Rewriting Textbooks With Single-Particle Tracking Microscopy

INTERVIEW WITH DR. ROBERT TJIAN

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Dr. Robert Tjian is a professor of biochemistry and molecular biology at the University of California, Berkeley. He received his PhD from Harvard University in 1976 and joined UC Berkeley as faculty in 1979. During his three decades at Berkeley, his research interests have revolved around gene regulation through transcription factors, which has led him to study cancers and cell differentiation. Dr. Tjian was named a Howard Hughes Medical Institute investigator in 1987 and served as president of the institute from 2009 to 2016. He also served as the director of the Berkeley Stem Cell Center and the faculty director of the Li Ka Shing Center for Biomedical and Health Sciences. In this interview, we discuss Dr. Tjian's current research on novel transcription factor mechanism modeling found through single-particle tracking microscopy.

BSJ: What are chromatin loops, and how do they fit into the model of DNA extrusion?

RT: One of the things that we discovered back in the '80s was that humans have a gene body, a promoter, and an enhancer. In simple organisms like bacteria or phages, the promoter, the enhancers, and everything that controls the gene are very close to each other on the DNA. In higher organisms like humans, the enhancer, which activates the promoter, can be thousands of kilobases away. How does that enhancer know which promoters it is supposed to talk to? The model that has come around to answer this question is referred to as "DNA extrusion." In this model, DNA, which is flexible, is presumed to form a "loop" that enables promoter-enhancer communication. The bigger the loop is, the further apart the distance between the enhancer and promoter.

Many papers from many labs write about how two proteins in particular, CTCF (CCCTC-binding factor) and cohesin, work together to form a protein complex that wraps around DNA and works as a kind of doorstop to stop DNA from looping continuously. When Joe Decker first discovered this mechanism, it was initially thought that this might explain enhancer-promoter relations. Our lab and Dr. Anders Hansen, the senior author of a paper we collaborated on, also wanted to better understand this possibility, so we started testing the importance of this function by making mutations to knock out cohesin or CTCF.[1] Surprisingly, when we did that, we got rid of the DNA loops, but the transcription was not affected. We now know that these loops are just structural components that help us condense chromatin. If you get rid of those loops, there is no effect on function. This is a really important lesson for people to understand: correlation does not give you causality.

BSJ: How did you discover the interaction between cohesin and CTCF?

RT: Here is where novel technology comes into play. When your BSJ predecessors interviewed me in 2000, my lab was working on in vitro biochemistry. Back then, there were only a few ways you could use to try to understand the biology within cells. One was to do a mutational analysis to assess the importance of the gene to a certain function. The other way was in vitro reconstitution, where we tear the cell apart, purify the components that make up the biological machinery we are interested in, put the machinery together in a test tube, and then observe the reaction that it causes. Until recently, that is what I did to understand functionality at a biochemical level. How-

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ever, I began to realize that through the reconstitution method, you cannot exactly replicate what is happening inside a cell because you have taken the biological machinery out of the context of its normal situation. I really wanted to study this machinery in the context of the living cell. In other words, instead of pulling it out of the living cell, getting rid of everything else and looking at it in isolation, what I really wanted to ask is: "How does this machine work in the context of the whole living cell or even in the whole organism, in vivo?"

Based on reconstitution experiments and genetics, we had found that cohesin and CTCF work together. They comprise a complex together that binds to DNA, but we did not really understand the dynamics of the reaction. In other words, we could measure the reaction based on where it started and ended, but we did not know the pathway in between. Based on the limits of available techniques, we had to study the biology in snapshots since we did not have a "camera" that could capture the movement of biological molecules in action like a movie. Nobody thought that we could ever achieve that because the methods available, like X-ray crystallography and cryo-electron microscopy, allowed you to see molecules, but those molecules had to be in dead samples that were frozen and blasted with an X-ray, or hit with an electron.

As a result, Eric Betzig, who was a physicist at the Howard Hughes Medical Institute (HHMI), understood that what we really wanted was a microscope that could measure the movement of molecules in live cells. In 2010, Dr. Betzig and I got together, and we figured out how to make such a microscope. I did not think that in my lifetime, I would ever be able to do that. For the first time in my life, I could actually watch transcription factors moving around, including CTCF and cohesin.

BSJ: How did this development affect the study of protein complexes?

RT: Later, Anders came from Harvard to study with me and Xavier Darzacq because he knew we had the one microscope in the world that could actually do this. He then measured the dynamic movement of CTCF and cohesin using the embryonic stem cell system. He observed residence time of the DNA binding event, which is basically how long something is bound to DNA before it leaves. We suspected that cohesin would probably bind in a pretty stable manner due to its ring-like structure that surrounds DNA, and we measured the residence time of cohesin to be 25-30 minutes. We expected the same range of residence time by CTCF because they are in the same complex, but he got a shocking finding: the residence time of CTCF was one minute.

