People & Ideas

Jim Kadonaga: Exploring transcription and chromatin

Kadonaga studies the RNA polymerase II core promoter and chromatin assembly.

By the 1950s, World War II had been over for several years, but its effects lingered on for families in the United States and elsewhere. For example, Japanese Americans were sometimes only allowed to purchase homes in certain areas, and, as a result, Jim Kadonaga grew up in a segregated neighborhood in East San Jose, California. Fortunately, says Kadonaga, his high school was a good one, staffed by dedicated teachers who supported and encouraged his interests. Kadonaga had a plan in place for college—although he actually departed from that path early on, ending up in unexplored territory.

Today, Kadonaga is one of the world's foremost molecular biologists. His lab at the University of California, San Diego (UCSD) has helped dissect the DNA motifs (1) and factors (2) that regulate the activity of the RNA polymerase II core promoter, which controls all gene transcription. However, DNA sequences aren't the only things that affect transcription, because in cells DNA is not naked, but is wrapped around histones to form chromatin. Transcription should really be studied in the context of this environment, says Kadonaga, so his group is also working to understand chromatin assembly and dynamics (3–5). We recently called Kadonaga to discuss what lies around the corner for him.

PLAN B

What was your first career plan?

My original plan, when I was in high school, was to go to an enology program and become a winemaker. I read all the wine books from UC Davis, and when I was a junior in high school, I bought 80 pounds of pinot noir grapes from Gilroy, California, and made five gallons of pinot noir wine.

Was it any good?

It was actually really good! The next year, I got more ambitious, and I bought a half ton of cabernet sauvignon grapes and made 50 gallons of cabernet sauvignon. Unfortunately, the cabernet was not as good as the pinot noir. [*Laughs*]

But then I took an evening extension course at San Jose State on winemaking and came to the realization that to be a winemaker, you need to have your own winery. I didn't, of course, have the capital to start up my own winery, so I decided that winemaking wasn't a good career for me. I ended up majoring in chemistry at MIT. It was my Plan B, I guess.

Somehow I never got back to making wine. Once you start up your own lab, there are so many things you have to do that you barely have time to do your basic job. Then, when you have a family and kids, there's always a higher priority.

How did you come up with your Plan B?

I was a chemistry nerd as a kid. But coming from the neighborhood I did, I had never thought of going to MIT until I attended a National Science Foundation summer program on chemistry. The other students at the program were going to really impressive schools, and I thought maybe I should, too. A friend of mine at the NSF program convinced me to go to MIT. I also liked the fact that I could buy wine in Massachusetts, as the drinking age was 18 at that time.

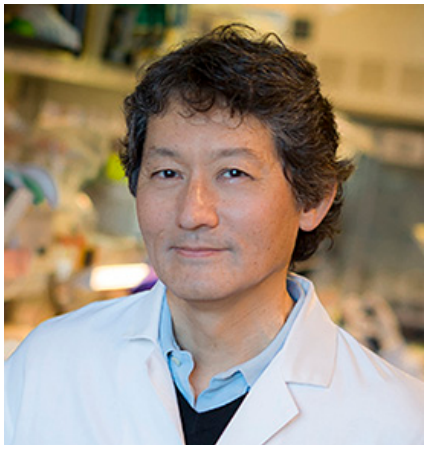
> "We're explorers. We don't know what's around the next corner."

AROUND THE CORNER

You first started working on transcription as a postdoc with Robert Tjian…

Tij had just discovered the transcription factor Sp1 when I joined his lab, and my project was to purify it. I developed a technique called sequence-specific DNA affinity chromatography to achieve that goal. By the time I left Tij's lab, we had purified and cloned several different sequence-specific DNA binding factors, and when I came here to UCSD, I started working on the basal transcription process.

In our work on transcription, we study core promoters. All of the signals that start from outside the cell, make their way into the nucleus, and tell a gene when to turn on, ultimately converge at the core promoter. This region is roughly from minus 40 to plus 40 nucleotides relative to the transcription start site.



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Jim Kadonaga

The best-known core promoter element is the TATA box. But in humans, only about 25% of genes have a TATA box or TATA-like sequence; therefore, about 75% of our genes are TATA-less. Hence, over the past 20-something years, we've been studying core promoter elements other than the TATA box. We've identified a few other motifs, including the DPE and MTE motifs.

One unexpected concept that came from this project was the finding that specific core promoter motifs can be associated with biological networks. For example, the DPE motif is present in the Hox gene network, whereas another core promoter element called the TCT motif is dedicated to translation.

Another surprising finding was that a transcription factor called TRF2 may have played a major role in the evolution of bilaterally symmetric organisms. TRF2 is related to a transcription factor called TATA box binding protein (TBP), but TRF2 appeared after the evolutionary split between bilaterians and nonbilaterian animals, but before the emergence of bilaterians. It probably arose from TBP via gene duplication and mutation. TBP binds TATA boxes, but TRF2 does not, and we postulate that the emergence of

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TRF2 led to the formation of entirely new transcriptional networks that facilitated the evolution of bilateral organisms.

