Scientists

Approaching the single molecule

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Paul Soloway's development of single molecule approaches to study epigenetics caught our attention. Curious to know more, *BioTechniques* contacted him to find out about the ambition, character, and motivation that led to his success.

What do you consider to be your most significant scientific contribution?

When I first began working in the field of epigenetics, we knew quite a bit about which enzymes control epigenetic regulation of the genome and where epigenetic marks reside, but had far less information on how specific regions of the genome are selected to receive those epigenetic marks. Even now, regulatory sequences for epigenomic states have not been well identified or characterized; in fact, there are only about four of such sequences known at the moment. One of my most significant contributions was identifying one of those sequences. We found a *cis*-acting regulator that is vital for placing epigenetic marks, particularly DNA methylation, in the genome. Since then, we have gone on to elaborate the mechanisms by which this *cis*-regulatory element controls local epigenetic fates.

What led you to focus on single molecule techniques?

Six years ago, Steve Levy, a postdoctoral research associate from Harold Craighead's

Engineering Physics lab here at Cornell, asked me if any of the methods they were developing could be applied to epigenetics. After our initial conversation, I invited him to give a group meeting in my lab, where he showed several fascinating videos of single DNA molecules passing through nanofluidic channels wherein they were manipulated, enumerated, or other properties detected by fluorescence. After a week of reflecting on what I had seen, I had an epiphany that set the stage for the single molecule epigenomic analysis we have done since.

Single molecule analysis allows us to query individual chromatin fragments for a plurality of epigenetic states, rather than looking at each individually. Right now, with chromatin immunoprecipitation (ChIP) we can use antibodies to isolate specific chromatin fragments containing an epigenetic marker or protein region of interest. For those interested in multiple epigenetic marks, data from independent ChIPs can be superimposed, and if there is evidence for the presence of an epigenetic mark in one ChIP and also in a second ChIP, one might infer that they actually coexist. But unless you do a re-ChIP experiment to demonstrate that a given chromatin fragment really possesses multiple epigenetic marks simultaneously, then the inference is just a leap of faith.

We have been developing specific methods to examine combinations of epigenetic marks as they truly reside on chromatin. By looking at one molecule at a time, we are able to directly measure epigenetic marks in a quantitative manner. In addition, we are also modifying some of our single molecule methods to sort molecules by epigenetic state, trying to improve the throughput, and implementing several sample processing steps that allow us to look at transcription factors in addition to epigenetic marks.

Do you have any pet projects outside your main research focus?

In 2003, we published an article describing a mouse that had a transgenerational epigenetic inheritance effect. At the time, we did not pursue that element of the story, but I have always wondered why it happened. So

in 2011, along with Hiro Sasaki from the National Institute of Genetics in Mishima Japan, we demonstrated that there's an RNA-mediated mechanism controlling the epigenetic state of a particular gene, introducing the possibility that RNA transmitted in the gametes allowed the presence of epigenetic marks even when the alleles generating them were not transmitted. We now have a few projects exploring the possibility of RNA-mediated control of transgenerational epigenetics states. This may end up becoming a main focus of the lab as things evolve, but for the moment, it's just a really intriguing side project.

What do you believe is the most important goal in epigenetics research today?

Our most important research goal is to be able to control the epigenome for therapeutic benefit. Of course it's clear that epigenetic influences aren't as potent as genomic influences when controlling phenotypes, but we really can't control our genetic state since it's fixed in the nuclei of all the cells in our bodies. Unfortunately, gene therapy approaches have produced less promising results than we had originally hoped, but the epigenome is highly variable and might be more amenable to manipulation.

We need to work towards the ability to modify the epigenetic states of specific genes and sites at will. At that point, we can monitor and correct sites where epigenomic modifications are sporadically acquired because of environmental and stochastic effects.

Hopefully, the single molecule technologies we are developing can leverage some of those efforts. In fact, I think that our instrumentation could provide very rapid quantitative and multiplexed data when thoroughly evaluating compounds for their epigenome modifying effects.

Interviewed by Kristie Nybo, Ph.D. Image courtesy of Deborah Mahoney.

BioTechniques 54: 183 (April 2013)
doi 10.2144/000114006