A Conversation with Jeremy Wilusz

Interviewer: Jan Witkowski

Cold Spring Harbor Laboratory

Jeremy Wilusz is an Assistant Professor of Biochemistry and Biophysics at the University of Pennsylvania Perelman School of Medicine.

Jan Witkowski: Could you start with a bit of background on your work?

Dr. Wilusz: I'm interested in unusual looking RNAs. We've known for 50 years what an RNA is supposed to look like: an mRNA [messenger RNA]. The whole point of making an mRNA is to get it to be translated. You need to splice the RNA. You need to add modifications to the 5′ and 3′ ends to stabilize it. That's obviously very important in how many mRNAs look. But do all RNAs look how we think they are supposed to? The analogy I give is microRNAs. Before 20 years ago, microRNAs didn't "exist." Clearly, they did exist; we just didn't know about them because no one thought RNAs that small could be functional. What drives our research is, can we find other unusual classes of RNAs? Can we find RNAs that aren't even linear, that are circular and have covalently closed ends or are processed in unusual ways that we would not expect?

Jan Witkowski: You've set out deliberately to look for these peculiar RNAs?

Dr. Wilusz: Back in my Cold Spring Harbor days, finding unusual RNAs was never our goal. We'd always set out to take loci that we knew were relevant in disease or developmental processes and understand all we can about them. About 10 years ago, we figured out that a very abundant RNA in cells that happens to be noncoding and misregulated in cancer didn't have the canonical poly(A) tail on its 3′ end, which is quite unusual. Once you remove a poly(A) tail from the transcript it should get rapidly degraded, yet here's a transcript that naturally never had a poly(A) tail but it's superabundant. Once we figured out that that existed and understood how its end got stabilized—that was all by accident, to be honest—it said to us, "If we go looking for things, what else can we find?" Nature is very clever and just because we figured out one way that it does its tricks, I'm sure there's others. Some of what we do now is very purposeful to look at high-throughput sequencing data in a different way or in a unique way, or do high-throughput screens to find things that we may not be expecting.

Jan Witkowski: You made a reference to circular RNAs. I would never expect to find a circular RNA.

Dr. Wilusz: Before a few years ago, not many of us thought anything about them. They're made from many genes through canonical splicing processes that were originally found here at Cold Spring Harbor and MIT. In normal splicing you'll take, for example, exon one and join it to exon two and then exon two joins to exon three. With these circles, instead of taking an end of an exon and connecting it to the next one, you'll connect it to the beginning of that exon. On the surface, it's the same exact process of splicing and joining those ends, but in some ways it's really unexpected. If you take a perfectly good protein-coding gene and splice it in that way such that only a single exon is included in the mature RNA, you have to wonder why you would do that because, for example, you may have removed the start codon. Even though this is from a protein-coding gene, there's no way that the mature RNA that you've made can possibly make that protein.

It's still fairly curious why these exist. There are some genes where the dominant thing that's accumulating from that gene is a circle, and it's unclear why. There are examples of circles that can bind specific RNAs or specific proteins and sequester them. There's thoughts that they could be translated, but there's still plenty of these where it's really not clear what it's doing. It's exciting for many reasons: not only understanding what these new RNAs that we really didn't know much about until the last few years are doing, but also how they are regulated. How does the cell decide to make a linear RNA or to make a circle?

Jan Witkowski: When it makes a circle, can it make a circle of the entire RNA?

Dr. Wilusz: Almost everything is possible except for the ends of the gene because at the 5′ end of an RNA you would have a cap structure and at the 3′ end you'd have the poly(A) tail, so you can't covalently close them. But there are clear examples where you'll have many different circles being made from a gene. You might have a single exon form a circle or you might have exons two and three together form a circle. How all that's regulated and what all that means is unclear. Even just identifying it is still a pretty new phenomenon, let alone understanding why cells are doing it or how it would be used in disease processes or in development.

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Jan Witkowski: Presumably, for the ones that are translated, the protein is going to be nonfunctional?

Dr. Wilusz: I don't want to necessarily say it's nonfunctional. What could be really interesting is, for example, if you have a two-domain protein and within your mature circle you might only encode half of that protein because you don't have the other exon. Let's say exon two encodes a DNA-binding domain, and exon three encodes the transactivation domain to promote transcription. If you make a circle that's only exon two and that gets translated, you would just have the DNA-binding domain. You might imagine that as some sort of a repressor or something like that. There aren't really clear examples of that yet, but it's the sort of thing that we're all thinking about how this could be working.

Jan Witkowski: You said that pretty much every gene can produce these circles. Are there exceptions to that?

Dr. Wilusz: It's thought that at least 15% of genes are making circles in a given cell type. We did deep sequencing and looked at hundreds of millions of reads and we often got one or two reads that supported formation of a circle from a gene. It thus still could be that the output of a gene—99.9% of it—is a linear RNA and there's only a little bit of a circle. There's still a debate over if that little bit of circle is functional or not, but there are a lot of genes that are doing this. We've tried to focus on the genes where you're making a lot of the circle—where it's more circle than linear—because it's really confusing why that would be. Why, if this gene encodes a kinase that's a perfectly good protein, would you "waste" an RNA and make a circle?

