Dr Petr Leiman discusses his latest research into the function of a special organelle in bacterial viruses that is surprisingly analogous to structures used by bacteria to attack enemy cells.

What is the current focus of your research?

Bacterial viruses, or bacteriophages, have a special organelle called a tail with which they attach to a host cell and deliver their genetic material into its cytoplasm. The tail is a complex multicomponent ‘nanomachine’ created by a precise arrangement of dozens of different protein molecules. Bacteria employ similar organelles to attack and kill competitors or hostile neighbours, such as other bacteria or cells of the human immune system. One of the most fascinating things about these nanomachines is how much they resemble machines from our macroscopic world. A phage tail-like structure can be compared to an extended spring, which contracts at the right moment to pierce the membrane of the target cell.

Your work explores proline-alanine-alanine-arginine (PAAR) repeat proteins, which are found in the type VI secretion systems of some bacteria; what are these proteins and what is their function?

The type VI secretion system (T6SS), along with other systems related to phage tails, breach the membrane of the target cell with the help of a special spike-shaped protein. Some of these spikes carry a sharp tip protein. Most of the tip proteins contain the PAAR amino acid sequence motif and form a smooth extension of the spike. In T6SS, the PAAR repeat proteins often contain additional toxic domains, whereas in phages they make the tip sharper and equip it with a metal atom close to the membrane-attacking apex. T6SS is known to deliver toxic proteins into neighbouring competitor cells, and the association of these domains with a PAAR repeat domain that forms the tip of the membrane-piercing spike, is a brilliant way in which these machines have evolved to be efficient.

This investigation is focused on both T6SS and R-type pyocins. How do these assemblies work together to aid infection of host cells?

The target organisms of T6SS and R-type pyocins are different. T6SS is primarily an interspecies weapon (eg. *Pseudomonas aeruginosa* against *Escherichia coli*) whereas R-type pyocins are used in intra-species competition (eg. by different strains of *P. aeruginosa*). In principle, *P. aeruginosa* can attack its neighbours with its T6SS and pyocins at the same time.

You have drawn comparisons between these systems and bacteriophage tail structures; what are the similarities and differences between these structures?

T6SS is a tubular organelle located inside the cytoplasm of the predator cell. A cell carrying a T6SS organelle can fire it repeatedly by reassembling the organelle again and again, thus sending numerous toxins out and into other cells. On the other hand, R-type pyocins, which are similar to bacteriophages, have to break the original cell to come out and become active. A cell essentially has to sacrifice itself in order to release about 200 pyocin particles, which will then diffuse out and kill other cells not related to the original mother cell. Each pyocin particle is then able to kill its target cell in a single shot-type of action; unlike T6SS, there is no recycling. Interestingly, the targets of R-type pyocin-like particles are not limited to prokaryotes. R-type pyocin-like particles are used by certain species of *Serratia* and *Photorhabdus* bacteria to kill insect larvae.

What are your hopes for the next stage of this research?

At present, our understanding of how phage tail-like organelles work is very fragmented. Some of our ideas are borrowed from macroscopic analogies, such as those of the extended spring and membrane piercing. We do not know whether these are really applicable. Neither do we know how much energy is stored in a tail-like structure or how much of that energy can be used for doing actual work – such as translocating proteins across a lipid membrane – or even how much energy is lost in vibrations and heating of the surrounding solvent. The answers to these questions, which lie at the interface of physics and chemistry, are to be found in the structure of the phage tail-like organelles that we hope to obtain. More importantly, with the atomic resolution description of a phage tail-like nanomachine, there will be a wealth of biological questions and medical applications to be discovered.
An offensive organelle

A team of researchers based in the Laboratory of Structural Biology and Biophysics at the École Polytechnique Fédérale de Lausanne, Switzerland, is conducting cutting-edge research into the structure and function of macromolecular machines involved in the infection of bacterial and eukaryotic cells.

Many of the world’s deadliest diseases are caused by bacterial infections. Although immune system cells attack potentially threatening microorganisms, and several decades of research have led to enormous advances in antibiotics, bacteria keep fighting back. New strains are evolving and developing resistance to antibiotics that were previously effective. With bacterial drug resistance on the rise, there is a constant and urgent need to develop new antibiotics.

One promising potential weapon in the fight against infectious strains of bacteria is bacteriophage therapy: making use of viruses that infect and replicate within bacteria. Bacteriophages in the Caudovirales group have a tail that functions as a nanomachine; attaching the virus particle to the host cell surface, to create an opening in the membrane and deliver DNA into the host cytoplasm. This starts the virus replication cycle that can kill the host bacteria. However, before this mechanism can be safely and successfully implemented as a therapy, there is an urgent need to understand how the process of virus attachment to the host cell is regulated and whether it can be manipulated.

Dr Petr Leiman, a prominent researcher at the École Polytechnique Fédérale de Lausanne (EPFL), Switzerland, has made important advances in understanding the function, structure and assembly of bacteriophages and bacterial secretion systems. He heads up the Laboratory of Structural Biology and Biophysics at EPFL and has an excellent track record of publications in high-profile scientific journals. At present, he is working on an exciting project with the aim of gaining detailed information about the function of two biological assemblies: the type VI secretion system (T6SS) and R-type pyocins. As a cytoplasmic organelle – that is, a macromolecular assembly situated in the cytoplasm of a cell with a specific function – T6SS is a complex molecular machine that injects toxic effector proteins into target cells and resembles a bacteriophage tail-like cell-puncturing device. R-type pyocins, which are also related to bacteriophage tails, are employed by certain bacteria to kill competing strains of the same species.