That was a revolutionary and foundational discovery, and it started to change our entire view of how protein complexes bind. Everything we ever thought we understood about protein and macromolecular interactions is probably wrong. The differences in timescales are probably in the order of several magnitudes. Certain transcription factors that we thought had a residence time of 20 minutes, 30 minutes, an hour, or a day instead bound to DNA for 300 milliseconds. It is as if we can just take the textbook and throw it out the window.

BSJ: In your paper with Dr. Anders Hansen, "Distinct Classes of Chromatin Loops Revealed by Deletion of an RNA-Binding Region in CTCF," you discuss how chromatin loops are controlled by an internal RNA-binding region (RBri). What is the exact role of this region?

RT: It is general knowledge that most molecules in aqueous solution move by Brownian motion. That means any molecule has total freedom to diffuse throughout the volume of its vessel. There should be no constraints on the movement of a molecule; it should be able to travel and cover the entire volume of its vessel, the cell. The speed with which the molecule moves depends on its size, temperature, and the viscosity of the solution. That is classical Brownian motion, and everything that we ever imagined about molecules in aqueous solution is governed by this principle. However,

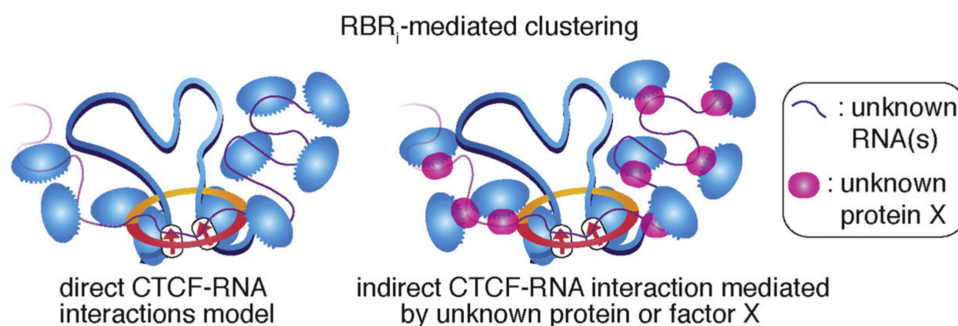

Figure 1: RNA binding region (RBRi) on CTCF mediates clustering of CTCF in DNA.[1] *CCCTC-binding factor (CTCF) is a highly conserved transcription factor that binds DNA and brings it together to form DNA loops in the DNA extrusion model. Both models above refer to the RBRi-dependent CTCF loop class.*

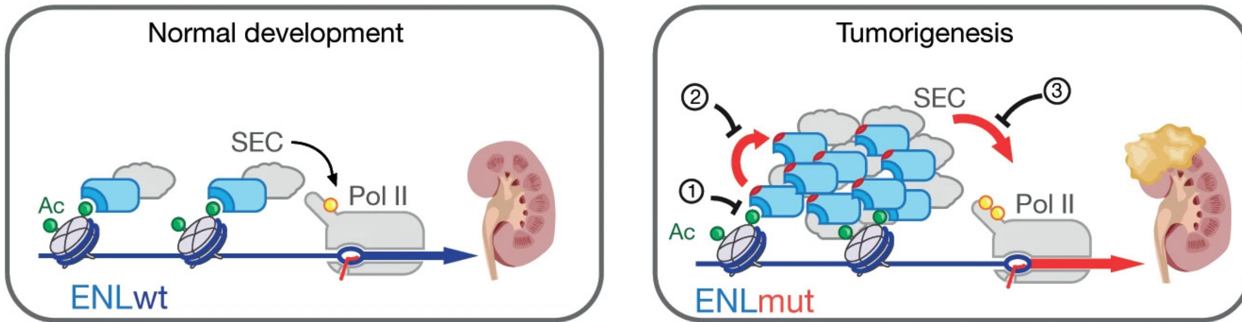

Figure 2: Gain of function mutations in ENL protein promote the development of Wilms' tumor.[2] *Eleven nineteen leukemia (ENL) proteins allow for appropriate transcription levels for normal kidney development. When ENL is mutated in a certain position, it increases self-association and activation of DNA Polymerase II, resulting in aberrant gene activation that contributes to the development of Wilms' tumor.*

when Anders tracked the movement of CTCF, he was shocked—it was moving by non-Brownian motion. This means that when we mapped out the angles and trajectories of the way CTCF travels in the live cell, it preferred to go back to where it came from rather than go somewhere else. This is called anisotropic diffusion. Very few people have ever seen such a thing in a living cell. Anders then did the classical genetic experiments to find the part of the molecule that is causing it to travel in this fashion, and it was the RNA binding domain.

BSJ: Your results demonstrated that the loss of CTCF RBri deregulates 5,000 genes after four days, possibly leading to the development of diseases such as cancer. Are there any functional similarities among these genes?

