# Chemical switch triggers critical cell activities

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The freeze-frame image of a molecular relay race, in which one enzyme passes off a protein like a baton to another enzyme, has solved a key mystery to how cells control some vital functions, according to investigators at St. Jude Children's Research Hospital. A report on this work appears in the January 14 advanced online publication issue of *Nature*.

The St. Jude discovery explains how a simple chemical link between molecules called a thioester bond acts like a switch to control the handoff of a protein called NEDD8 like a baton from one enzyme called E1 to a second enzyme called E2. When attached to E2, this thioester bond allows E2 to bind to a third enzyme called E3, which then helps E2 hand NEDD8 off to the ultimate target molecule at the end of the race. In the cell, this NEDD8 relay race triggers a number of biochemical reactions, one of which takes the brakes off cell division, allowing cells to multiply, according to the researchers. These thioester bonds are chemical links between two biological molecules that form when a sulfur atom on one of the molecules binds to an atom that is part of the other molecule.

Understanding how the thioester bond switch works is important not only because it explains a critical step in the NEDD8 hand-off from E1 to E2, but also because enzymes related to E1 and E2 run similar relays with other important protein batons, said Brenda Schulman, Ph.D., associate member of the St. Jude Structural Biology and Genetics and Tumor Cell Biology departments. "Our study shows that this simple

switch could control comparable relays in charge of several different biochemical activities that keep cells alive and functioning normally." Schulman, a Howard Hughes Medical Institute (HHMI) investigator, is senior author of the Nature report.

Scientists already knew that E1 momentarily juggles two NEDD8 molecules at once, holding them in two different "hands." They also knew that E2 takes the NEDD8 that is in E1's left hand; and that the other NEDD8 then hops over from the right hand to E1's empty left hand. That leaves the right hand free to grab yet another NEDD8. By continually passing the NEDD8 proteins from the right hand to the left hand and on to E2, E1 keeps a relay of these batons flowing. In turn, that keeps the specific biochemical cascade triggered by NEDD8 in action.

But in order for the handoff to work, E2 must first insert itself into a docking site on E1, next to E1's left hand, so it can grab NEDD8, Schulman explained. However, the E2 docking site is initially turned away from E1: so if E2 hopped into the docking site at that point, it would be too far away from NEDD8 to grab it, she noted. And that was the puzzle the St. Jude researchers solved.

Specifically, the St. Jude study showed that when NEDD8 forms a thioester bond with E1's left hand, it squeezes itself next to the E2 docking site, which is facing away from NEDD8, according to Danny Huang, Ph.D., HHMI postdoctoral research associate and Harold Hunt, HHMI research technologist,. These researchers in Schulman's laboratory did most of the work on this project.

In such close quarters, NEDD8 bumps the docking site, making it rotate like the paddle wheel on a riverboat that carries a notch on one of its paddles. As the docking site rotates, it carries the notch all the way around to the other side, next to NEDD8. As soon as the notch of the docking site is next to NEDD8, the notch catches E2, so E2 is bound to

the notch, right next to NEDD8. NEDD8 then breaks its thioester bond with E1 and reforms it with E2. As soon as NEDD8 breaks its thioester bond to E1 and forms a thioester bond with E2 instead, E2 falls away from E1 taking NEDD8 with it. Then E2 can interact with E3 to pass NEDD8 onto the target.

The investigators were able to make sense of this combination of relay race and rotating paddle wheel by using a technique called X-ray crystallography. In X-ray crystallography, scientists bombard crystals of proteins with X-rays and use the patterns formed by beams bouncing off the crystals to create computer-generated 3-D images of the molecules.

The goal was to create a freeze-frame image that captured the fleeting moment when E2 is next to NEDD8, just before it falls off and carries NEDD8 with it. To do that, Schulman's team first had to prevent E2 from binding with NEDD8, so it would not fall away from E1. The investigators used a mutated form of E2 that binds to the docking site and gets near NEDD8, but cannot form a thioester bond with it. The team reasoned that if E2 could not bind to NEDD8, it would be stuck on the docking site with nothing to do and nowhere to go. An X-ray crystallography image could then capture that moment.

Source: St. Jude Children's Research Hospital