Jan Witkowski: Are the RNAs that make the circles defective in some way? Is this a way of weeding out messenger RNAs that have some defects?

Dr. Wilusz: It doesn't seem like it. If anything, what would be confusing about a model like that is that once the circles are produced, they're actually very stable because they're covalently closed molecules and most decay happens from the ends of RNAs. In some ways, circles are really clever. Once they're made, they're resistant to the main degradation enzymes. Introns that are spliced out can form lariats, which are a form of circle, but for the most part those are very rapidly debranched and degraded. The cell has figured out that lariats are junk or useless and so they get rid of them very rapidly. If you thought the same thing about these circles coming from exons, you would have thought the cell doesn't want these to accumulate either.

Jan Witkowski: You're working on genes where the circular form predominates. Is there anything particular about that class of genes?

Dr. Wilusz: Not necessarily. A lot of circles are expressed in the brain more than other places and it's unclear why that is. In general, alternative splicing patterns are often more complicated in brain than in other places. Another idea that's out there is that things like neurons are not cycling cells, so you can just simply have them accumulate with time. For example, circles accumulate with aging. What's not clear at the moment is if this is a bad thing. Is this a cause of aging or is it actually just a consequence that is insignificant?

Jan Witkowski: What proportion of the RNA in a neuron is going to be in circles? Presumably, a small fraction?

Dr. Wilusz: Most of the RNAs are ribosomal RNAs and things like that, so it's still a small proportion for any given gene. But others have shown maybe several hundred genes where the circles are more abundant than the linear mRNA from that gene. It's really curious why that could possibly be the case.

Jan Witkowski: If there's a slowing down of pre-mRNA processing, the number of circles goes up. Is that a close linkage?

Dr. Wilusz: It's unclear. What's exciting about this whole field is it's still quite new. We've gone after circles by trying to understand biogenesis: understanding what the sequences are that are important for them and the proteins that are important for all of this. I would have thought—as I think many would—that if you inhibit splicing, all splicing will be inhibited. Instead, we often find that if we knock down core spliceosome components, circles go up when you inhibit general splicing. It seems that cells now shift to making more circles than they do linear RNAs, and it's curious why that would be. It probably has something to do with how the spliceosome is assembled and regulated. It gives a way that the circles can be regulated and, potentially, function differentially depending on the circumstance.

We've also shown for some transcripts where you have what's called "readthrough transcription," where a gene doesn't stop transcribing where it's supposed to but keeps going further and further, that can also lead to circles. In that case, it's unknown whether the circle is actually a very important functional consequence or a way to say, "This polymerase is just going out of control; let's process it in a way so that we can terminate it and get this thing restarted." We like looking for these unusual RNAs because it tells us something about these processes that we didn't know before.

Jan Witkowski: Do you do that by serendipity or do you devise screens that enable you to detect these things?

Dr. Wilusz: It's a little bit of both. In science, you try to be smart about it, but also weird things happen. We did try to do high-throughput screening approaches where we would design reporters and then try to figure out all the factors that are regulating those reporters. Often, the hits that we get have nothing to do with what the original screen was for. For example, we had knocked down, individually, 10,000 different things but when we knocked down this complex called Integrator it caused the levels of our reporter RNA to go up more than anything else, even though our original screen had nothing to do with finding Integrator or anything like it.

Jan Witkowski: What is Integrator?

Dr. Wilusz: Integrator is a complex of 14 subunits. It's actually an endonuclease. It's supposed to cleave snRNAs [small nuclear RNAs] as they're being made and release them from the polymerase so that they can then form a snRNP [small nuclear ribonucleoprotein] and function in splicing. We've found that Integrator also regulates this mRNA that we were studying. We were confused for a while why this would be because we were very much thinking this would be an snRNA effect or something like that. Instead, we find that Integrator can cleave a nascent mRNA just as if it was an snRNA and terminate transcription.

But whereas, when you cleave an snRNA, you release the mature snRNA that functions in splicing, when you cleave an mRNA you're only making a portion of that mRNA. You're not making the whole thing. When that happens, you actually now terminate transcription. It's a way to turn off that gene. What's really interesting there is the question of how is that controlled? It's a really nice way to keep the gene "off" because you're always cleaving the RNA as it's being made but when you do actually want to make this RNA, how do you tell Integrator, "Stop, let's make the whole transcript."

Jan Witkowski: So, making circles is a two-step process. Integrator does this cutting?

Dr. Wilusz: This is unrelated. This has nothing to do with circles. Making circles is all done by the spliceosome. This Integrator thing is completely different. It's interesting, because, to go back to circles for a second, how circles ultimately get degraded is unclear. They can't be degraded from ends, but presumably there's some sort of endonucleases that will cleave them to degrade it.

In total, it's complicated. You take any given gene and a lot of things can happen to it. You can make your normal mRNA. You can also make a circle. You can also never make it even to that stage because you prematurely terminate by having Integrator come in. There's a lot happening there. That's why we go after these different angles. In my lab, we have very diverse interests. In some ways, it becomes hard for me to keep track of all of it because we study transcription, translation, splicing, and all these different stages. But we're still learning a lot.