That work took you places you didn't expect to go…

I like to discuss with students something I call "the landscape of research." I have a little sketch of it that I show in class, and the point of it is that we are only studying a small fraction of what we really can know. It's somewhat arbitrary what we actually study.

We're explorers. We don't know what's around the next corner, or what we'll do when we get there. It's funny: years later, former graduate students from UCSD like to talk about the landscape of research. It also often manages to make an appearance in the graduate student skits at our annual departmental retreats.

UNWINDING AND REWINDING

But transcriptional regulation isn't your only focus…

Our chromatin project was very controversial when we started working on it. In the late 1980s and early 1990s, people hated chromatin. They felt that if you were working with chromatin, you were setting yourself up to study artifacts.

However, I strongly felt it was important to study transcription in conjunction with the natural state of the DNA template—which, of course, is chromatin. In our initial round of experiments, we were able to show that you can recreate in vivo phenomena better in vitro if you use chromatin templates instead of naked DNA templates. But, in those experiments, we were making chromatin by using nonphysiological methods. Those methods yield randomly distributed nucleosomes that do not resemble the evenly distributed nucleosomes seen in vivo. I believe that we should always strive to improve the quality of our experiments, and it was obvious we could make better chromatin. With that in mind, we started looking at the process of chromatin assembly.

> "It was obvious we could make better chromatin."

Starting with a crude extract from *Drosophila* that could make chromatin, we eventually—after about nine years—managed to fractionate, purify, and clone the factors that assemble chromatin.

These include ACF?

Yes. ACF is a motor protein that makes nucleosomes. It's a pretty simple protein with two subunits: the imitation switch (ISWI) ATPase, and another protein that we called ACF1. ACF acts catalytically, so one molecule can make about 100 nucleosomes in vitro. It is somewhat rare and was difficult to purify. When we did—from 2 kg of *Drosophila* embryos!—we were finally able to assemble periodic nucleosome arrays with purified components. Today, we use purified recombinant proteins for chromatin assembly.

ACF can also reposition nucleosomes deposited by histone chaperones or polyanions…

Yes, ACF can reposition randomly distributed nucleosomes into periodic arrays. That particular activity had led some people to think that ACF is only a nucleosome positioning factor, but it's also a nucleosome assembly factor. And that relates to our most recent work.

We found that when a chaperone deposits histones onto DNA, the resulting histone–DNA complex is a particle that we call the prenucleosome. Then, the ACF motor protein converts the prenucleosome into a canonical nucleosome. The prenucleosome is a stable isomer of the nucleosome that only associates with only about 80 base pairs of DNA; in contrast, a canonical nucleosome is associated with about 147 base pairs of DNA. Multiple lines of evidence suggest that prenucleosomes are present at the "nucleosome-free regions" of active promoters.

Do nucleosome assembly and positioning occur by the same mechanism?

Not necessarily. There's another ATPase, termed CHD1, which is able to make nucleosomes. In collaboration with Greg Bowman at Johns Hopkins, we identified a mutant version of CHD1 that can make nucleosomes but not move them. Therefore, nucleosome assembly and repositioning activities appear to be separable.

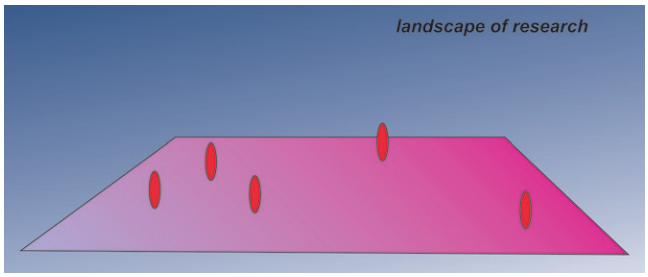

IMAGE COURTESY OF JIM KADONAGA

On the landscape of research, the pink trapezoid represents what can be known, and red ovals depict what we've actually studied.

You've worked on other proteins related to ACF and CHD1…

The HARP protein is a distant member of the same family of proteins as ISWI, CHD1, and SWI2/SNF2. Mutations in HARP are associated with a human disease, so we thought that we would look at the biochemical functions of the HARP protein. Timur Yusufzai, when he was a postdoc in my lab, did almost every imaginable chromatin remodeling assay with HARP but did not observe any chromatin-related activity. Then, he found that it binds to fork DNA better than it does to single- or double-stranded DNA. This suggested that HARP could be a helicase. But when he tested it for traditional unwinding helicase activity, it had none. Timur started wondering if it might rewind DNA. Eventually, he developed an assay for DNA rewinding activity and found that HARP is a DNA rewinding motor.

That was really unexpected. Now, it appears that a DNA rewinding motor might be important, for example, when dealing with DNA damage, where it may act to rewind DNA forks in front of a stalled DNA polymerase. From a more general perspective, it was exciting to find a new enzyme that affects the structure of our genetic material.

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