'Insulator' helps silence genes in dormant herpes virus

May 2 2007

By adulthood, most people have suffered at least one bout of painful cold sores brought on by the Herpes simplex virus 1, also known as HSV-1. After the initial infection, the virus usually remains in the body, hiding out in nearby nerve cells where the victim's immune defenses cannot reach it, causing no symptoms at all.

In order to escape detection by the body's immune system, the latent virus works to silence genes that would cause it to replicate. In this dormant state, only a tiny fragment of the virus genome – a single gene called the Latency-Associated Transcript gene (LAT) – remains active. Scientists have long puzzled over the mechanism used to keep this small region of the genome going while nearby genes remain quiescent.

Now, scientists at The Wistar Institute have discovered a molecular mechanism that keeps HSV-1 activation restricted to a single gene for months or even years. The researchers have identified an "insulator" – a stretch of DNA about 800 base pairs long – that serves as a physical barrier between active and inactive regions of the virus genome. Base pairs are the nucleotides on each side of the rungs that connect the strands of the DNA ladder.

"By establishing an insulator in early latency, the Herpes virus can protect this one small region of the genome from silencing, allowing infected cells to survive," says study senior author Jumin Zhou, Ph.D., an associate professor at The Wistar Institute.

The findings, appearing in the May issue of the *Journal of Virology*, mark the first time an insulator has been identified in a virus and may lead to ways to develop strategies to manipulate the virus.

Insulators, also known as boundary elements, are DNA segments that work to prevent a gene from being influenced by the activation or repression of its neighbors. About a dozen different insulator elements have been identified in organisms as varied as yeast, fruit flies, and humans.

Not simply passive barriers, insulators help organize and regulate gene activity by marking boundaries on chromatin, the condensed genetic material that forms chromosomes. By establishing chromatin boundaries, insulators can limit the range of action of other DNA elements that work to activate, or "turn on," the genes.

Recent studies on the LAT region of the HSV-1 genome have shown that nearby regions of the genome contain modifications indicative of silenced chromatin. The patterns found resemble well-studied regions where insulators are found in both yeast and chicken, namely the yeast mating loci and chicken globin locus.

To see if insulators play a role in silencing viral genes during the latent phase, Zhou and his group studied cells infected with HSV-1. The studies showed that during a latent period, the virus binds to a host protein called CTCF, a protein known to act as an insulator in mammals and in fruit flies. What's more, the findings revealed that the viral DNA binds to CTCF in the same manner as the host DNA binds to the protein.

"By binding in this manner, we believed the CTCF protein was interacting with other viral proteins to form a type of insulator in the virus structure," Zhou says.

To verify that it was an insulator at work, the researchers then inserted copies of the structure into fruit fly embryos to see if they could block the activity driven by gene-activating elements called "enhancers" during development.

"If the element we were testing was an insulator, then only one enhancer would be affected, and that's exactly what we found," Zhou says. Further studies showed that the insulator element blocked enhancer activities in the eye tissue of fruit flies and in human cells in culture.

"Based on these findings, we were able to identify this element as a kind of chromatin insulator that helps HSV-1 maintain a balance in its life cycle."

The study also showed that HSV-1 chromatin is organized in a manner very similar to the host chromatin, a similarity that may work to the virus's advantage, says Shelley L. Berger, Ph.D., the Hilary Koprowski Professor at The Wistar Institute and co-author on the study.

"This means that the virus can take advantage of the many regulatory schemes that the host has worked out for its own chromatin and not have to reinvent the wheel by making its own proteins and unique structures," she says.

The researchers now plan to study the HSV-1 insulator in mice to see how the mechanism works to block the communication of gene-activating elements such as enhancers and promoters. The group is also working to identify any additional proteins that may play a role in establishing the insulator.

Knowing what genes the virus uses to hide and re-emerge could give pharmaceutical companies targets for designing drugs that disrupt those mechanisms. The studies also have implications for treating and

manipulating other types of viruses, Zhou says.

"This study provides one of first examples of how viral chromatin is organized in a very similar way to host chromatin," Zhou says. "Learning more about the similarities and differences in these chromatin structures may help finding ways to develop therapies that can target the virus and not the host."

Source: The Wistar Institute