Malcolm Gefter, they were focused on prokaryotic systems or single-cell eukaryotes. David Botstein was just starting to do yeast. But it was all focused on the power of microbial genetics. Single-cell colony formation. All that stuff.

So lymphocytes are the one kind of cell that acts like microbes even though they are mammalian cells. And the final connection that made it so interesting was that these viruses that I had been working with were called murine leukemia viruses.

They caused leukemia by immortalizing immature T-cells. They are famous for doing this, and I had been working with these viruses for my whole PhD. But what was different about these viruses from other cancer-causing viruses was that other cancer-causing viruses pick up cellular oncogenes and that is what makes them oncogenic – that's was causes the cancer, when they transport them to other cells. These viruses that I was working with did not have that property; they did not pick up an oncogene from the cell. They were basically just going into the cell and doing something which must be taking advantage of some feature of the cell's own biology.

And so these were turning immature T-cells into tumor cells. They must be taking advantage of a feature of the immature T-cell, which was bringing the cell close to the edge of cancer already, so the virus didn't need to introduce an oncogene. It could just do something smaller than that. That made me interested specifically in T-cell development, and so I went into immunology really not for medical reasons and not to study host responses to antigen, but because they were the cells which were, first, able to do things on their own and, second, which were obviously flirting with the edge of cancer. Right on the edge. But normally they would pull themselves back; when they had this virus in them they couldn't pull back and then they would fall into cancer. This has actually turned out to be a pretty close to correct view of these cells. I have remained fascinated with this question, learning more and more and more about the development of these cells. It is a long story of how I got into this, but then I have been working on the same thing ever since.

As a woman in molecular biology

UD: May I ask a social question? MIT at the time, probably today, was a male dominated institution. Did you encounter any problems because of this?

ER: Well, they probably had problems with me also. We all came from these "tiger mothers" a little bit - I didn't realize then that it was a phase of culture at that time. But this whole generation of women after World War II who decided to stay home and raise children instead of having a career themselves, some of those were very fierce and ambitious women. My mother would never have done that if she had lived in a different decade, but she lived in that time, and so she brought my whole family up on a diet of "not Mozart, but Beethoven". There was this heroic sense of one's potential and we were all going to be heroes. We had a sex-blind upbringing; we were all going to be heroes, we were all going to be pioneers, we were all going

to be discoverers, we were all going to be Einstein, we were all going to be whoever.

UD: So it was your mother's influence which gave you the strength?

ER: Well, and my father. He brought me up as a son. He taught me advanced math and logic to the point that I got in trouble with my teachers in school. And we did a lot of things as a family away from the rest of the culture. So I'm sure I wasn't alone because a lot of the women of my generation came into Harvard and MIT very ambitious, very confident, and nobody knew what to do with us. But we were also expecting to be heroes. We were not expecting to be embraced; we were expecting to be heroic. Of course we ran into all kinds of problems, but I don't think that we had this victim complex that people have now. We were proud of taking on heroic challenges and overcoming them. We were proud of being the first. We didn't want there to be a lot of others, we wanted to be the first. We wanted to break through.

And we loved torturing the men. Because they didn't know what to do with us. We made all kinds of mistakes, but we loved confusing them about how they were supposed to respond to us. There were no codes, and it was a very wide-open time.

UD: It fit the codeless culture of molecular biologists, didn't it?

ER: Yes, it was very exciting; it was really fun.

UD: I remember that when we met some years ago, you talked about Francis Crick having had very nasty attitude towards women colleagues.

