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INTERVIEW

The people behind the papers – Zoran Gajic, Diljeet Kaur, Julie Ni and Sam Gu

Endogenous siRNAs play important roles in silencing 'non-self' DNA elements but how the pathway distinguishes between self and non-self is unclear. In a new paper, Sam Gu and colleagues identify siRNA suppression as a mechanism for silencing 'self' siRNA. We caught up with the first authors, Zoran Gajic, Diljeet Kaur and Julie Ni, and the corresponding author, Sam Gu, Associate Professor at Rutgers University, to find out more about their research.

Clockwise from top left: Sam Gu, Julie Ni, Diljeet Kaur and Zoran Gajic

Sam, can you give us your scientific biography and the questions your lab is trying to answer?

SG: I was first introduced to *C. elegans* and small RNA biology during my graduate research training with Dr Alan Zhehler at the University of California Santa Cruz. After receiving my PhD, I joined Dr Andrew Fire's lab at Stanford University for my postdoctoral training, where I explored the impact of small RNA on chromatin and transgenerational epigenetic regulation. My lab at Rutgers University investigates how germ cells distinguish self and nonself genetic material, how transgenerational epigenetic information is regulated to maintain genomic stability and adapt to environmental changes, and explores novel epigenetic structures at RNA and chromatin levels using *C. elegans* as the model system.

Zoran, Diljeet and Julie, how did you come to work in Sam's lab and what drives your research today?

ZG: I first met Sam in freshman year of undergraduate at a mixer. While talking with him about his work, I was intrigued by epigenetic systems but, more importantly, by the insightful ways Sam dissected their inner workings. I knew then that I wanted to work with Sam to try to learn to ask questions like he does. It's the chance to ask novel questions and work towards their nuanced solutions that motivates my work today.

DK: When looking for research opportunities as an undergraduate student, I reached out to Sam because of my interest in epigenetics and small RNAs. From this, I was able to work in Sam's lab during the summer after my sophomore year, and I continued to do so for the rest of my undergraduate career. During my time in Sam's lab, I fell in love with the process of scientific research. I learned that while research can certainly be frustrating at times, I ultimately find the process to be very rewarding. At that time, I decided that scientific research is something that I would like to pursue as a lifelong career.

JN: I joined Sam's lab as a senior research scientist and became Assistant Research Professor this year. Sam and I co-mentored undergraduate students in the lab. It was a great pleasure to work with Zoran and Diljeet. I would say helping students mature as scientists and exploring the small RNA biology, which is full of surprises, are the two main motivating forces for my research activities.

What was known about the control of unwanted gene silencing before your research?

Before this project, we knew that many transposons in the *C. elegans* genome produce a lot of siRNAs. Interestingly, many germline genes also produce a low level of siRNAs. We asked: what happens when a germline gene fragment is inserted into a transposon? We initially expected that the target germline gene would be silenced by the ectopic siRNAs produced from the transposon. Surprisingly, we did not observe any silencing of the target gene. This unexpected result prompted us to examine the siRNA levels and eventually led to the discovery of siRNA suppression described in this paper.

Can you give us the key results of the paper in a paragraph?

C. elegans germ cells use a sequence homology-based mechanism to distinguish self-targeting and nonself-targeting siRNAs. Self-targeting siRNAs are repressed by the mRNA of the target gene. Nonself siRNAs are permitted to reach high abundance since their target mRNAs are very few. siRNA suppression requires P granules (cytoplasmic RNA/protein condensates involved in mediating RNAi). Ectopic siRNAs, when not suppressed, can silence the target gene. These results provided a mechanistic insight regarding

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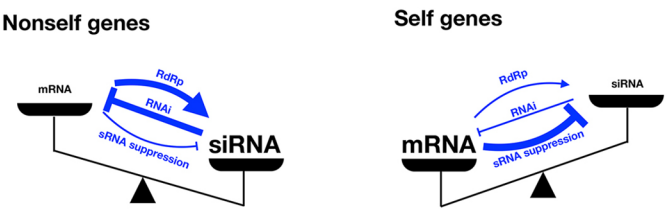

A model of the complex interactions between mRNAs and siRNAs

specificity of siRNA turnover: they explain why siRNA suppression happens to self-targeting siRNAs but not to the nonself-targeting siRNAs. The siRNA suppression pathway further increases the complexity of the relationship between mRNA and siRNAs: mRNA is the target of siRNA for RNAi; mRNA is also the template of siRNA synthesis by RNA-dependent RNA polymerase (i.e. mRNA is necessary for RNAi). On top of that, our work shows that mRNA can suppress homologous siRNAs.

