Novel effectors and terminators of CRISPR-Cas antiviral signaling

Just as we suffer from viruses, so do bacteria: the viruses that plague bacteria are known as bacteriophages, or simply phages. And just like us, bacteria have an 'immune system' to help them to deal with the phage threat. Like ours, bacterial immunity also has generic and pathogen specific pathways. Studies of both pathways have had a major impact on the development of molecular biology. The work on generic immunity, which gained momentum in the 1960s and 1970s, has led to the discovery of restriction-modification systems. Shortly afterwards, the enzymes discovered in this context became key tools for molecular cloning, i.e. the *in vitro* manipulation of DNA. The technique is used for a variety of purposes, including the genetic modification of bacteria in such a way that they produce eukaryotic proteins for research and biotech applications, substantially reducing the consumption of animal material.

In this century, the focus has been on pathogen specific immunity of bacteria. The key finding was that a significant part of pathogen specific immunity is due to so-called CRISPR systems. These systems store the memory of previous infections in a 'blacklist' of fragments of their genetic material and generate an immune response when the genome of the current invader matches any of the fragments on the list. As for restriction-modification systems, the discovery of CRISPRs has led to a revolution in biology. This time, CRISPR effectors have greatly simplified *in vivo* genetic manipulation, which has led to a breakthrough in genotype-to-phenotype research studies and is already being used for biotech, diagnostic and medical applications.

Initially, it was thought that CRISPR anti-phage immunity was based only on the destruction of invading nucleic acids, so that the same protein or protein complex could detect and attack the invader. It has now become clear that bacterial immunity is even more similar to human immunity than previously thought. Our collaborators in Lithuania have discovered that many CRISPR systems synthesize second messengers, that act as 'danger signals' to trigger a broad range of anti-phage responses. This has stimulated a lot of work around the world on both the sensors of these danger signals, and on their signaling pathways.

In this project, we will focus on the second messenger arm of CRISPR systems. In particular, we want to build on the discovery of the Lithuanian team that some effectors directly attack mRNA (the instruction for protein synthesis) at the ribosome (the site of protein production). We will work on previously undescribed proteins that we predict will act by this mechanism. Moreover, we will study CRISPR effectors that we expect to sense the 'danger signals' and interfere with phage replication by ribosome based mechanisms, but without destroying mRNA.

Bacterial responses to the 'danger signal' of infection come in two varieties. In some cases, bacteria simply undergo programmed cell death, in order to deprive an invading phage of metabolic resources, and save the colony. However, many other mechanisms aim to overcome the infection without sacrificing the originally infected cell. In such cases, it is necessary to switch off the 'danger signal' after some time. In this project, we will also work on proteins that we predict to be signal terminators. In some cases, our preliminary data suggest a dual role of the proteins as signal terminators and anti-phage effectors. In these cases, we will test both activities.