ER: Oh, he could be a jerk. He's a jerk. He was ridiculous! But you could see that it was a cognitive problem. Really, that he could not actually – because he had this reputation as a ladies' man. So he was supposed to be very gallant, but only to women he didn't think were scientists. So he was a scientist to scientists but a ladies' man to ladies. But there was a categorical contradiction in his mind. And so when I was a faculty member at the Salk Institute briefly before coming here, I remember sitting next to him and having a scientific discussion across him. And I remember him looking at me with this weird expression. He could not talk science with me. Everyone else could, but he could not. He just stared at me. I was a cutish little thing; I was small and peppy and slimmer than I am now. But obviously I didn't fit into one of the categories that he could handle. But you realize that we took a certain amount of pleasure in upsetting people.

UD: Yes, I understand that you didn't consult with women's rights organizations.

ER: No, no. In fact I could not understand this when it started happening, because I could not understand how these women could be so weak. And yet it has really been different. We obviously made mistakes all over the place and we made weird choices, but we were very proud of ourselves and we loved being proud of ourselves.

UD: How was David Baltimore? How do you remember him?

ER: He is a very complicated person. He is very tense, very smart guy. He was exhilarating in terms of how quick he was. When I was in his lab, I wanted constantly to prove to him that I was smarter than he was. Needless to say, this did not endear me to him! But it was incredibly exciting because he created a very critical, very dynamic atmosphere. I think in retrospect, though, he was never very interested in talking with me about the aspects of an experiment when you don't know yet how to make it work -- if you are starting on something really new and there is a lot of biology to learn.

UD: What background did he have in science?

ER: Virology and biochemistry.

UD: Not physics.

ER: Not physics and not really biology. And not genetics either – really biochemistry of nucleic acids.

But he was incredibly exciting and the people in his lab were phenomenal.

Molecular biology and immunology

UD: I'll come back to your work of today later. Now I would like to ask a few questions about the recent history of immunology, in particular, how did molecular biology influence immunology, and how did immunology influence molecular biology? All those exceptions in immunology rendered molecular biology much more complicated, didn't they?

ER: Oh no, no, no, it was wonderful. It was very, very early. Susumu Tonegawa found out about the rearrangement of the immunoglobulin genes in 1975, '76, and this was before cloning. And he could do this even with the incredibly primitive methods at the time. He did get the Nobel Prize for it, so he's easy to find. But the interesting thing about this was – there were a couple of things. As eukaryotic molecular biology developed, it was breathtaking how fast things happened. So

splicing just came in 1974. And as late as when I was a graduate student, one of the things that we were taught, in 1972, ’73 was the colinearity of the gene with the protein and the transcript. But splicing already in 1974 broke this. This came from virology; it came from adenoviruses. This was one of the major things that was unthinkable in mammalian cells, but viruses are allowed to be weird. So it was accepted in the viruses, and then after cloning came in and you could get the equivalent molecules from the genome, you could see that this was a general phenomenon. So that was one violation, that was ‘74.

In ‘75 Susumu Tonegawa discovered that not only is there splicing at the RNA level, but there’s actually rearrangement at the DNA level. He was expecting that he was going to get another case of splicing, but instead what he found was that the DNA from these B-cell leukemias that were clonal had actually a different DNA structure from the DNA in all other cells in the body. So this was very early – this was super-early. I think, it actually was one of the things that catalyzed the interest both in immunology and in the power of molecular biology and what you can do. And then when cloning came in then you start to ask these questions. OK, what about spliced RNA structure versus the gene, and what is a gene – is the gene invariant except in lymphocytes and so on. So that was an unbelievably exciting time. So ’73, ’74, ’75, ’76, ’77 – amazing times to be in this field. The excitement, of course, from immunology was to understand the nature of these rearranging structures. So molecular biology and that Tonegawa result created an explosion of interest in the immune system.

For Baltimore’s lab, by the way, its greatest years were after I left – in the ‘80s. Because they then embarked on an absolutely beautiful, beautiful project to find the enzymes that were responsible for causing these DNA rearrangements, which they actually found. Then they found the transcription factors that regulated the expression of these genes in B-cells. And I think he shouldn’t have gotten the Nobel Prize for what he got it for -- he should have gotten it for that. That was a brilliant decade of work. Almost all of my best colleagues in my field today came from his lab or Phil Sharp’s lab, his neighbor, in those years.

