Structural studies of herpesvirus proteins involved in DNA replication

Herpesviruses are among the most common human pathogens. They cause life-long infection and occasionally become activated to cause symptoms. Herpesviruses are especially dangerous for people whose immune system is not functioning properly. Infection with some herpesviruses is also associated with other serious diseases, including cancer. The best studied herpesvirus is herpes simplex virus type 1 (HSV-1), which causes cold sores on or around the lips.

One step that is required for the production of new viral particles is making new copies of genetic material of the virus. In herpesviruses, similar to cellular organisms, genetic information is encoded in a DNA molecule that forms a double helix that is composed of two strands that consist of nucleotides (i.e., individual letters of the genetic alphabet). Copying a double-stranded DNA usually begins at a special site within DNA. Starting from this location, the two DNA strands need to be separated, and then each of them serves as a template (i.e., a blueprint that provides the basis whereby a new strand can be built by adding matching nucleotides). This complicated process is performed by a group of proteins that together form a tiny piece of machinery, termed the replisome. Herpesviruses possess their own set of six or seven proteins that are needed to create a new copy of DNA. Although all of these proteins have been extensively studied, their molecular mechanisms are still not understood, and unclear is how their different functions are coordinated to efficiently produce new viral DNA.

In this project, we will perform studies that seek to reveal how the herpesvirus DNA genome is multiplied. To visualize this process, we will determine three-dimensional structures of proteins that are involved in it. We will determine the positions of thousands of atoms in each protein using two methods: X-ray crystallography and cryo-electron microscopy. Studies that use these methods can produce very detailed three-dimensional pictures of proteins with each atom visualized. We are particularly interested in seeing these proteins bound to fragments of DNA to understand how they recognize their preferred forms of DNA molecules. We will also obtain structures of complexes that are composed of several proteins to determine how they are arranged within the complex. The binding of one protein with another or with a nucleic acid molecule often makes it change its shape and thus some of its properties. Our structural studies will explain the role of these changes in the process of DNA multiplication. Once we determine how these proteins interact with each other and with DNA, we will be able to understand how they assemble on DNA to form the molecular machinery that synthesizes two new strands of DNA in a synchronized manner and without errors.

Based on these structures, we will reveal how the replisome works and how the activity of its components is regulated and coordinated. The results of this project will be a valuable resource for future efforts that seek to produce new anti-herpesvirus drugs. The multiplication of viral DNA is absolutely necessary for the virus to proliferate. If we understand this process better, then we will be able to discover new ways to block it and combat the virus. Furthermore, the multiplication of herpesvirus DNA is in many ways similar to DNA multiplication that occurs in our own cells and other organisms. The key difference is that the DNA multiplication protein machinery in higher organisms is extremely complex and difficult to study. The replisome of a herpesvirus is composed of only six proteins. Because of this, it is both a convenient and a valuable model system for studying DNA multiplication.