Biologists describe details of new mechanism for molecular interactions

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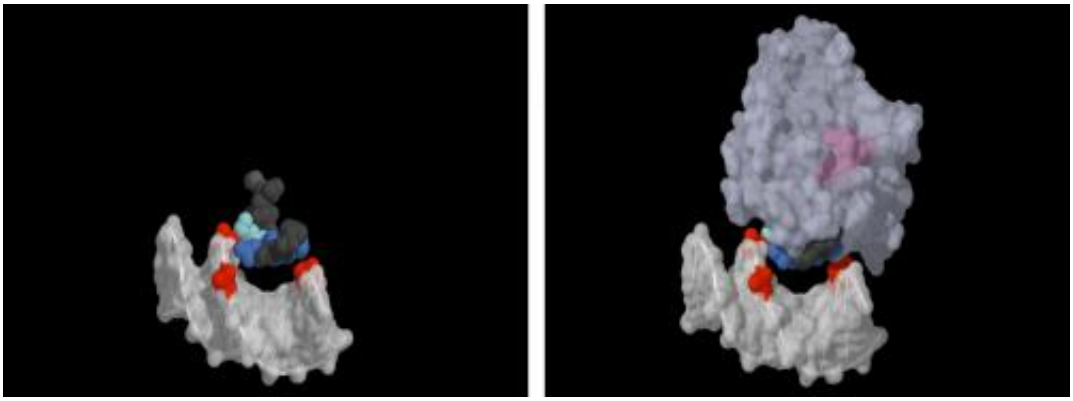

Model of the "molecular sled" mechanism. Left: The sled (dark gray and blue) sits in a groove on the DNA (white with four phosphate groups colored red). The blue parts of the sled are what interact with and slide along the DNA. Right, the adenovirus protease (light gray) is attached to the sled, with its active site (pink) exposed to interact with other proteins.

(Phys.org)—Scientists at the U.S. Department of Energy's (DOE) Brookhaven National Laboratory, with collaborators from Harvard University, the University of Madrid, Princeton University, and the University of Zurich, have discovered a new mechanism that may alter principle understandings of molecular interactions within a cell's nucleus. The discovery illustrates how two proteins of the human adenovirus use DNA as an efficient form of transportation inside a newly synthesized virus particle. The proteins use what the scientists are calling a "molecular sled," which slides along the DNA double helix-

much like a train running along its tracks-to find and interact with other proteins. In a series of four papers published back to back online October 7, 2012, in the *Journal of Biological Chemistry* under the title "Regulation of a viral proteinase by a peptide and DNA in one-dimensional space," the group, led by Brookhaven biophysicist Walter F. Mangel, has raised the possibility that all proteins in the nucleus of cells interact by sliding on DNA in this fashion.

The papers are a continuation of research by Mangel and his various collaborators that explores how a protease (an enzyme that cleaves other proteins) is involved in the replication of adenoviruses. The adenovirus protease shares common features found in the proteases of many other viruses, such as HIV, and some bacteria, such as Chlamydia. This makes the results from studying adenovirus-whose different types cause a wide variety of diseases from common colds, to pink eye, blindness, weight gain, and diarrhea-highly applicable to different areas of human health. Ultimately, the Mangel group plans to use what they learn to develop new antiviral and antibacterial agents.

At the time Mangel's group began working on adenovirus, it was known that its protease is responsible for the final stage of virus development. "The last step in formation of an infectious adenovirus particle is the activation of the protease and its cleavage-or cutting out-of some of the proteins used to assemble the virus particle," Mangel explained. Much in the same fashion that gothic cathedrals were built around a wooden frame, adenovirus uses "construction" proteins to build its frame. When a cathedral is nearly finished, the internal scaffolding is removed, leaving a fully functional building. Similarly, as shown in these papers, the adenovirus protease navigates the viral DNA in the new virus particle, cutting out the "construction" proteins.

In the early 1990s, the Mangel group observed the viral protease interacting with DNA, which surprised them because proteases had

never been known to interact with DNA before. Subsequent research characterized the binding of the protease to DNA. But, the group still did not know why the protease bound to DNA. In these four back-to-back papers, they finally describe the process in detail, and postulate an explanation.

Pathway to discovery

To elucidate the process, Mangel and his group placed a purified protease and one of the adenovirus proteins it cleaves in a test tube. Surprisingly, they did not interact; no cleavage occurred. Thinking this indicated that more than these two components are required for cleavage to take place, they mixed in disrupted virus particles with the two purified proteins. This resulted in the protein being cleaved. But when they purified the co-factor-the substance in the virus particle whose presence is essential for the protease's cleaving activity-they found out it was the virus' own DNA.