So again, he was really following the notion that you could look at these immunoglobulin genes with a kind of self-contained quality that he had brought to thinking about viruses. He basically transported that to these rearranging genes. Let’s look at cells for which their whole role is just what they do to these genes. Zoom in. Now at that time when I started working on T cells, he was less enthusiastic about that. Because T-cells don’t operate that way. T-cells have

rearranging genes, but most of what they do is much more complicated than what B-cells do. David was mostly interested in B-cells because he could use the zoom in strategy to distill the whole function of the cells into effects on a very defined set of genes. That was another thing that we didn't totally agree about.

One of the other things that influenced immunology at that time, and that's very weird, is the Vietnam War and the whole Cold War, because the entire focus was on these rearranging genes which coded for receptors ***against*** whatever the cell is recognizing. These phenomena were always described in terms of foreign, pathogenic agents. This was how foreign antigens were recognized. Now, antigens really are just anything in the universe that these cells might happen to have a receptor to recognize. But the whole rhetoric of the field was built up around these wartime metaphors – "foreign antigens" – and there were these specialists who were all targeted on specific foreign antigens, foreign antigens, foreign antigens.

UD: But metaphors alone don't drive research, perhaps they helped to receive more funding?

ER: But this was the one toehold that people had on the field. So it has become interesting since then, because the whole paradigm has shifted and people have realized two major things that were not at all appreciated at that time. I think the combination of wartime metaphors and the fact that it fit with these receptors, drove the science, and this is exactly what these receptors are for. They are carefully selected in the developing immune cells so that any immune cell that has a receptor that would recognize yourself is killed. So only the immune cells that have receptors for "foreign" are allowed to live and defend your body. This part is true, but we now know about additional types of immune cells and cases where more violent response is not better. More recently, the emphasis in the field has shifted toward understanding how the immune system normally prevents itself from unleashing responses that are too destructive for the host, how we manage to avoid autoimmunity or chronic inflammatory disease. The immune system in normal people is amazingly self-restrained.

UD: This fit is amazing.

Fritz Melchers, a pupil of Max Delbruck, told me recently that research in the 1970s with the aim to find the T-cell receptor led to a crisis in immunology. Many people claimed to have found it but nobody really had. Only with new molecular biological techniques was it found later on. I would like to know whether you know of more dead ends in molecular immunology that is research in which people

really went into the wrong direction until the problem was solved in a very different way.

ER: Wonderful. The biological questions were all good questions, and they went on really illuminating what people have done except for this paradigm about foreignness, which I'll come back to later on. But the T-cell receptor was difficult to get because people really wanted it to be related to the immunoglobulin. And, in fact, it was. In fact it is. But there were two problems. One was that when T-cells develop, their mature function does not involve making hundreds and hundreds and hundreds of thousands of the T-cell receptor and spewing them out in the world. It means making just enough to use as cell surface receptors. And you can get away with a very low level of RNA to do that. And with early technology it was very, very difficult to find anything that was expressed unless it was at a very high level. The other thing was that, honestly, the relationship between the T-cell receptor and the immunoglobulin – you can see it at the protein structure level, but it is not high enough percentage identity at the sequence level to be detectable by nucleic acid hybridization. I think it's probably less than 30% identity at the amino acid level, and even 100% identity at the amino acid level can be 30% mismatched at the nucleotide sequence level. So it was just way beyond the threshold of what could be detected and that's why couldn't find it with that old technique.

UD: But they claimed to have found it - that was what is so interesting.

ER: Well, they tried and there was some bad work that was done and some of the stuff on possible "Suppressor Factor" was horrible. That was the other thing. They knew that there were a lot of complicated responses of T-cells that didn't just go in a linear way. You didn't just have a situation where the more T-cells you added the more response you got. You had all kinds of suppression effects. And looking back on what we know now about these populations, it's amazing that they got anything to work at all. They would take I don't know what from the supernatant of these cultures and they thought they had suppressor T-cells.

