# the Beautiful Biology of an Evolutionary Arms Race

## INTERVIEW WITH DR. KIMBERLEY SEED

BY: ANDREW DELANEY, LAURENTIA TJANG, AND ANANYA KRISHNAPURA

***Kimberley Seed, PhD, is a professor in the Plant and Microbial Biology Department at UC Berkeley. Dr. Seed received her PhD in Microbiology and Biotechnology from the University of Alberta and completed her post-doctoral training at Tufts University School of Medicine. Her research focuses on understanding the host-pathogen co-evolutionary arms race between bacterial immunity and phage genome dynamics. In this interview, we discussed the beautiful biology of mobile genetic elements and how the presence of these genes in bacteria can defend against phage transmission.***

BSJ: Your lab's research focuses on immune strategies employed by the bacteria *Vibrio cholerae* as well as the viruses (i.e. "phages") it is infected by. What are phages, and what motivated you to choose *V. cholerae* as your model organism?

KS: Phages are viruses that only infect bacteria. Viruses are fascinating for many reasons. They are master manipulators of the cells; their whole propagation depends on getting into a susceptible host cell to reproduce more and more viruses. I have been interested in phages since I was a graduate student. When I started my postdoc research, I started working with cholera. It is a good model organism because it is very well-studied and is genetically tractable (the organism's genome can be manipulated). You can grow it in the lab, work with it fairly easily, and it is not super dangerous to work with. In addition, the literature has suggested that phages were really important in controlling *Vibrio cholerae* levels for over the last 15 years at least, if not over 100 years. The hypothesis is that if you have lots of phages killing the *V. cholerae*, then you get less cholera disease. This hypothesis has been put forward to explain why we see cholera disease in certain areas go up and down every year. We do not understand what triggers cholera outbreaks and what causes them to end, so people think phages might have something to do with the fluctuating cycle of *V. cholerae* levels. At the time I started my lab, people did not really understand these viruses and their interactions with cholera bacteria, so that is where my lab comes in.

BSJ: In many of your papers, you refer to mobile genetic elements as sequences that can have significant impacts on the fitness of organisms. What are mobile genetic elements, and what are some examples of how they have affected the co-evolutionary arms race between *V. cholerae* and phages?

KS: Mobile genetic elements are segments of the genome that can move as a unit between cells. It is a way for a bacterium to acquire multiple genes or multiple traits at once, unlike drift or mutation. It is like a fast-forward form of evolution for an organism where it can acquire a whole new biosynthetic pathway or new defense system at once. Generally speaking, the field as a whole is starting to see a pattern where most phage defense systems are encoded on mobile genetic

elements. Phages can quickly become resistant to these defense systems and counter adapt, so it is beneficial for bacteria to be able to rapidly respond and evolve via these mobile elements. The fact that they are mobile means that you can just swap them around and keep trying until something works. However, because of this, they usually have high fitness costs to the bacteria since it needs to acquire and maintain this big block of genes. Because of this cost, they can often end up lost from the bacterial genome. This is probably the main reason why the presence of mobile genetic elements tends to fluctuate in bacterial populations, especially for cholera.

PLEs (phage-inducible chromosomal island-like elements) are one such example of mobile genetic elements that my lab studies. PLEs are a major driving force in phage resistance. I discovered PLEs when I was a postdoc; there was initially nothing known about them, but now my lab is responsible for analyzing and figuring out any molecular details about them. The other mobile genetic elements we study are called SXT ICEs (integrative and conjugative elements). They have been well known for a number of years in the literature because they confer multidrug resistance to antibiotics. They are five times bigger than PLEs and include many more genes.

**BSJ**: How exactly do PLEs inhibit phages?

**KS**: PLEs function like parasites of phages. They live in the bacterial genome, and they divide and replicate along with the bacterium. In the absence of phage infections, they are pretty quiet. When a phage infects the cell though, they can sense the infection. In response, they excise from the bacterial chromosome and start replicating and stealing proteins from the phage. They are parasites of the phage because they hijack important aspects of the phage's lifecycle. They have evolved ways of tinkering with the phage's capacity to replicate and package its own genome such that at the end of the infection cycle, more PLEs are released encapsulated in phage proteins compared to the actual phage genome. They then spread their genome to neighboring bacterial cells, ultimately making use of phage particles to spread and replicate. In many ways, PLEs are really just a parasite of phages, but we think of them as a defense mechanism for *Vibrio*. By inhibiting a given phage from producing new progeny viruses, PLEs are protective to the population of bacteria.

**BSJ**: Phages and CRISPR are two terms that have received a lot of concurrent attention recently. How is it that bacteriophages can take advantage of CRISPR to overcome PLEs?