RT: Our results demonstrated that there were certain classes of genes that were really dependent on CTCF and its RNA binding domain, and there were some genes that were not. This indicates to us that there is probably more than one mechanism involved in chromatin loop control. The thing about biology is that, due to billions of years of evolution, things are never as simple as you think.

Currently, this research is being continued by Andres at MIT. In time, I think this research is going to have a definite impact on processes like drug discovery, but we are still too far from being able to say that right now. There will need to be more research into what other complex is taking over the RNA binding function outside of CTCF's RBri.

BSJ: You have also published research discussing the role of a chromatin reader mutation in causing Wilms' tumors.[2] What are Wilms' tumors, and how do they relate to leukemia?

RT: Wilms' tumor is a very devastating children's kidney disease. Eleven nineteen leukemia protein (ENL) is a regulator that can affect many genes, and mutations in this factor can help lead to many different diseases. My colleague Dr. Charles David Allis discovered ENL's influence on leukemia, but realized that it is also affecting a lot of other functions. With regards to Wilms' tumor, many different gain of function mutations in ENL can turn on genes that should be turned off, leading to the development of cancer.

BSJ: What is special about the target genes of ENL gain of function mutations that their upregulation promotes oncogenic activity?

RT: ENL is basically functioning like an oncogene. It is turning on genes that are causing the cells to replicate faster and causing carcinogenic functions. ENL is a chromatin reader, so I think a lot of people are interested in how it controls gene expression and how chromatin marks are read by ENL. It is measuring methylation or acetylation on chromatin, specifically on histones. There is a relationship between the marks and how genes are either turned on or repressed, but it is still very mysterious as to why, in a particular cell type—in this case the kidney—this particular set of mutations has oncogenic effects, but in other cells, it does not happen.

BSJ: We interviewed you in our journal's 2000 issue, "Special Report on Biotechnology," about your new biotechnology company Tularik, Inc. In your opinion, how do you think the field of biotechnology has evolved since then?

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RT: The field of biotechnology continues to explode. The first wave of biotechnology was in the late '70s and was led by Genentech and Chiron. At the time, the field was called biologics, where you express and purify proteins like insulin or growth hormone. Tularik was founded in the '90s during the second wave, where we began using molecular biology to find bioavailable drugs. Since then, of course, the development and use of so many other techniques, such as antibody treatments, CRISPR, RNA inhibition, and non-coding RNAs, has resulted in many different local modalities of drugs.

The latest company that I started is called EIKON Therapeutics,

which was formed in 2019. Within months after the start of the company, COVID happened, but it did not slow us down. EIKON Therapeutics is now booming. The company centers on single-particle tracking microscopy, which is what allows us to watch individual molecules move in live cells. When drugs hit their target, they can change the target's speed or binding capability. Thus, single-particle tracking microscopy gives us the ability to directly observe whether the drug is actually hitting your target. We are the only company in the world that can do that right now. I think of EIKON as Tularik 2.0. Tularik changed the whole model of drug discovery, and EIKON is doing it again, but with completely different technologies. Most current pharma are based on biology, but half of EIKON consists of engineers because we have to build a microscope, have robots, and use machine learning and AI to interpret the data. Single-molecule tracking data is not something you, as a human being, can analyze, so machines have to do the interpretation as you are generating, literally, terabytes of data per day. Data processing is four orders of magnitude faster than what we did in the lab. The field of biotechnology is changing very dramatically.

BSJ: In our previous interview with you in 2000, you also expressed that the scientific field is completely dominated by speakers of the English language. Considering the growing emphasis on diversity more recently, has this changed at all?

RT: That is a tough question to answer because I live in a privileged bubble here in the Bay Area and at Cal, the number one public university in the U.S. So it is really hard for us to understand the challenges faced by people, including scientists, in other parts of the world. The field is still dominated by English. Even when my partner is French, everything we do is in English. I am afraid that English is still the dominant scientific language. I think this is true in many science fields even outside biology, such as physics, chemistry, and computer science.

BSJ: In your opinion, what is the most important step you have taken in your scientific research role?

RT: I have been an independent scientist ever since I started here when I was 28 years old. When I came back to Cal from being president of HHMI, I did something very unusual. I combined my lab completely with a young faculty member, Xavier Darzacq. There are huge advantages of this fusing process. While one might see imbalances as I am a senior faculty member while he is a junior, we turned this unbalanced situation to our advantage because we completely trust each other. It allows him to learn about Cal much faster and allows him a much bigger budget when we fuse our lab budget into one. On my side, I have a young colleague who brings a completely new skill set to the table; he understands machine learning and the microscopes we hope to use and develop. All the grad students and postdocs in my lab now have two mentors that teach them very different things, and the diversity of technologies we have in the lab expanded by a factor of three.

I think co-mentoring and teamwork like this is a trend for the future of academia. Our combined science is greater than the sum of each of our work separately by a large measure. We are also able to recruit better people and inspire different generations of scientists. Fusing our labs is probably one of the most revolutionary things I have done.

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