Now there are suppressor T-cells, but they completely misidentified them. They didn't have any methods for studying them. No one had ways of cloning T-cells. No one had ways of understanding what T-cells' functions were because they had nothing to do with secreting their receptors. So there was a lot more cell biology to learn about T-cells before people could reconstruct this. And these problems got solved later when people were able to clone out individual T-cells and look at how that T-cell clones' DNA differed from other cells in the body and also what genes

T-cells express that are different from the genes that other cells express with a subtractive hybridization method like the one developed in Eric Davidson's lab to isolate the genes. So Mark Davis and Steve Hedrick, who used subtractive hybridization to clone the T-cell receptor genes, were really important for the field, and also the Kappler and Marrack lab, who took this other approach of directly finding the proteins that formed these clonally specific receptors by making monoclonal antibodies against the cells. This was also an incredibly important approach.

What Kappler and Marrack did turned out to be very important technology. If you don't know anything about distinguishes one cell from another, one way to do it is to look at all the genes they express that are different from each other. But in those days they didn't have very good techniques for that. So what they did was they said, "look, this is going to be a cell surface receptor". And the monoclonal antibody-making strategy of Cesar Milstein and George Kohler meant that you could immortalize cells that made an antibody with a particular specificity. Now it was a reagent and you could use that forever anywhere in the world in an unlimited quantity to always identify the same molecule. So it became possible then to ask, "If you make 1000 different monoclonal antibodies against immune cells, 10 microtiter plates full of them, which ones recognize T-cells but not B-cells? Which ones recognize T-cells and not fibroblasts? Then you could start zeroing in on them. And eventually they found some that were specific for some T-cells and they realized these are recognizing clonotypic T-cell receptors.

UD: Which role did or does, the so-called Lamarckian concept of Linus Pauling in the 1940s play? He claimed that antibodies received their specificities by a special alignment or shaping to the antigen.

ER: It died. It really died.

UD: Yes? Dan Tawfik, in the Weizmann Institute, quotes this research somewhat approvingly. I think, it was simply bad experimentation of Pauling."

ER: You know, it was so hard because every immune cell population, until recently, was very heterogeneous. And so you had mixtures of cells that had different receptors and mixtures of cells that had different functions. And the behavior of the population was extremely complex and affected by many, many, many different complicating factors. So people did many experiments, but really they could not have been good experiments in those days. They were these frontier-breaking experiments; they were trying to explore the unknown. But they never were going

to be able to get the right answers completely at that time. And it's just one of those things that just needed more stepwise work, one thing building on another. So these populations of antibodies were certainly very different in what they recognize, but until the importance of cloning the cell was identified, you could not really tell what the structure of the antibody was that was doing the recognition. What has happened since then is that it has become clear that there is a kind of templating that goes on, but it is a totally different kind of templating. It's these rounds of somatic mutation that the B-cells go through when they're already responding to the antigen, each round followed by selection of the cells with better antigen recognition. So it's not a structural thing, but it gives the output that you would get – if you didn't know the sequence of the protein it gives you an antibody that looks as though it's been molded to fit the protein better.

UD: Pauling did it in the pre-molecular time.

ER: Right, and they couldn't.

UD: There were chemists who clearly showed it did not work.

ER: In these labs, these are sometimes ideas that people have and they just -. It's funny because all this happened long before I got into the field.

UD: Another question: How important are changes in chromatin structure like histone modifications -

ER: Huge.

