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Following a Hitchhiker for New Clues to Viral Replication

Howard Hughes Medical Institute researchers have discovered how the virus that has a causative role in Kaposi's sarcoma, a cancer associated with HIV infection, hitches a ride inside cells to ensure its survival.

The researchers said their findings promise greater understanding of how the virus, Kaposi's sarcoma-associated herpesvirus (KSHV), persists in the multiplying cells of growing tumors. More broadly, however, the researchers said their studies dramatically change how they view nucleosomes - bead-like structures of DNA and histone proteins that form the core of chromosomes. Once thought to be a simple scaffold around which DNA wound itself, the new findings indicate that nucleosomes are active docking stations for a number of regulatory molecules that affect gene function.

The research, which was published in the February 10, 2006, issue of the journal *Science*, was a collaboration between the laboratories of Kenneth M. Kaye at Harvard Medical School and Brigham and Women's Hospital and Karolin Luger, a Howard Hughes Medical Institute investigator at Colorado State University. Co-lead authors of the paper were Andrew Barbera in Kaye's laboratory and Jayanth Chodaparambil in Luger's laboratory.

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- Karolin Luger

Kaye's laboratory had previously investigated the function of a protein known as latency-associated nuclear antigen (LANA), which the Kaposi's virus uses to latch onto the nucleosome of dividing chromosomes. They had shown that the KSHV virus uses LANA to ensure that its own DNA enters new cells along with dividing chromosomes.

Kaye and his colleagues genetically altered the LANA protein, and attached tracer molecules so that they could see how their changes affected LANA. The tracers permitted the researchers to see which amino acids were critical for LANA binding chromosomes. This work led to the discovery that LANA docked onto particular histone proteins called H2A-H2B.

Next, Luger and her colleagues used x-ray crystallography to analyze structural details of the binding between a key docking segment, or peptide, of LANA and H2A-H2B. X-ray crystallography is a widely used analytical technique in which x-rays are directed through crystals of a protein. The resulting diffraction pattern is then analyzed by researchers to deduce the protein's detailed atomic structure.

“We found a very strong, very specific binding between the LANA peptide and H2A-H2B,” said Luger. “The LANA peptide recognizes one particular region on the nucleosome and has evolved to fit that surface very precisely.

“What's even more intriguing is that - while the nucleosome is usually depicted in drawings as just a disk with DNA wrapped around it like twine -- we found it to be really highly structured and contoured,” said Luger. Thus, the nucleosome surface offers a complex interface for regulatory molecules such as LANA, Luger added.

But the findings have broader implications for understanding the cellular role of nucleosomes. “This really changes the way we think about nucleosomes,” Luger said. “We've always pictured them just as little spools that DNA wraps around, and that they are no more than a nuisance that DNA-replicating enzymes have to deal with. Now, we see them as much more dynamic protein assemblages that can actually serve as binding platforms for all kinds of cellular factors.

“We've had a hypothesis all along that the acidic region on the nucleosome and all the canyons on its surface must be good for something, because they are highly conserved throughout many species,” said Luger. “Now we have found a first instance where this region is really utilized for a purpose. And I would think there are many more such proteins that will interact with a nucleosomal surface and not just with histone tails, which have been the major focus of research on such interactions,” she said.

Luger and her colleagues are now examining how the LANA docking protein affects the way nucleosomes arrange into higher-order structures. These effects could have implications not only for understanding viral replication, but for other interactions between regulatory molecules and the nucleosome, she said. “LANA serves as a very useful and unexpected tool that will change how we think about nucleosome higher order structure,” said Luger. In particular, she noted, LANA appears to interfere with the interactions between proteins that attach to nucleosomes, which should offer insight into the normal interactions of those proteins.

Luger speculated that the new information about LANA binding could help efforts to design molecules that inhibit the binding and thwart propagation of the KSHV virus. “Such efforts could prove fruitful, because it has been shown by Kaye and his colleagues that when they delete the specific binding segment of the LANA protein, or even make a point mutation in that segment, the virus is no longer able to segregate,” she said.