QnAs with Howard Y. Chang

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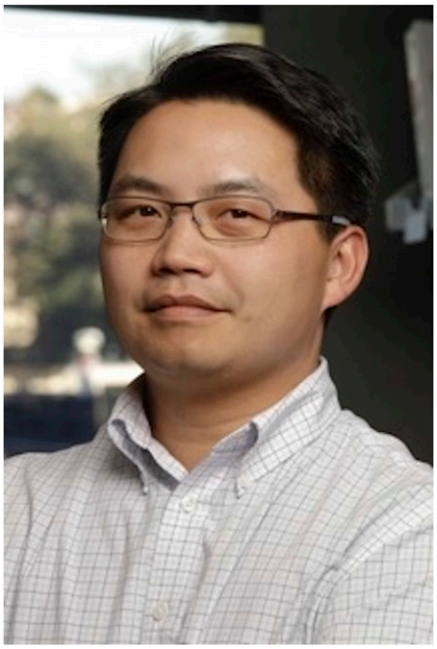
According to the traditional paradigm in molecular biology, genetic information contained in DNA is transcribed into RNA, which goes on to make proteins. A notable exception to this paradigm is long-noncoding RNA (lncRNA), which is not involved in protein synthesis. Although lncRNAs have been known for decades, recent discoveries have shown them to be an integral part of cell function. lncRNAs outnumber protein-coding genes by a factor of three, and various mutations in lncRNAs have been implicated in cancer pathology. The number of functional lncRNAs is an active area of study. Howard Y. Chang, a genome scientist at Stanford University, has enriched researchers' understanding of lncRNA function. Chang has shown that lncRNAs can act as guides or scaffolds for complexes of other biomolecules and are often essential to gene regulation. Chang has also developed genomics technologies that have facilitated the study of lncRNAs and other biomolecules. For his accomplishments, Chang was awarded the 2018 National Academy of Sciences Award in Molecular Biology. PNAS spoke with Chang about the work that led to the award.

PNAS: What are some of the functions that lncRNAs perform?

Chang: Broadly speaking, many lncRNAs have a role in gene regulation. Chromatin is the DNA–protein complex where genes reside, and a lncRNA could control genes by controlling chemical modifications on the chromatin. By doing so, they could control the accessibility of the chromatin to the gene-transcribing machinery, and therefore affect gene activity. And because gene expression is so fundamental to many processes in the cell, we see lncRNA in many different aspects of biology. For example, we know that lncRNAs are important for cancer initiation and progression. If we program cancer cells to express a certain lncRNA at a fairly high level, then those cancer cells are much more able to spread to other parts of the body. We know that there are certain lncRNAs that are involved in keeping cells in a stem cell state that allows them to divide in an indefinite fashion. When immune cells in your body are fighting infections, they're using lncRNAs to control their gene activity states.

PNAS: What are some clinical applications for lncRNAs?

Chang: One of the first lncRNAs that we studied [is] called HOTAIR; we discovered that it was highly elevated in about a quarter of breast cancer patients. And the patients who have those high levels of HOTAIR turn out to have a much worse prognosis: they're more likely to die of the disease and more likely to have the disease spread to other parts of the body (1). So that immediately says that the level of HOTAIR could be a prognostic factor to understand whether a patient is in a high-risk group or a low-risk group, without even knowing how it worked. This principle has now been extended to different human cancer types: that's just for HOTAIR. But there are many other lncRNAs that perhaps could be used to track cancer progression. In 2013, the [Food and Drug Administration] approved a test for prostate cancer that involves measuring a particular lncRNA. Another very exciting area is the field of RNA chemical modification, in which we discovered that chemical modifications of RNA change the RNA's shape. And because the RNA's shape is so important for RNA function, that's another strategy by which one could manipulate RNA function.



Howard Y. Chang. Image courtesy of Howard Hughes Medical Institute.

PNAS: Your award specifically cited technologies you developed to help study lncRNAs. How do these technologies work?

Chang: In every human cell, there's about 2 meters' worth of DNA that's packed into a 10-micrometer

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nucleus. So, most of your DNA is really tightly wound up and not accessible to enzymes that actually want to read it off; only the active elements are accessible. A lot of the work of biochemists and genomic scientists is trying to find those active elements. ATAC-seq [Assay for Transposase-Accessible Chromatin using sequencing], which I coinvented with my Stanford colleague Will Greenleaf, basically uses an enzyme—derived from bacteria—that copies and pastes DNA. Because this little copy-and-paste machine can only copy and paste accessible sites, in a single step you covalently tag the regions that you want to identify and can sequence them. This allows about a million-fold improvement in the sensitivity and a hundred-fold improvement in the speed of doing this kind of work. In the past, sometimes people needed millions of cells to do these experiments. This technology opened the door for a lot of scientists to characterize their favorite systems and answer a lot of interesting questions. ChIRP-seq [Chromatin Isolation by RNA Purification] has to do with looking at what the RNA of interest is interacting with at a biochemical level. It allows us to retrieve the particular RNA that we care about and all its associated molecules and figure out what those associated molecules are.

PNAS: How important is technology development for scientific discovery?

Chang: We oftentimes see that we have some question in mind, but there's a technical bottleneck. The reason why the question is still open is because people didn't have a way to solve it based on existing tools. Developing a technology to solve it helps address our own biological questions, but often has additional catalytic effects, letting many other people advance their science and also revealing the next level of interesting questions.

PNAS: What are you currently working on?

Chang: We're working on a special flavor of lncRNAs, called circular RNAs. lncRNA doesn't have to be like a long string; it can also be joined head-to-tail, so that it becomes a circle. We discovered that there is an immune system that cells have that decides whether a circular RNA is from its own production or from a foreign invader (2). And if it's a foreign invader, the cell will mount a very strong immune response. So we're now very interested in understanding how self-versus-nonself is decided in the RNA world.

PNAS: What was your reaction upon learning that you had won the National Academy of Sciences Award in Molecular Biology?

Chang: I was both delighted and shocked. Several of my scientific heroes, including my former mentors, received this award, so it's really a special honor. And after the initial shock, I was really happy for the recognition. I think it helped highlight the work that some of my own trainees have done, and hopefully this will bring them some recognition as well.

1 Gupta RA, et al. (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 464:1071–1076.
2 Chen YG, et al. (2017) Sensing self and foreign circular RNAs by intron identity. *Mol Cell* 67:228–238.e5.