UD: -in the development of the immune cell? I thought it is one of the fields where the impact is-

ER: Enormous, enormous, enormous. It's really obvious. And the great example is that both B-cells and T-cells use exactly the same enzymes for rearranging their receptor genes. They have exactly the same specificity, they recognize exactly the same nucleotide sequence. Yet B-cells use these enzymes to rearrange immunoglobulin genes and T-cells use them to rearrange T-cell receptor genes. The difference comes because different parts of the chromatin are open. So in T-cells the chromatin around the T-cell receptor genes is open, and in B-cells the chromatin around the immunoglobulin genes is open. Now the reason for that is because of the transcription factors that are expressed in B-cells versus T-cells early on. This is all early in their development, so they start out with the same precursors and then the B-cells turn on some transcription factors; T-cells turn on others. And they start to work to make different parts of the genome accessible.

But this is not back from the embryo; this is relatively late in development. And then both of them, in parallel but through slightly different machinery, turn on the same enzymes. But because the T-cell transcription factors-

UD: And how do they know?

ER: The genes that code for these enzymes use enhancers that are actually using some transcription factors that are shared between B and T cells. So both cells turn on the RAG-1/RAG-2 recombinases. But where those enzymes get targeted then is different in the B-cells than the T-cells, because the B-cell transcription factors open up different parts of the genome than the T-cell transcription factors. And the T-cells rearrange T-cell receptor genes and B-cells rearrange B-cell immunoglobulin genes.

So it's a very lovely thing and people are learning more and more about how that works. But the other thing is that you can see that the boundaries of the domain that's available to be rearranged are set by these histone modification marks. And the CTCF, which is the looping factor, defines all these regions of the DNA as being within one domain. Everything outside has different rules at that moment. The immunoglobulin gene complex has magnificently beautiful domain boundaries and I think a lot of the elegant and informative work about this really came from the B-cell immunoglobulin field. And then the T-cell stuff afterwards. But the B-cell immunoglobulin data are just gorgeous. I mean, these modifications are like little walls around the regions of the genome that are open for rearrangement and keeping away the ones that aren't. It's very, very nice. So this system has been a great piece of validation for these histone marks.

UD: And what regulates those marks?

ER: The transcription factors basically change the marks.

UD: Where is the overall beginning of the regulation?

ER: This is my territory. So you start with the stem cell. It is capable of giving rise to both B-cells and T-cells and many other kinds of cells like the innate immune cells which are now very, very hot. Because people suddenly realize that our macrophages and granulocytes that fight inflammation are also an incredibly important part of our system.

But T- and B-cells start out with precursors that have many shared properties; they can give rise to lymphocytes and they have some transcription factors in common. The cells that are going to become T-cells become different from the others

because they migrate to the thymus which gives them signaling from a pathway called the Notch pathway. And it's that experience that changes them. At that point, instead of having the same transcription factors that B-cells turn on, they turn on different transcription factors, GATA 3, TCF-1, and that puts them on a different pathway. It also squelches the transcription factors that B-cells would turn on.

So right now you have two kinds of cells that have this shared heritage, but now they're expressing – the B-cells go on to express the transcription factors that they would have expressed by default, while the T-cells are turning on these special ones from the thymus influence. And in parallel they start working on activating different sets of genes. But because you have different combinations of factors – the T-cell combination includes GATA-3 and TCF-1, the B-cell combination includes EBF1 and Pax5 – and those don't overlap even though the other factors do, that targets even the shared factors to different parts of the genome. And some of the parts of the genome that they open up, then, are these regions that code for all the possible elements of the immunoglobulins or all the possible elements of the T-cell receptors.

It is at that time when the difference between these cells is really highly established. Then the transcription factors, probably the ones that they both originally had in common, get, for some reason, deployed now to turn on the recombination machinery (RAG-1 and RAG-2). And it's still not clear why this step waits so long, and why it waits to the equivalent stages in what are now two different programs. At that point the products of those genes are turned on and they start the rearrangement work on whatever is open, immunoglobulin or T-cell receptor genes. But that decision has already been set up for them by the action of the different transcription factors, so these enzymes don't have extra degrees of freedom. So it's very interesting, this whole thing, because these cells have a chance to develop a difference in what genes are permitted to be rearranged, but then they can go back and use common parts of the toolkit to work on those different genes, and then finish their development.