**KS**: When I was a postdoc, I discovered this phage called ICP1 that normally encodes a fully functional CRISPR-Cas system. It was a huge surprise when I discovered it because CRISPR is supposed to be in bacteria, but I found it in a phage! It turns out that the phage's CRISPR-Cas system functions in the same way that CRISPR-Cas works in bacteria. Its job is to use sequence-guided nucleases to chop up a target that is otherwise dangerous to the phage. Rather than a bacterium using the system against the virus, this virus now uses it against its parasites, PLEs, in order to regain the upper hand. The data suggest that CRISPR systems in phages are very rare. Because of that, I think the overall hypothesis is that they emerged

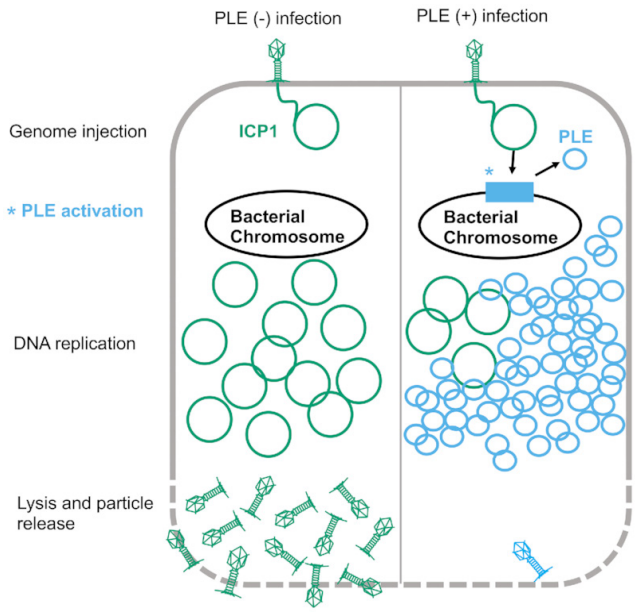


***Figure 1: Bacterial PLE defense system.*** *PLEs are mobile elements that act as bacteriophage satellites ("viruses" of viruses) encoded in the* V.cholerae *genome. PLEs are activated by phage infection and block phage DNA replication during infection. This process prevents the transmission of phages.*

in bacteria on multiple independent occasions and then have been hijacked by some viruses for their own purposes in the same way that viruses will hijack and reuse other cellular components for their own purposes. Since CRISPR-Cas systems are usually quite big, for many viruses, it is too big of an investment to be able to encode that much DNA. But if the going gets tough enough, some viruses, like ICP1, will find a way to encode it.

**BSJ**: What other pathways does ICP1 implement to combat PLEs?

**KS**: We found another nuclease called Odn which functions in a similar way as CRISPR-Cas systems for the phage. It targets PLEs to stop them from stealing proteins from the phage, but it does not have the adaptability of a CRISPR-Cas system. It can only target a single, predetermined target. The downfall for the phage is that PLEs can mutate. If a phage relies only on Odn, and if PLE ends up losing the site for Odn cleavage, PLE regains the upper hand. What we envision with ICP1 phages that have a CRISPR-Cas system is that this system was selected in evolutionary history because, at some point, the phage was not able to use Odn anymore to cleave the intended targets.

Both CRISPR-Cas systems and Odn make use of nucleases, but we have discovered one other mechanism in the lab. It is a small protein in phage that can overcome some PLE variants. It does not look like a nuclease on its own, unlike CRISPR and Odn, but rather it interacts with a PLE protein that is a nuclease. Normally, that nuclease is very specific, and it only cuts certain segments. Our data collectively suggest that this phage protein turns that protein from a good, well-behaving nuclease into a very badly behaved nuclease