The binding of ectopically produced germline siRNAs to the 'non-self' Argonaute protein HRDE-1 suggests that the siRNA sequence is not important for specificity. What do you think is the mechanism behind this selection?

We suspect that different classes of siRNAs (e.g. transposon versus germline genes) are produced by distinct biochemical pathways, which may specify which Argonaute proteins siRNAs are loaded into. Future studies are needed to investigate this important question.

What implications could your work have for mRNA-based therapeutics?

mRNA-based therapeutics are based on the simple expectation that the transfected mRNA molecules will translated into proteins by ribosomes. Our work reminds us that mRNA can potentially engage in biochemical pathways other than protein synthesis. Can mRNA regulate the steady-state level of siRNAs in human cells? Future investigation is needed to test this hypothesis and explore other unexpected consequence of mRNA-based therapeutics.

Our work reminds us that mRNA can potentially engage in biochemical pathways other than protein synthesis

When doing the research, did you have any particular results or eureka moments that have stuck with you?

DK: I was particularly intrigued when we found that the *Cer3::gfp* insertion in the *oma1::gfp* genetic background caused siRNA suppression of *Cer3::gfp*. Since the *oma1::gfp* translational fusion is present in the mature *oma-1* transcript, this gave further support to the our hypothesis that the target germline gene mRNA is mediating the siRNA suppression phenomenon.

And what about the flipside: any moments of frustration or despair?

ZG: I'll answer the above two questions together: a few months into the project, we were reading out the siRNA levels of the first construct that contained a self gene inserted into a retrotransposon. I was perplexed when I saw that the siRNAs were depleted only in the region corresponding to the self gene. I remember sitting at the bench, confused and worried that this meant the end of our initial goal of silencing target genes using endogenous retrotransposons. I told Sam about the observations, and where I saw the end of a project, Sam saw an exciting new avenue to pursue. After discussing this, we set off towards a new goal to understand siRNA suppression.

DK: There were many moments of frustration during this project. Various experiments that I tried did not work and had to be troubleshooted. For example, there was one round of small RNA-sequencing which did not produce very high-quality data and had to be re-done, which was very frustrating at the time. However, the moments when I was able to obtain interesting and significant data made it all worthwhile.

What is next for you after this paper?

ZG: I am working to complete my MD-PhD at NYU Langone School of Medicine and am currently working to enhance anti-tumour checkpoint blockade and CAR T-cell immunotherapies utilizing high-throughput technologies.

DK: Given how much I enjoyed doing research in Sam's lab, I decided that I want to become a research professor myself. In pursuit of this goal, I am currently a second-year PhD student in the University of Pennsylvania's Genetics and Epigenetics program. Here, I am continuing to study epigenetics, but now from the perspective of mammalian DNA methylation and its role in cellular state, phenotype and genomic mutagenesis.

JN: I look forward to working with Sam and our new undergraduate team to continue our research.

Where will this story take your lab next?

SG: We are hoping to learn more about siRNA suppression: what is the underlying mechanism? For example, does it occur through siRNA turnover or inhibition of siRNA biogenesis? What proteins are involved biochemically? Does siRNA suppression occur in the nucleus or cytoplasm, or even more specific subcellular compartment? We also want to know the functions of siRNA suppression? We are currently seeking ways to determine the molecular and cellular phenotypes of siRNA suppression-defective mutants.

Finally, let's move outside the lab – what do you like to do in your spare time?

ZG: I enjoy spending my time outdoors, from taking care of animals to hiking and skiing, I love experiencing the beauty of the natural world.

DK: In my free time, I enjoy running, hiking, listening to podcasts and spending time with family and friends.

JN: Family time, gardening and community work.

SG: BBQ for family and friends, and woodworking.

References

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