So this business of how they become different but maintain some elements of what they have in common is absolutely beautiful. To me, this is one of the most exciting things about the field that I study. These cells also have a big overlap with the cells that go on and become macrophages, and some of the B-cells keep that overlap for a long time; they have some shared features with macrophages all the way out into their functional roles. T-cells keep features shared with macrophages

for a while and then they shut those off. That is another thing that is different. So there is this whole general immune cell precursor population that then subdivides and specializes through the kind of processes that Eric Davidson studies in his embryos.

Perspectives

UD: What is your next aim?

ER: My next aim is to explain all things I was just telling you. That is, we think the T-cell case is a very good illustration because some of the features are easier to observe carefully than in the B-cell case. And we have very beautiful ways of tracking how a certain gene's activity in one cell affects the ability to turn on other genes in those cells. But it's not as easy a system as Eric's system.

UD: That became clear at the conference. I remember that you said that your system is not so hardwired as his.

ER: There are things that are hardwired about our system, but there's also this long period when the cells are delaying the ultimate decision of what they will be. And they keep open these options. I think that that's partly because the blood cell system is trying to balance production of many, many cells all the time with the decision of which fates are the most important for the body to focus on at that time. And so it's useful for the organism to have some flexibility and to extend the proliferation of the offspring of the stem cell. Let that happen for a while before you absolutely say, "OK, you guys have to go to law school, you guys have to go to medical school."

UD: But how can this flexibility be selected in evolution?

ER: Well, it's ancient in evolution. I think part of it is to set it up so that the different cell types can use some overlapping properties. Let me say how it's different from the embryos. When Eric's embryos make a boundary between two cell types, the boundary sets up so that the genes that are expressed in one type basically shut off everything that would normally happen in the other, and vice versa. So you cannot have a cell that is expressing both. But one of the things that the blood system in general takes advantage of is, it says, "those are shared functions, yes." The factors that are expressed in this cell type can block the expression of the factors that drive genes used in the other. But we're not going to make that absolutely an intrinsic property of that transcription factor. We're going to add another component to it;

we're going to make it depend on another protein that has to bind to the transcription factor. And then you could express the transcription factor with or without that other protein, or with more or less of it. So that the one transcription factor can actually have more gentle effects on the genes coding for the others, letting the cells keep more options open, at different stages in differentiation. This is one mechanism. I think there's still a lot of interest in understanding whether that's really in general the answer to the question. That is, is the flexibility in development really always because these are collaboration-dependent repression events, but direct activation events? So you can imagine how that might work. And, I think, one of the reasons we're trying to connect the activities of these factors with where we see them binding their DNA is we really want to understand more of the rules that govern when they work as unequivocal activators, when they work as conditional activators, when they work as unequivocal repressors, when they work as conditional repressors.

I think it's a very exciting frontier for molecular biology because it's actually talking about – our lives depend on the cells getting these decisions right, in the right balance. But it's an area of molecular biology that you don't really see people talking about when they're just thinking about these all-or-none choices – like I'm going to be a gut cell or I'm going to be muscle cell.

UD: It looks like a real challenge to the established molecular biology.

ER: Yes, but it pushes you into new areas, so it's exciting.

I have to finish my metaphor about the war. After the Vietnam War, and after people realized that these immune cells – some of them could actually exist with receptors against self – and suddenly, "Oh my God, how can we have subversives in the body?" And so one of the things that people suddenly realized was that suppression has to be real, but they gave it a new name. There have to be some cells which prevent their neighbors from attacking yourself. That is, some T-cells whose job it is to prevent other T-cells from making a mistake and attacking your own body. And as people stopped having infections so much and started having autoimmunity, people realized that you can get sick from not having suppressor cells as well. And so the whole field of T-cell immunology has shifted to having a huge emphasis on "how do you restrain immunity?" So in the Vietnam War days and the Cold War days, it was, "how can we be strong enough - have a huge strike force to fight off the foreign enemy?" And now what everyone is saying is, "how can we prevent autoimmunity?" The whole field has changed.

