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Q&A

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Haifan Lin: peeling back layers of stem cell control

Monya Baker[1]

Self-renewing enthusiasm spans from the fly ovary to new RNA-protein guides for gene expression

Haifan Lin directs the Stem Cell Center at Yale University in New Haven, Connecticut, where he uses genetics, cell biology and biochemistry to probe how a new class of small RNAs and proteins direct epigenetic factors to their proper places. This work began with laser beams and mutant flies, which he used to gather some of the first evidence for the stem cell niche. Lin recently spoke with Nature Reports about his life and research.

Haifan Lin, director Yale University's Stem Cell Center

Harold Shapiro

How did you start studying stem cells?

As a graduate student in the 1980s, I worked on how mitosis initiates in embryos. I thought that was an important first step in life.

At that time stem cells were mostly just a concept. I thought that stem cells needed to be really well defined, both their identity and their mitotic behaviour. And to get to the mechanism of stem cells, I really needed to work on the genetic level. That's why I started to work on Drosophila. The structure of its organs is so distinct and simple; we took advantage of that.

So you started looking for stem cells in fruit flies?

When I started in Allan Spradling's lab as a postdoc, the exact location of stem cells was unknown. We knew from previous studies that stem cells were located somewhere in the tip of the ovary.

We first devised a method to allow me to transplant the tip of the ovary from one fly to another fly, and that would initiate oogenesis. I was able to regenerate ovaries in male flies. I called these male flies Mr. Mom. It was pretty clear the structure contained stem cells.

I started with laser ablation to directly identify stem cells. Once you know the structure contains stem cells, then you can use the laser beam to kill specific cells there, and when you kill stem cells, that will stop the entire egg-production process[1].

That approach also provided evidence for the existence of niche cells. The fly ovarian tip is made of two types of cells. There were always two to three germline stem cells at the top of the tip, and right next to these cells are somatic cells. We became suspicious; why are those cells always associated with the stem cells? When I did laser ablation to kill some of these cells, I could change the behaviour of the stem cells. At least in the fly, that was the first evidence that stem cell behaviour can be regulated by neighbouring niche cells.

So you went from identifying stem cells to identifying genes?

An advantage of studying flies is that the fly genome is only one-tenth [the size] of the mammalian genome, so if you identify a stem cell gene in the fly you know immediately that it's important. We are using a billion years of evolution to select the important genes.

We thought about the work of **E.B. Wilson**, who introduced the concept of asymmetric division: during each division a cell makes one identical copy of itself and one that can go on to differentiate[2].

To me this was a century-old hypothesis that needed to be checked. We could show that germline stem cells divide very clearly to make a daughter cell that can change and also maintain a copy of itself.

The next question is obvious: how is stem cell division controlled by genes? We decided to take a systematic approach. If you knock out a gene that's important for the self-renewing ability of stem cells, that will cause the stem cell to differentiate. Alternately, if you find a gene important for asymmetry, it will not abolish the cell's ability to divide but it will now become tumourous. There's a third class of genes. When you have a cellular process that has two opposing processes, yin and yang, there must be a mechanism to coordinate these opposing activities. So if we hit one of those genes, both processes will be screwed up. So we were looking at the fly's ovary for a defect; by screening through more than 50% of the fly's genes, we found those three classes of genes.

In the 1990s, we were able to find about a dozen genes that are really affecting stem cells, either self-renewal or differentiation. Piwi was one such gene. When

I looked into the mutant flies, the ovaries were so tiny. Usually the ovary is about the same size of the abdomen, but when I dissected that fly ovary, it only had a few cells inside. I knew I'd found something really good.

I looked to see if it was a problem with male flies; the testes were so tiny, I screamed out "What a wimpy testes!" People suggested that I name the gene wimpy testes; I thought this doesn't sound very elegant, so I named it *Piwi* [*P element–induced wimpy testes*].

Piwis are interesting because that was really the first gene family that was found to be important in stem cell self-renewal across diverse organisms. We found there were highly conserved homologs in humans (*hiwi*) and even in *C. elegans*, and we identified known plant genes such as *Argonaut* as its homolog[2]. [Lin and others later on identified related proteins in mice, called *miwi* and *mili*; in frogs, called *xiwi* and *xili*; and in planaria, called *SMEDWI-2*.]

What do piwis do?

We approach all the stem cell functions from an epigenetic standpoint [from the idea that modifications to DNA activate and inactivate genes]. If you think about epigenetics, it's very important to think about how you regulate chromatin structure. And there is some proposal that transcriptional factors may be guiding this process, but it can't explain the whole picture.

The one big question in epigenetic studies that is not well addressed is how do these factors know where to go in the genome? *Piwi* and piRNA together will guide epigenetic factors to specific sites in the genome. We have evidence that they are wonderful guiding mechanisms[3,4,5]. This can apply to all sorts of genomes. It may not be just in the stem cells but also in the niche cells.

Piwis bind to their own class of small RNA?

Yes, they bind to a new class of RNA, which are generally longer than microRNAs and small interfering RNAs. They represent a distinct small RNA pathway. Since they are interacting with piwi protein, but not with Argonaut, we call them piRNA for piwi-interacting RNAs.

And piwi-piRNA complexes regulate genes by interacting with chromatin?

