QnAs with Jeannie T. Lee

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The role of RNA as an intermediary between DNA and protein synthesis has long been recognized, but the work of National Academy of Sciences member Jeannie T. Lee continues to reveal that long-noncoding RNAs (lncRNAs) are not merely passive genetic messengers. Instead, these RNA molecules can exert powerful control over genes, rendering, for example, an entire chromosome inactive. For two decades, Lee, a Harvard Medical School Professor of Genetics and Howard Hughes Medical Institute Investigator, has been linking lncRNAs to epigenetic regulation, a field that concerns modification of gene expression rather than alteration of the genetic code. In 1999, Lee and her colleagues identified Tsix, a model antisense RNA that controls Xist (inactive X-specific transcript) RNA. Both Tsix and Xist are involved in regulating X chromosome inactivation (XCI). In 2006, Lee's laboratory discovered the process of X chromosome pairing and described how it aids in choosing X chromosomes for inactivation. Her team has additionally determined how cells "count" the number of X chromosomes using lncRNA.

Moreover, Lee's laboratory has defined more than 100 proteins that interact with Xist RNA to initiate and spread gene silencing. In her Inaugural Article (1), Lee elucidates how Xist RNA, which coats and shuts down X chromosomes, is marked to be expressed from the paternal copy in the early embryo. Lee spoke with PNAS about this work and its relevance to treatments for human disease.

PNAS: What led to your decision to focus your work on XCI, lncRNAs, and particularly, Xist?

Lee: My graduate work on Fragile X Syndrome, a developmental disorder caused by a gene mutation on the X chromosome, got me interested in XCI because, at the time, the transmission of the Fragile X Syndrome repeat-expansion phenotype was thought to result from X inactivation in the female germ line. Females are the only ones that can pass this phenotype to offspring. In late 1990, I heard an amazing talk from Hunt Willard's laboratory at the American Society of Human Genetics meeting. They had discovered an RNA—they called it Xist (inactive X-specific transcript)—that is produced only from the

inactive X chromosome and coats that chromosome. There was no proof that Xist was required and no knowledge of how Xist would be working, but I was fascinated by this noncoding RNA that might be doing the work of silencing a whole chromosome. At the time, I was finishing my PhD and transitioning back to medical work, but Xist continued to fascinate me. Three years later, I resolved to work in mice as a model system for human disease, and studied many different topics, but I found myself continually drawn to noncoding RNA, and that was, in the end, what I chose to work on.

PNAS: Your Inaugural Article (1) sheds further light on the mechanism of Xist imprinting, whereby it is paternally marked to be expressed in female embryos. Can you explain what your investigation found?

Lee: Genomic imprinting has always fascinated me. In some mammals, the X-chromosome is imprinted to be expressed only from the maternal copy. This is due to the fact that Xist is imprinted to be silent on the maternal X, and is additionally imprinted to be expressed from the paternal X. The Inaugural Article (1) deals with the latter, the mechanism of paternal Xist imprinting. We and others have proposed that paternal imprinting might be traced to the silent "XY" body in the male germ line. It has been established that the X and Y chromosomes are inactivated during male meiosis, and the mechanism of inactivation requires the absence of homology—and hence, pairing—between the two chromosomes. We have been suspecting that the silent paternal X of daughters might be traced to this original silent state, and we set out to test whether the lack of pairing during meiosis might be responsible for Xist imprinting that would then lead to paternal Xist expression in the next generation. This is the case! We placed Xist on a transgene and inserted it into a nonsex chromosome. When the Xist transgene has a pairing partner, it is not expressed in the next generation. When it is unpaired, it is expressed in daughters, and the nonsex chromosome exhibits signs of inactivation.

Jeannie T. Lee. (Image courtesy of Jeannie T. Lee.)

This is a QnAs of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 14415 in issue 47 of volume 112.

PNAS: In earlier talks, you have mentioned that RNAs like Xist may control gene expression. How so?

Lee: Noncoding RNAs make up the bulk of what our genome transcribes, in terms of both the number of species and total mass. Their importance has, until recently, been hugely underestimated. There are going to be many more RNAs like Xist. RNAs, as a group, will likely have as many functions as proteins. RNAs and proteins can do very similar work. RNAs can even be catalytic. On the other hand, proteins are limited in one critical aspect: Unlike RNA, proteins cannot target factors to a single location in the genome and operate strictly at that site. This privilege belongs to lncRNA. Thus, lncRNAs have a unique role in epigenetic regulation.

PNAS: In 2008, your team found that Xist recruits a protein complex called PRC2 (polycomb repressive complex 2) to the X chromosome. Can you explain the function of this protein complex, and how your research on it gave you the idea that lncRNAs can be leveraged to treat disease?

Lee: PRC2 is a very important epigenetic complex with critical roles not only in embryonic development but also in disease, where its

subunits are frequently aberrantly expressed. Because none of its four core subunits is a sequence-specific DNA binding protein, there's been the question of how this complex can be targeted specifically to thousands of specific sites in the genome. We showed in 2008 that a locus-specific RNA, RepA, could target PRC2 to the X inactivation center. This work first demonstrated that lncRNAs play a role in targeting epigenetic complexes to a unique location. We then went on to show that PRC2 interacts with thousands of RNAs in mammalian cells, and we suspected that some of these transcripts are also involved in PRC2 recruitment. We proposed that some of these RNAs could be drugged by designing complementary antisense oligonucleotides against the RNA site bound by PRC2. By drugging them, we might affect PRC2 recruitment in a locus-specific manner and thereby turn up genes. This idea was the foundational intellectual property for RaNA Therapeutics, a company we launched in 2011. The potential to translate our scientific discoveries to the clinic has been enormously satisfying.

PNAS: What diseases could be better treated as a result of research on XCI?

Lee: The inactive X is in actuality a reservoir of 1,000 functional genes, so why not unlock it for therapeutic purposes? Our goal is to tap into these dormant genes to treat X-linked disease in women. In one approach, we identified more than 100 proteins that bind to Xist RNA. We could reactivate some genes on the X chromosome by drugging two or more Xist interactors. We are now drilling down to find optimal drug combinations to reactivate specific disease genes. We eventually hope to be able to treat Rett and CDKL5 Syndromes (two autistic disorders) and the Fragile X Syndrome, which would bring me full circle.

PNAS: Are there possible applications for cancer treatments?

Lee: Yes, because many cancers involve extra X chromosome doses. These include certain breast, ovarian, and blood cancers, as well as intestinal, male germ cell tumors, and more. In 2013, we showed that losing Xist expression is sufficient to cause blood cancer in mice. Perhaps someday we will stop cancer growth by targeting the X chromosome.

1 Sun S, et al. (2015) Xist imprinting is promoted by the hemizygous (unpaired) state in the male germ line. *Proc Natl Acad Sci USA* 112(47):14415–14422.