The other thing is that people suddenly realized how powerful these innate mechanisms are. The sad thing is that when people discovered these rearranging B-cell and T-cell receptor genes, they completely disrespected macrophages because they felt that macrophages don't do this, so they must only be stupid cells. Knee-jerk stupid cells. Well, it turns out that now that we know something about them, they do incredibly sophisticated signal processing to figure out exactly how they're going to respond in different situations. We would also die without them, but they are very sophisticated in what they do. People are really realizing that they also give clues to the B and T cells about whether a response is even warranted in these situations or whether it would be better not to do anything. They also play a huge role in picking up whether there's cell death or whether, basically, this is a cell type that should be left alone because it's just normal. You don't want pregnant women to kill their fetuses because they are foreign.

So now, suddenly, macrophages have the biggest respect in the field – no one cares about T-cell receptor and immunoglobulin rearrangement any more. Only old fogies (like me) care about that stuff. They don't even teach it in the immunology classes with very much significance any more. All the emphasis is on, "how do macrophages tell when to attack and when to hold back the response?" "When do regulatory T-cells succeed in repressing their auto-immune neighbors?" So the whole story has changed to one about maintaining health against having too much auto attack. This sort of disrespecting now of the immunoglobulin gene rearrangement is very sad to me because the rearrangements are a great story and had such great influence in the field. But it is quite ironic that these things keep changing around and everyone goes back to Metchnikoff's original pictures saying, "This wasn't about B and T cells, this was macrophages that were doing this response, and that was the basis of immunology."

UD: This looks like a fashion that reflects on developments in politics or society.

ER: Yes, it was very funny. Teaching this over a 30-year period, the fashions have changed so completely it has been very interesting.

UD: But you have been going more or less into the same direction, right?

ER: Yes, maybe I am actually learning some answers, which is a pleasure. It's an interesting system. These T cells can make so many decisions about growth and death, and carry out so many functions based on their own computation from environmental signals -- they are so fascinating, the more you learn about what

they do, the more the questions become interesting. So I haven't stopped being interested in them even though the answer fans out in a lot of directions.

UD: It will be interesting to see from where progress will finally come.

ER: People are doing a lot of things that are helpful. The trouble is that because these cells have this wonderful "microbiology" property of working as individuals, the population response can be dominated by a few cells that do the wrong thing. So it makes it very challenging for the medical profession when they try to – they say, "Most of these cells are this kind of cell. So we're going to block that kind of cell." But it might be that the ones that are causing the trouble are a minority and you don't even pay attention to those guys. And so you don't block them, or you don't stimulate them when they should be being stimulated.

Impacts on human immunology

I feel bad because there's a lot of emphasis now – suddenly everybody should start working on human immunology and take money away from what I work on, which is mouse immunology. But honestly, you should feel very happy about this, because what they're discovering is that there are reasons why some of these early translational approaches didn't work. The reason is that the human immune system and the mouse immune system are not identical at all. The elements are the same, but the immune system is a very evolutionarily flexible part of an organism. And many of the ways that human immune cells interact, exactly which factor has the dominance and which one is more recessive in humans is different from mice. So they're finding that if you know more about the human system you actually can make much better guesses as to what will work for people, and so I think you should feel at least somewhat optimistic that things are moving. Every day they say, "Oh my god, we always thought it would be this way, but in the human it's this way." So we now have to go back to the drawing board. Now that they realize it's different, they realize they have to make different model systems and they have these very fancy mice that are set up to have pieces of the human immune system in them. So they're trying to set up more and more tools for testing things in a humanized mouse.

