Podcast interview: Jeannie Lee

**PNAS**: Welcome to *Science Sessions*, the podcast of the *Proceedings of the National Academy of Sciences*, where we connect you with Academy members, researchers, and policymakers. Join us as we explore the stories behind the science. I’m Paul Gabrielsen, and I’m speaking with Jeannie Lee of Massachusetts General Hospital and Harvard Medical School. In a recent PNAS study, she and her colleagues found that segments of RNA called B2s and ALUs in mice and humans, respectively, are ribozymes, RNA sequences which act as enzymes and can cleave themselves when activated during stress. For making a significant contribution to their field, the study was awarded a 2020 PNAS Cozzarelli Prize in Biological Sciences.

Jeannie, introduce us to ribozymes. What role do they play in gene activity?

**Lee**: Sure, so, ribozymes are sort of a hark back to a very interesting time in the evolutionary history of life on Earth. So, it was once thought that only proteins can serve as these enzymes to catalyze biochemical reactions. It's now widely recognized that RNAs can also perform that activity. And in fact, it’s now believed that RNAs evolved before DNAs and proteins. So, RNAs are a little bit easier to evolve from the, sort of, the chemical soup in the early earth conditions than DNA and proteins. So, it's now known that RNAs can both store genetic information as well as carry out the chemical reactions themselves. And so, the first enzymes to evolve were really these RNA catalysts called ribozymes. So, since their discovery in 1982, only about 15 classes of ribozymes have been identified, and most of them in the most primitive of lifeforms, the bacteria and bacteriophages.

And only about four or so, give or take, ribozymes have been identified in mammals, but the functions of most of these modern-day ribozymes are not known. So, when we came upon this discovery that the B2s and ALUs can serve as ribozymes, we were very excited. And what we infer from this is that ribozymes have not largely died off since the early Earth. So, some of their functions have continued to evolve. And so, the ribozymes that we're working with must have emerged very recently with mammals about a hundred million years ago or so. And this activity allows cells to adapt to stress.

**PNAS**: What are short interspersed nuclear elements, or SINEs, and where are they found in the genome?

**Lee**: They are a fascinating class of genetic elements. So, there are many different kinds of retrotransposons and transposable elements in our genome. And one of the most abundant forms is the SINE, the short interspersed nuclear element. And among the SINEs, one of the most interesting is the B2 element, which is the subject of the paper that was given the Cozzarelli Prize. So, these B2s are very short—they're about 180 to 200 nucleotides—but there are about 350,000 copies of these in the genome.

Interestingly, though, our cells keep them quiet, right? For most of our lives, they're very quiet, not really doing much. However, there is a big burst of B2 activity in germ cells. And then again, in the early embryo, but then thereafter, they're mostly quiescent. That is, until the cells encounter a stressful event like thermal stress or heat shock, or a chemical insult or inflammation or even cancer. And under those conditions, the B2s become massively upregulated. But nobody understood why. They thought, “Well, maybe these are just parasitic elements that are trying to get the heck out of our genome under times of stress.” Okay. So, the
question of why they’re massively upregulated is something that we have been looking at. And the discovery that they are ribozymes really emerged serendipitously out of that research.

**PNAS:** Tell us more about the two SINEs you focused on in your study, B2 and ALU.

**Lee:** They evolved in mammals, the B2s evolving in rodents like mouse and rat, [and] ALUs, which are close cousin[s], evolving in primates, so in monkeys and in humans. So, we humans have ALUs, the mice have B2s, but they’re very similar to each other. Although they share no sequence similarity, they’re both SINEs. So, these B2s and ALUs were recently evolved elements. They occurred only in the last hundred million years or so in mammals.

There are 350,000 copies of B2s and up to a million ALUs in our genome. So, ALUs occupy something like 11% of our genome, if you can believe it. So, there are more ALUs than there are protein-coding genes, right?

Environmental stress needs to be dealt with very quickly, within a matter of seconds to minutes, because they tend to be life-or-death situations. And B2s and ALUs, by virtue of the fact that they’re preformed and prebound, and by virtue of the fact that they can very quickly cleave themselves in times of stress, and by their sheer numbers, are a perfect way for cells to rapidly respond to a harmful environmental stimulus and avert a potential organisal catastrophe.

