QnAs with Chaitan Khosla

Beth Azar, *Science Writer*

Stanford University chemical engineer Chaitan Khosla has spent his career at the intersection of chemistry and medicine. One of the first researchers to discover the molecular assembly lines bacteria use to build antibiotics, he has methodically investigated the structure and biochemistry of polyketide synthase (PKS) enzymes. His efforts are ultimately aimed at identifying and creating medically useful antibiotics for human and animal diseases. Along the way, Khosla has explored the biochemistry of celiac disease. Elected to the National Academy of Sciences in 2020, Khosla describes in his Inaugural Article (1) a genetic element that appears to supercharge PKS evolution, possibly allowing bacteria to quickly develop new antibiotics for changing environments. Khosla talked to PNAS about his discovery.

PNAS: Your Inaugural Article (1) examines PKS evolution; how can this lead to medically important discoveries?

Khosla: If you're trying to understand dinosaurs, it's much more powerful when you have a gazillion dinosaurs, each slightly different, because then you have a continuum of evolutionary function to study. That is what these assembly line PKSs offer. There are literally tens of thousands of them and each does something slightly different. So, you can look at five different assembly lines doing exactly the same thing but with distinct sequences. Or you can study five different enzymatic assembly lines with single functional differences relative to others; they also have comparably different sequences. Studying evolution from a sequence, structural, chemical perspective, when both structure and function are gradually evolving, is a powerful way to get insight into mechanism.

PNAS: Researchers have known about PKSs for decades; why the belated focus on PKS evolution?

Khosla: When I started my independent career in 1992, there was only one assembly line PKS known, for erythromycin. The only way one discovered them was from deliberate cloning and sequencing. From 1992 to 2018, we went from one assembly line to a couple hundred known PKSs, of which dozens gave us very important

Chaitan Khosla. Image credit: Stanford University Department of Chemistry.

antibiotics. After the human genome was sequenced, sequencing machines looking for new projects turned to bacteria. They could sequence an entire bacterial genome in a day. PKS sequences proliferated, but no one had the patience or interest to study them individually. People just dumped them into GenBank. Therein lies the opportunity. We could finally start looking at evolution.

In 2010, as my [laboratory] began systematically cataloging the information, a paradox I'd seen in the mid-90s when the second, third, and fourth PKSs were sequenced became more obvious: Certain assembly line modules that catalyze different chemistry on assembly line A are closer in sequence to each other than

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article, e2100751118, in vol. 118, issue 26.

Published June 23, 2021.

their counterparts on assembly line B that catalyze exactly the same chemistry. When postdoc Aleks Nivina came to my [laboratory], I introduced her to this puzzle. This paper highlights the most important conclusion so far (1). There's a very strong correlation that wherever this evolutionary weirdness occurs there is also a very unusual genetic element that she called GRINS (genetic repeats of intense nucleotide skews).

PNAS: What exactly are GRINS?

Khosla: Watson and Crick taught us that in DNA, A bonds to T and C bonds to G, and, therefore, in double-stranded DNA there must be exactly the same amount of A as T and C as G. For single strands of DNA, there's no law to determine the ratios of these nucleotides. However, if you look at vast amounts of DNA in the databases, you see the ratio of G to C and A to T is about 50:50 on the same strand; deviation from this ratio is called "skew." Aleks observed that in PKSs the ratios of nucleotides can occasionally be dramatically skewed. And what Aleks and her collaborator Sur observed by doing this over literally thousands of assembly lines in the database was that wherever they see that evolutionary weirdness that I referred to earlier, they also see this skewing.

PNAS: Does this correlation mean something?

Khosla: We have a hypothesis that GRINS act like nature's medicinal chemists. I'll use an example to explain. Take seven assembly lines that make seven closely related but slightly different molecules. If you look at the assembly lines and ask where the GRINS elements are, you see that wherever there are small chemical changes in the PKS products, there are GRINS elements in the DNA. From that we hypothesize that, just as medicinal chemists make small changes to molecules in the hope it will produce an optimal product, nature does the same with these antibiotics. We leave the reader with two questions. One: Why is it more efficient to do this kind of late-stage medicinal chemistry by using GRINS genetic elements instead of traditional evolutionary mechanisms? Two: What chemical or biochemical processes give rise to these skews in the first place?

PNAS: Is this approach to increasing genetic diversity similar to how the human immune system promotes genetic diversity of antibodies?

Khosla: At the genetic level, no. But there's a good evolutionary analogy. Most of the DNA in our bodies has evolved based on well-established mechanisms of how DNA changes, except for the DNA in our B cells and T cells, where nature created a very special set of DNA evolutionary rules for a very special need, namely that an adult body needs to very quickly completely change its immune chemistry in response to something like SARS-CoV-2. We can't wait generations; we need to do that in days. In the context of PKSs, maybe this antibiotic arsenal was so advantageous to bacteria that nature created a different set of evolutionary rules to evolve their DNA.

PNAS: What is next on your research agenda?

Khosla: First, I will see where Aleks would like to take this in her own career. It is generally my practice to let my postdocs tell me where they want to go, and then I decide to do something that is complementary but not competitive. If Aleks is more interested in understanding what GRINS do for PKS assembly lines and for the bacteria that express them, I'd focus more on understanding what genetic and biochemical mechanisms give rise to GRINS and facilitate their spreading.

1 A. Nivina, S. Herrera Paredes, H. Fraser, C. Khosla, GRINS: Genetic elements that recode assembly-line polyketide synthases and accelerate their diversification. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2100751118 (2021).