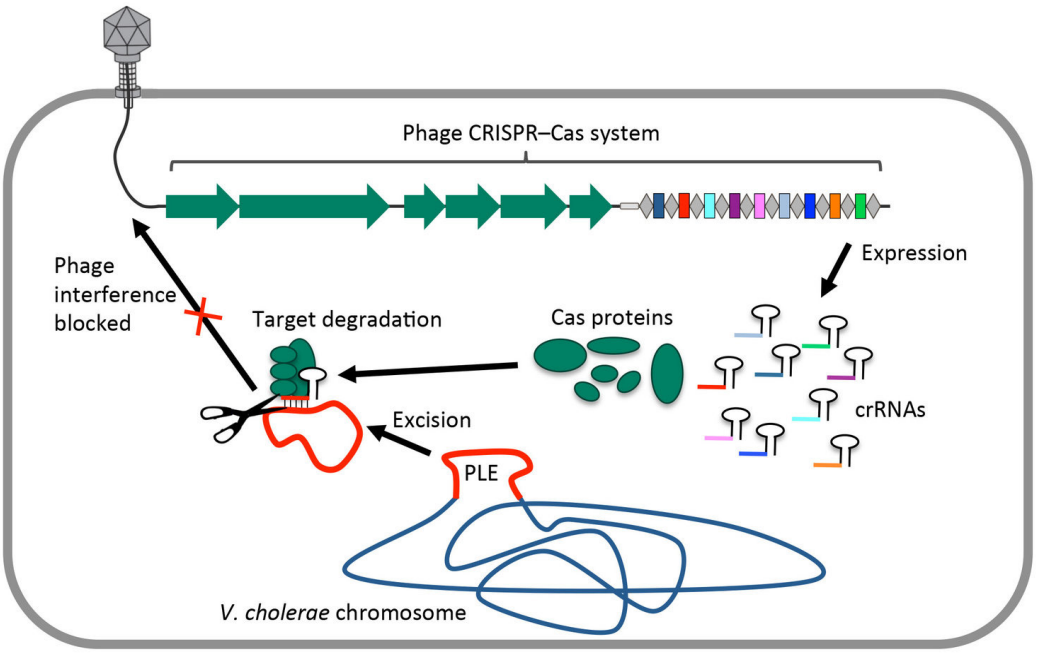


***Figure 2: Phage CRISPR system against bacterial element PLE.*** *In this diagram, the bacterium uses PLE to interfere with phage replication, while the phage uses a CRISPR-Cas system to block PLE functioning.*

that targets the PLE. In this way, the phage protein manipulates PLEs into turning on themselves.

**BSJ**: Your lab has conducted much of its research on a second type of mobile genetic element found in γ-proteobacteria: SXT ICEs. How prevalent are these elements in these bacteria, and how do the phage defense systems encoded by these SXT ICEs compare to those from PLEs?

**KS**: Most epidemic strains of *Vibrio cholerae* encode SXT ICEs. They are very common, but they come in different "flavors," encoding different antibiotic resistance or phage defense profiles. The major difference between how PLEs function and how these SXT ICE phage defense systems function is that SXT ICEs are constitutively on, essentially guarding the bacterium from diverse phages or other mobile elements that are trying to compromise the cell. SXT ICEs are much more promiscuous and less specific to a given phage. They operate by blocking DNA replication. Once the phage gets its DNA into the cell, SXT ICEs have ways of either recognizing the foreign DNA right away and cutting it or they stop the phage DNA from replicating. In this case, the individual cell survives the infection, whereas PLEs only protect the bacterial population as a whole rather than the initially infected cell.

**BSJ**: Could the phage resistance conferred by SXT ICEs have implications on the study of antibiotic resistance in bacteria?

**KS**: SXT ICEs in cholera can confer antibiotic resistance. In fact, they were discovered because of that phenotype. When antibiotics started being used against cholera, cholera strains with SXT ICEs were selected for. Now, it is at the point where basically all strains of epidemic cholera have these SXT ICEs. They are undoubtedly responsible for antibiotic-resistant pathogens.

We mentioned in a recent *Science* paper that SXT ICEs can sense when a cell has been infected by a phage. So, in some cases, it might make more sense for SXT ICEs to leave their infected hosts and try to gain access to other cells. Similarly, a PLE excises from the genome, steals phage material to package itselfs, and then leaves the original cell. However, after phage infection, PLEs only transmit when the cell is dying or dead, whereas SXT ICEs can transmit while the original cell is still alive. This means that if you infect the bacteria with a phage, you can stimulate the transfer of an element that will confer antibiotic resistance. I think what is really important as people start to consider phages for therapeutic applications is that we think critically about the consequences of our actions. If we do not actually understand what those consequences are and fail to ask, "What are the risks if we want to replace antibiotics or use phages in conjunction with antibiotics? Could we actually be making the problem worse?" the consequences could be catastrophic. At this point, we may not necessarily have the answers to these questions because we have not studied these interactions in depth.

**BSJ**: Since phages were discovered in the early 20th century, scientists have continuously proposed or discovered potential uses for these viruses in a multitude of fields. Were there any scientists in particular whose work inspired you to pursue the research you conduct today?

