Podcast Interview: Christine Dunham

PNAS: I'm your host Prashant Nair, and welcome again to Science Sessions. Proteins carry out virtually all the functions in living cells. In most plant and animal cells, they're made in factories called ribosomes, where the genetic code embedded in DNA is translated into linear strings of amino acids that make up the proteins. The code's translation is a minutely orchestrated process involving multiple players. Among them is messenger RNA, which, as the name suggests, is an intermediary that serves as a physical transcript of the code. The transcript is decoded by transfer RNA molecules, which scroll through messenger RNA, reading its content as a series of triplets and adding the appropriate amino acid in the correct sequence for each triplet. So, the fidelity of protein translation depends on transfer RNA's ability to correctly read the triplet code. But some transfer RNAs can shift out of frame while reading messenger RNA. This property, called frameshifting, has been exploited by researchers to design proteins with desirable attributes not found in nature. Christine Dunham and colleagues at Emory University explored how transfer RNA frameshifting occurs. Their work, which earned the 2018 PNAS Cozzarelli Prize for the year's best article in biological sciences, provides a structural explanation of frameshifting. Dunham explains why she was drawn to frameshifting.

Dunham: The ribosome has a pretty tough task. It takes the information that is encoded in one polymer, so [the] nucleic acid, and has to translate it into a different type of language, and that's proteins. The ribosome is this large macromolecular machine that performs this task, and any sort of deviations, or let's say, any sort of inhibition of protein synthesis, leads to cell death. So, for example, antibiotics. More than 60% of all clinical antibiotics inhibit the ribosome.

We're really interested in understanding how the three-nucleotide codon of the genetic code is synthesized into proteins, and whether you can deviate from that and how the ribosomes adapt to this type of dysregulation that occurs.

PNAS: One type of dysregulation occurs when certain transfer RNAs called frameshift suppressor tRNAs alter the reading frame on the messenger RNA, reading the code in a series of quadruplet nucleotides instead of the canonical triplets.

Dunham: So we didn't understand what the snapshots of the ribosome looked like as it was undergoing frameshifting, as it's being moved through the ribosome.

PNAS: To understand the mechanics of frameshifting, Dunham's team used X-ray crystallographic analysis of ribosomes, messenger RNAs, and transfer RNAs. The analysis revealed that the tRNA undergoes conformational rearrangements at two specific sites when reading the code. These rearrangements loosen the ribosome's grip on the messenger RNA, allowing the tRNA to shift reading frames. Frameshift suppressor tRNAs are far from a minor curiosity of nature. They've been used in biotechnology to design proteins with novel functions. Dunham explains the relevance of her work to biotechnology.

Dunham: Frameshift suppressor tRNAs are used in synthetic biology to attempt to incorporate unnatural amino acids into proteins. The real goal is to incorporate unique types of chemical moieties that you can use in, say, fluorescence imaging or other sorts of technical advances, and that's really, I think, the holy grail of synthetic biology. We think that our research that we've published in PNAS will help the synthetic biology community better engineer tRNAs so that they can be more readily accepted by the ribosome and not ejected.

PNAS: Besides unraveling the structural details of frameshifting during protein synthesis, Dunham's work raises interesting questions about the evolution of the genetic code itself.

Dunham: So the question is really has the genetic code evolved to be three nucleotides or has the ribosome evolved to only read three nucleotides, and I would argue it's the latter. If you look at how these tRNAs fit with the mRNA pairs on the ribosome, the ribosome is architecturally different depending upon the biology of each of the tRNA binding sites. And so what we think is happening is that, and as many others have noted, the ribosome intimately interrogates the tRNA–mRNA pairs in the decoding center, and then almost, for the most part, doesn't even touch the tRNA. That inability to monitor the three-nucleotide code is what gives rise to these frameshifts, we believe. How this works on an evolutionary timescale, we don't really know, but we think our results in the bacterial system that we use would be applicable to eukaryotic systems as well. We don't think that that has changed at all. If you look at the most important parts or regions of the

ribosome across all three kingdoms, it's evolutionarily conserved. That's why we think these results are applicable to all three kingdoms of life.

PNAS: I asked Dunham about her reaction on learning that the article had won the Cozzarelli Prize.

Dunham: I was shocked and really, really proud. I think of this work as the best work that's come out of my laboratory. This paper to me is the culmination of last 10 years of work in my lab. I mean, I know it's only two structures, but it's been the question that's really driven me for a long time. It's been, I think, a fascinating question, and we're really only on the tip of understanding it.

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