New insights into how cells accessorize their proteins

September 18 2008

St. Jude Children's Research Hospital investigators have gained new insight into how the cell's vast array of proteins would instantly be reduced to a confusion of lethally malfunctioning molecules without a system for proteins to "accessorize" in order to regulate their function.

Just as eyeglasses improve vision, a coat provides warmth or an umbrella wards off rain, cells use a set of proteins called ubiquitin-like proteins (UBLs) as accessories that adapt their function as needed in the cell. Now St. Jude scientists have discovered how the function of a protein called cullin-RING changes when it wears the UBL accessory called NEDD8.

The researcher's findings, published in the Sept. 19, 2008, issue of the journal "Cell," reveal that NEDD8 changes the shape of cullin-RING to activate it to perform its function. The researchers found that when NEDD8 attaches, it transforms cullin-RING into a kind of molecular valet that can then attach a different accessory (ubiquitin) onto other proteins to foster the myriad of biochemical reactions that enable life.

"The ubiquitination machinery is critical for the cell's proteins to be able to function as necessary in a given environment. One of the major functions of ubiquitin is to mark a protein for disposal when its job is done," said Brenda Schulman, Ph.D., associate member in the St. Jude Structural Biology and Genetics and Tumor Cell Biology departments and Howard Hughes Medical Institute (HHMI) investigator. "Understanding ubiquitination can give us important knowledge about

such biological processes as cell division, embryonic development and immune function." Schulman is the paper's senior author.

"Basic insights into ubiquitination could ultimately have clinical application, because defects in the machinery have been implicated in cancers, neurodegenerative disorders and some viral infections," said David Duda, Ph.D., the paper's co-first author and HHMI research specialist in Schulman's lab.

Schulman and her colleagues study the enzymatic machinery that manages the accessorizing process, whether the accessory is NEDD8 or ubiquitin. Both molecules depend on specialized cadres of enzymatic valets that attach them to their correct targets.

One question the researchers addressed about the NEDD8-attaching machinery was how NEDD8 can make a considerable molecular leap to cullin-RING from the previous enzyme in the cadre that manages the attachment process. Also, the researchers wanted to understand how, once NEDD8 attaches to cullin-RING, it activates the enzyme to attach ubiquitin to its target protein. In this activation process NEDD8 also somehow thwarts the action of another molecule called CAND1, which normally inhibits cullin-RING.

"It is important to solve these lingering mysteries to give greater insights into this critically important process in the cell," said Daniel Scott, Ph.D., the paper's co-first author and HHMI research specialist in Schulman's lab.

In their experiments, the researchers crystallized cullin-RING proteins both with and without modification by NEDD8 attachment. They then subjected the crystals to structural analysis using X-ray crystallography. In this widely used technique, X-rays are directed through the crystal of a protein to be analyzed and its structure deduced from the pattern of

diffraction of the X-rays.

Comparison of the two structures revealed that, when NEDD8 attaches to cullin-RING, it dramatically modifies the conformation of the enzyme, causing it to blossom from a "closed" to an "open" state. This open state eliminates the binding site for the inhibitor CAND1, activating cullin-RING to then put ubiquitin onto a different target protein. The open state also frees a key component of the cullin-RING, called RING, making it flexible enough to function, helping cullin-RING transfer ubiquitin to its target protein.

Schulman said that their findings with NEDD8 and cullin-RING have broader significance for detailed understanding of the workings of the intricate ubiquitination machinery.

"The whole process of attaching ubiquitin—or any of this family of UBLs—to their targets involves moving a protein onto other proteins to regulate their activity," she said. "And in many cases, this process is probably regulated by just the kind of conformational change that our findings reveal. So, we believe that these findings offer overall insight into how conformational change can activate these kinds of ligases."

Source: St. Jude Children's Research Hospital