**KS**: I have to be honest: I am not inspired to do the research that I am doing because I want to use phages to treat bacterial infections. I am inspired by the fact that there is this dynamic

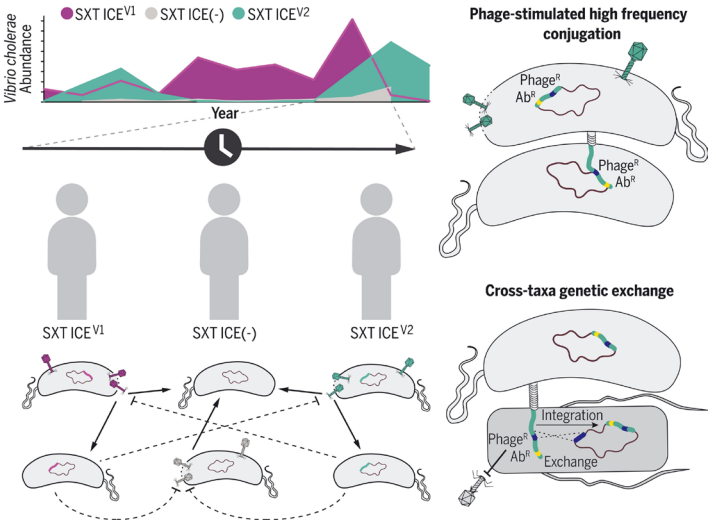


***Figure 3: SXT ICEs over time.*** *SXT ICEs are responsible for phage resistance in* V. cholerae. *SXT ICEV1and SXT ICEV2 refer to bacteria containing these two variants of SXT ICEs, while SXT ICE(-) refers to bacteria where SXT ICEs are absent. The abundance of SXT ICEs in bacterial populations fluctuates with time for reasons that are still unknown.*

evolutionary back-and-forth between bacteria and their viruses or sometimes viruses and their viruses. This relationship is just so fascinating and reveals totally unexpected mechanisms, like the CRISPR-Cas systems in a virus. My research has some direct implications for people who are interested in phage therapy because my work can help us answer many relevant questions: "Will this organism be effectively killed by the phages being administered? How can we engineer or select phages that can overcome this phage defense system?" However, I personally am inspired by the plain beauty of these systems and the discovery of novel biology; I cannot say that I was primarily motivated by some scientists who studied phages for their application in other fields. I am much more inspired by simply discovering amazing biology and being willing to follow that biology and see where it takes me. My postdoc mentor was someone similar who was super excited about any kind of science, and his enthusiasm for all cool ideas and discoveries definitely has made a huge impact on my scientific career.

Frankly, you never know where the next big discovery is going to be. CRISPR would not be something we all know and use in our labs and medicine unless people were just fundamentally interested in this back-and-forth war between phages and bacteria. For example, when scientists first studied CRISPR, it was not because they thought they were going to generate a genome editing tool that would change the field of biology and medicine. They were studying it because their bacterial cultures were dying of phage infection in a dairy industry setting. They were trying to make yogurt, and it was not working because phages kept killing all their hosts. They asked themselves, "How do we get these phages to stop killing our hosts? What are these spontaneous mutants that are resistant to viruses?" Well, they were CRISPR acquisition mutants, and that is what led to the discovery of CRISPR. In essence, you never know when the act of following beautiful biology will reveal something amazing. Your mind is much more open when you are in that state of following biology rather than solving particular problems.

**BSJ**: Can you provide any insight on the future trajectory of your research?

**KS**: I think one of the things I really love about our research is that we rely on sampling that we do in collaboration, primarily in Bangladesh, with Dr. Munir Alam's lab. We get stool samples from cholera patients every year, and it is like we go on a treasure hunt, except in stool. One of the things we found last year that was really surprising is that although it seems like CRISPR is much more effective than Odn and the phages we saw needed to have CRISPR because some variants of PLEs present were resistant to Odn, we actually found that CRISPR and PLEs disappeared and Odn was once again favored in the *V. cholerae* and phage populations. I was very nervous about that because my career is largely based on studying PLEs. However, we now have the next cohort of samples, and I am happy to say that a new PLE has emerged. This PLE is resistant to CRISPR, resistant to Odn, and resistant to this other protein mechanism that we have identified. Now, the battle seems to be back in PLEs' favor, and we are very eager to see what phages are going to do in nature to circumvent this, because they will. I think just following this biology—this coevolutionary arms race—in almost real time is so amazing because you never know what you are going to find. We were not initially looking for the SXT ICEs. We did not have a hypothesis that SXT ICEs were responsible for this phage-resistant phenotype. We found it by looking in the stool and asking, "Well, why are you not infecting these bacteria anymore?" I truly enjoy looking into these genomes, seeing what gets selected for, and trying to reconstruct what could have happened that led to the current situation.

## REFERENCES


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