Understanding secretion systems

Secretion systems are used by bacteria to gain advantage in a given environment by secreting toxic effector proteins that can inhibit or kill neighbouring cells, including cells of the human immune system. T6SS and R-type pyocins consist of an internal tube and an external sheath, and function in a similar way. When targeting susceptible cells, the sheath contracts and the internal tube pierces the target cell membrane. The similar biological organisation and functional mechanisms of tailed bacteriophages, T6SS and R-type pyocins, strongly imply that they share a common ancestor.

T6SS resembles a microscopic spear with a metal-hardened and poisoned tip, which is surrounded by a sheath and assembled like a stretched-out spring.

While T6SS appears to be the most versatile of all known secretion systems, it is only recently that researchers at the EPFL have uncovered key information about the way it selects effector proteins for secretion and subsequently delivers them into the target cell. Crucially, Leiman and his team – working in collaboration with Dr John Mekalanos from Harvard Medical School, USA – have demonstrated that the structure of T6SS is inextricably linked to its function. By utilising a range of techniques, including X-ray crystallography, bioinformatics and in vivo experiments on pathogenic bacteria, the researchers have discovered a new class of proteins: proline-alanine-alanine-arginine (PAAR) repeat domains. These create a conical extension on the tip of the T6SS spike, which tapers to a one-atom diameter apex. Amazingly, many such tip proteins contain a centrally positioned zinc or iron...
The central spike complex of a T6SS organelle. The PAAR repeat domain is in yellow; the spike is in red, green and blue; and the toxic domains are in orange. The structure was obtained by X-ray crystallography and molecular modelling.

atom. Furthermore, tip proteins often carry extension domains with toxic function. T6SS therefore resembles a microscopic spear with a metal-hardened and poisoned tip, which is surrounded by a sheath and assembled like a stretched-out spring. If a bacterium carrying T6SS is attacked – by immune cells, for example – the spring-like sheath contracts and the toxic spear is driven out of the bacterium, piercing the target cell’s membrane and poisoning it. The researchers have hypothesised that the shape and structure of the tip is related to the target organism that can be attacked.

INNOVATIVE METHODS

The team is utilising a range of innovative methodologies and equipment to achieve their research aims, as undertaking structural studies of these macromolecular machines is a highly technical procedure that requires a hybrid approach. Firstly, X-ray crystallography is employed to determine the structure of the individual component proteins; and electron microscopy of specimens, preserved in their native state by cryo-fixation, is used to ascertain larger complexes or the whole assembly. Next, the data from both of these techniques are combined to give a detailed, high-resolution description of the nanomachine as a whole. Preparing the best possible sample at this stage is vital because it directly impacts the quality of the structural data. Conducting these experiments involves a high level of expertise, with the successful study of large biological complexes necessitating specialist knowledge of X-ray crystallography, electron microscopy, biochemistry, molecular biology and genetics. Indeed, when it comes to selecting the most important proteins from a large pool, the researchers often have to make educated guesses by drawing on their vast swathes of existing knowledge.

EXPLOITING ORGANELLES

In addition, Leiman and his colleagues are seeking to discover how to manipulate organelles. For example, if the properties of R-type pyocins can be exploited, there is a chance they could be used as a new type of antimicrobial. Leiman’s laboratory collaborates with AvidBiotics Corp (a small biotech company based in San Francisco, USA), which showed that replacing one of the pyocin-component proteins could cause the pyocins to kill a different bacterial target cell.

To explore the possibility of manipulating organelles, the researchers are working to obtain more information about their highly complex structures. With each tail-like organelle consisting of 1 million atoms or more, one of the team’s objectives is to comprehend the structure at the atomic level of detail: “We want to understand how this huge structure behaves in solution and how it changes its conformation upon interaction with the target cell surface,” explains Leiman. “This information will make it possible to control the function of T6SS. Furthermore, we are trying to harness the efficiency of the pyocin particle to retarget it from its natural host, Pseudomonas aeruginosa, to other types of pathogenic bacteria.”

FUTURE APPLICATIONS

Looking ahead, the hope is that the research conducted in Leiman’s laboratory will have clinical implications. Organelles such as T6SS and R-type pyocins could prove to be promising weapons in the fight against infectious bacteria – and could perhaps be used to complement or even replace traditional antibiotics. If the parts of the organelles most effective at killing bacteria can be isolated, a whole new class of antibacterial agents could even be created.

To facilitate the transition of their research into clinical practice, Leiman and his team are currently focusing on building a more robust understanding of T6SS. While knowledge of this system is incomplete and fragmented, it is known that T6SS can secrete a large number of toxic effector proteins that kill many different types of cells. Drawing on their extensive knowledge and expertise, the researchers at EPFL aim to design molecules that can regulate the activity of T6SS by turning it on or off. These experiments, conducted with ingenuity and enthusiasm, herald exciting opportunities for treating a range of diseases.