Yes. We've started to accumulate evidence that when piwi binds piRNA, it interacts with chromatin that's a matching DNA sequence. That leads to the recruitment of other genetic factors. This might be a new mechanism to tell epigenetic factors where to go in the genome. People have done a wonderful job studying what epigenetic factors [do] once they reach their target site, but [we] don't know how they reach their target site.

So you started out looking at fly phenotypes, and now you are looking at RNA-protein 3D structures?

This reflects my whole approach to research. I always start by finding a great biological question. Then I find the mechanistic factor, and then I look for a function. The biological question to me was stem cell renewal, and in order to study that, I had to study genes. And to study the mechanistic question, you had to get into a molecular level. What is the protein; what is the inhibitor of the protein? That led me to study the piwi proteins and also RNA.

It just seems so difficult to move between so many techniques.

When I moved to mice, it was like a new world. My first experiment about mice was humbling. I didn't know which was a female mouse and which was a male mouse! So many people said, "Don't you want to get your tenure first?"

I figured that if I didn't try, I may have missed something that was important to answer my biological question. I give my lab members a lot of credit for going through this very tough transition. People in my lab often work only on mice or flies, but their knowledge is on both sides. One of the most satisfying things I've heard is that often when my students go to other labs for postdocs, the PIs [principal investigators] tell me, "wow, your students know a lot." That's from all the juggle they had to go through.

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Haifan Lin
Yale University

How did you become a scientist?

Many people become biologists because they are fascinated by life. That was not true for me. I always liked building things; I imagined building big ships or being an architect. At that time in the '70s, genetic engineering became known in China, and that was portrayed to be the science of the future. What caught me was more the word 'engineering' than the genetics.

At that time the Fudan University Genetic Institute was led by a famous professor, C.C. Tan, who was a student of Thomas Morgan [the scientist who won a Nobel Prize in 1933 for showing that genes reside on chromosomes]. Even during the Cultural Revolution, he [Tan] was the one who truly guarded the Genetics Institute at Fudan so that it wasn't affected by political influence, like the Communist Lysenko 'voo-doo genetics' that doesn't believe in genes.

How did the Cultural Revolution affect your career?

When the Cultural Revolution started, I was five years old. I finished high school when I was 15. At that time, when you graduated, you were supposed to be sent to the countryside for re-education, but there was an age limit, and I was too young. I was hanging around, and my mother said, "You cannot be a street bum." So I passed the exam to become a temporary school teacher. My students were 12–13; I was 15, but my whole heart was in it. I organized them to build ship models, and I asked each one to donate one or two books; I built a little library for my class and they would stay there after school instead of making trouble on the street.

At that time, all the colleges were shut down unless you went to the countryside first. In 1978, there was the first nationwide college entry exam. I took the exam and I got in. It was so competitive; there were ten years of high school graduates stuck together. So the admission rate to get into college in China then was 1%; now it is about 50%. I was very lucky to get into Fudan University.

For my graduate training, I went into the CUSBEA [China-United States Biochemistry Examination and Application] program, which was the first organized program by US universities to recruit graduate students from China. The exam was held in a huge hall, hundreds and hundreds of people. And then there was an interview.

At the time, Chinese tended to be facially non-expressive; they seldom laughed. They almost had a poker face. This American psychology professor who interviewed me, even today after so many years, I remember how I was so amazed — I didn't know a human being could be so expressive.

After my PhD from Cornell, I was interested in stem cells and development. I interviewed in Allan Spradling's lab at the Carnegie Institution [in Maryland]. It's a tiny place; each lab should have no more than six members, so they emphasized intellectually original research. Allan was always in the lab even though he was so prominent. I used Andy Fire's laser ablation to identify stem cells; I watched and helped him build that.

What's the best advice you've ever received as a scientist?

If I had to pick, the best advice was from Allan Spradling. He told me that a great scientist should identify a new and important question and work on it as hard as he can. He always said that science has fashions, that fashions come and go, but truly original discoveries will have a lasting effect. How important your discovery is depends not on where it's published but on how many people still remember your paper five years later.

What advice would you give?

I would give the same advice.

Tell me about what you're doing for stem cell research in China now.

There are lots of young, talented students in China. And many American scientists of Chinese origin have been setting up labs in China. I don't have much time, so I do short courses in China. I've also done a bioethics course in China. Also, I can get involved with Chinese policies, so I've been involved in implementing an American-style peer-review system. I am now an international advisor to the Chinese Academy of Sciences to establish systems for faculty promotion.

"[Allan Spradling told me] how important your discovery is depends not on where it's published but on how many people still remember your paper five years later."

Haifan Lin
Yale University

What about Connecticut?

I feel very fortunate to be in such a wonderful state. The citizens of Connecticut are very progressive and they have a high level of understanding of science. When stem cell research passed as a bill in the Connecticut legislature, it passed 30 to 2. It's an amazing majority, and one of the representatives who voted no eventually changed his mind.

I have been trying my best to unite with sister institutions in Connecticut, to have lots of collaborations rather than generating competition for money. Connecticut is a small state, and this is all taxpayers' money, so we share a lot of stuff. We even critique each other's papers sometimes.

Any advice for the field?

Stem cell researchers should not see themselves as in an isolated or distinct science. They should really think in broader terms. We can learn from other disciplines and also provide feedback to other disciplines on the way forward. If your discipline becomes isolated, you become limited in your thinking. You become a little community that is not as viable as an integrated one. We need to be more integrated into other disciplines.

Related articles

Haifan Lin, director, stem-cell programme, Yale University, New Haven, Connecticut

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