**PNAS:** Tell us about your previous work with the EZH2 protein. How does it interact with SINEs?

**Lee:** Okay. So, the story really began about 12 years ago when we got interested in RNAs that interact with EZH2. Now, EZH2 is a subunit of a larger complex called Polycomb repressive complex 2. We now know that this complex can interact with a very large ensemble of transcripts, and among the transcripts is this B2 element. But there was something really unusual about the way this transcript came into contact with EZH2, as opposed to all the other transcripts that we had studied. And that is, when it comes into contact with EZH2, it seems to break down into multiple fragments.

That was really surprising. And we started to wonder whether this disintegration had anything to do with the stress response that other people had reported previously. So we asked the simple question, which is, do these B2 RNAs interact in any way with our genome? And the answer is yes, when our cells are unstressed and are in the resting position, B2 RNA binds to a subset of stress response genes.

But, when the cells are stressed, the EZH2 protein gets recruited within a matter of seconds to minutes of the insult, binds to B2 RNA, and induces it to degrade and be released from the gene. And that act is what allows the genes to deal with the sudden heat shock stress or chemical insult. So, we look at B2 RNA as a sort of a speed bump, if you will, for the copying machinery, which is called RNA polymerase II, that turns DNA into RNA.

So, these are findings that we published in 2016, but those findings raised a lot of questions in the field, the most significant of which is the fact that EZH2 is not known to be a nuclease. It is a very famous histone methyltransferase. But it has never been ascribed a nuclease function either for the RNA or the DNA, meaning that it is not known to be an enzyme that cuts either RNA or DNA.

So, the finding was so surprising that some prominent scientists in the field believed that we had done the biochemistry incorrectly and were looking instead at some sort of artifact. However, we knew that something was up because EZH2 had this effect only on B2 RNA.
So, we reasoned that B2 may have an intrinsic nuclease activity. And indeed, it turned out that the reaction did not absolutely require EZH2 protein because when we treated the reaction with the protease, another enzyme which essentially digests away any protein that's in the reaction, B2 was still cut into pieces. So, that left us with the unavoidable conclusion that the RNA must be cutting itself.

So, it turns out that contact with EZH2 accelerates the self-cutting behavior by approximately a hundredfold. So, we believe that EZH2 is serving as a molecular chaperone, if you will, which is a fancy way of saying that it is a helper that enables B2 to fold into a cleavage-competent state. And we believe that the presence of this EZH2 chaperone is what ultimately stabilizes the catalytically active conformation of this B2 RNA.

**PNAS:** Why was it surprising to conclude that B2 is a self-cleaving enzyme? And why is that significant?

**Lee:** The observation that B2 and ALU are self-cleaving ribozymes was very surprising in the sense that these RNAs had been studied in other contexts, but nobody had ever seen their induced degradation, or had they suspected a ribozyme activity of any sort. And also, I should mention that the B2 and ALUs are recently evolved elements, probably occurring only in the last 100 million years or so with the evolution of mammals.

**PNAS:** You write in your study that B2 and ALU can be classified as "epigenetic ribozymes." What does that mean?

**Lee:** Yeah, epigenetic is a term that refers to events in our cells that are not directly controlled by our genes. So that’s what “epi” is, above genes. So, we call the B2s and ALUs epigenetic ribozymes because to our knowledge, they’re the only ribozymes that appear to be regulated by this epigenetic factor EZH2. And also they appear to be the only ribozymes again, to our knowledge, that directly regulate transcription. To our knowledge, no other ribozyme interacts with the gene so directly and with RNA polymerase II.

**PNAS:** How did you feel when you heard your paper had won a Cozzarelli Prize?

**Lee:** We were, of course, exhilarated by this finding, and the Cozzarelli Prize is really the icing on the cake. So winning this award has been a really nice validation of the work, and my coauthors and I are extremely honored and would like to thank the National Academy for this incredible honor.

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