UD: It's so amazing what is possible now.

ER: Yes, human immune systems in mice. So I think that they're finally getting better ways of asking the right questions to help. It's just like when you think about the

Pauling hypothesis about – we didn't know enough at that time to understand how it could work. And it turned out that it involved a lot of cell biology that was being interpreted as though it was protein chemistry. It wasn't even molecular biology; it was cell biology and molecular biology affecting the protein chemistry. And there was no way that Pauling could have known how this could work at that time, so-

UD: No, he couldn't have known. But still, I am very critical of-

ER: He was wrong, but -

UD: No, not because he was wrong, but because-

ER: He was too arrogant? -

UD: Yes, he did not react to the critical responses. And there were people who clearly showed that it did not work. Without knowing why not, that's another story. But they showed it didn't work.

ER: Well, that's a classic thing. There are famous people now who are the same way. "I published it, therefore it must be right."

UD: Pauling loved the idea so much; he was always in love with his ideas. And often they were right of course, but -

ER: - not this time.

UD: But not this time.

ER: They are making a lot of progress, very interesting, on what they're learning about how these systems where you can really model more of the real human response – what real human T-cells are going to do, and the human innate cells that are very important for this response. It is potentially very valuable. They are learning lots of things that are totally surprising, and violating a lot of dogmas.

One thing I just learned in the last year – it turns out that the antigen presenting cells in the liver – we thought that they were constantly being produced from the same blood cells because they're macrophages and macrophages come from the blood. It turns out – no one knew this until just this year – that a lot of the macrophages in the liver, the Kupffer cells, actually come from a very special set of blood stem cells - only stem cells that were produced in the embryo before birth. The stem cells that we are making right now in our bone marrow are not helping to produce those Kupffer cells in the liver. Maybe some of the pathology of some virus diseases affecting the liver comes from harm to those liver macrophages that can't easily be replaced. But then maybe if you know this, maybe you can learn

what is different about those and find a way to make them in culture and then put them back in. You can imagine that maybe this is one of the reasons your immune cells can't normally defend you in a situation like this, because if the virus is killing those special macrophages, the new ones aren't substituting for them.

UD: Because they would have to be produced in the embryo -

ER: So you may have to mimic that program. These cells stay there for the whole life and no one knew that. People are absolutely amazed.

Also, the macrophages in your brain, the microglia. It turns out that those also come from the first wave of blood cell development. And obviously that is not helpful in itself when you're an adult, but it means that you can now say, "OK, what's different about them? How can we modify the adult type to make them like that type so they can fulfill that role?" And now you've got something rational that someone can try to do, and it may be quite simple. I think people are able to ask questions that might be much more helpful now.

UD: Involving different lines of research.

ER: Well, bringing them together. Bringing the cells, and the embryology, and the molecular biology together. The best way to change a cell so that it acts like a different kind of cell is by gene modification. Now that people can do that, you can imagine them repairing cells which have been damaged by putting in cells which you have modified, in culture, to match the right set of gene expression patterns. I think they are going to be able to do a number of things that would help. I think this would be fantastic.

UD: It is really like a detective story. Or like many of them together.

Can you imagine that one day the whole molecular biology of immunology will be much simpler? Can be reduced to a few basic mechanisms?

ER: I think a lot of the complication has to do with controlling it. Controlling it not to be always activated, but activated at the right time. And that makes it kind of difficult, because it can't know all of the circumstances. You can't inherit a gene that tells you all the time, "Don't attack this cell," because sometimes you want to attack it when it's got a virus in it. But not even all of the viruses. Some kinds of viruses it's better not to attack. Leave the cell alone; the virus is not doing so much harm – leave it alone. There's a lot of conditionality, and I think that a lot of the complex parts of the system have to do with sensing that and making the right choices.

UD: I thank you very, very much for sharing with me this fascinating information and thoughts of yours!