"That was a complete surprise," said Mangel. "Even more puzzling, we found out that the two proteins both needed to be on the same DNA molecule in order to interact," he added. "What was happening?" The simplest, but unprecedented, interpretation of these results was that the protease was sliding on the DNA to look for and cleave its proteins.

The breakthrough began when Mangel contacted Sunney Xie, a Professor in the Department of Chemistry and Chemical Biology at Harvard University. Mangel said he had some indirect evidence that the protease might slide on DNA as opposed to diffusing in any direction inside the virus particle. Xie, whose lab is famous for doing research on single biomolecules and single cells, agreed to help investigate, and Paul Blainey, a graduate student, became interested in working on the project. Brookhaven scientists Bill McGrath and Vito Graziano, who work with Mangel, labeled the protease with a fluorescent dye and Mangel took it

to Cambridge to see if it would slide.

"The first day, we saw no sliding on DNA and we were so disappointed," Mangel said. He and Blainey agreed to try one last set of experiments the next morning, in a more acidic solution. As soon as the first images of single molecules of proteases and DNA appeared on the computer screen, it was clear there was a massive amount of sliding of the enzymes along DNA, Mangel said. The group then went on to show that the protease finds its targets by sliding along DNA inside the virus particle as well, not just in test-tube experiments.

Another surprise came when they started to determine what enables the protease to slide on DNA. They found that the protease alone does not slide. It must first be attached to an 11-amino acid fragment from a different adenovirus protein. Interestingly, the fragment by itself slides on DNA. Could this fragment be a "molecular sled," which can slide along the DNA "track" carrying any cargo, not just the protease?

Proof of this 11-amino-acid fragment acting as a vehicle came when Mangel's group attached other proteins to the sled, even ones that by themselves do not bind to DNA, and discovered that those slid too. The concept of the "molecular sled" is the subject of a provisional patent that was just filed with the intent that the idea can be commercialized to deliver desired cargoes (for example, healthy genes for gene therapy).

New cellular transport system

The four articles contain the first examples of proteins sliding along DNA solely to interact with each other, rather than the DNA. This raises the possibility that all proteins in the nucleus of cells interact by sliding on DNA.

Four amino acids of the 11-amino-acid "molecular sled" contain a

sequence that enables nuclear proteins to enter the nucleus of cells. That sequence appears also to be where the "sled" attaches to DNA. "Since most, if not all, proteins that enter the nucleus contain the DNA-binding portion of the sled, and since the molecular sled slides on DNA, then perhaps most if not all proteins in the nucleus interact with each other by sliding along the DNA," Mangel said.

The high concentration of DNA inside a virus particle (and the nucleus of a cell) may offer an explanation for why such sliding takes place. In both environments, the DNA is so densely packed, there's no room for simple diffusion. "If one were to take the DNA from the nucleus of one human cell and stretch it out, it would be 7 feet long," Mangel said. "How can the protease find the proteins it has to cleave in such a DNA dense environment? Most everywhere it would move, it would bump into DNA, not its target proteins," he said.

But a protein sliding along the DNA could easily move around to find its targets. Such a mechanism might also greatly improve the efficiency of molecular interactions.

Normally in solution, for two molecules to bind to each other, they must collide at a specific speed into specific sites on their surfaces. In most collisions the molecules recoil and move apart, Mangel explained. But if both molecules are bound to DNA and one or both slide on the DNA, then the speed at which collisions occur is mostly determined by the speed of the sled, and the angle of the collisions is fixed by the interaction of the proteins with DNA.

"This could give rise to chemistry that is far more efficient, in which almost all collisions by sliding lead to binding," Mangel said.

To explore the process further-and how universal the sled mechanism might be-the group is now studying nuclear proteins that have nothing to

do with DNA metabolism to see if they also slide on DNA.

More information:

- Scientific paper: Regulation of a viral proteinase by a peptide and DNA in one-dimensional space. I. binding to DNA and to hexon of the precursor to protein VI, pVI, of human adenovirus
- Scientific paper: Regulation of a viral proteinase by a peptide and DNA in one-dimensional space. II. adenovirus proteinase is activated in an unusual one-dimensional biochemical reaction
- Scientific paper: Regulation of a viral proteinase by a peptide and DNA in one-dimensional space. III. atomic resolution structure of the nascent form of the adenovirus proteinase
- Scientific paper: Regulation of a viral proteinase by a peptide and DNA in one-dimensional space. IV. viral proteinase slides along DNA to locate and process its substrates

Provided by Brookhaven National Laboratory

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