

AUGUST 15, 2002

## Researchers Identify Enzyme Involved in Protein Degradation

When the cell wants to dispose of unwanted proteins, it marks them for destruction and shreds them. Researchers are learning a great deal about this process, and they have identified an enzyme that the cell's shredder, called the proteasome, uses to strip off the tag that marks proteins for destruction.

The scientists have also discovered how the signalosome, another component of the cell, snips off a molecule similar to the one that marks doomed proteins. The findings have broad significance because the type of cleavage reaction that removes the tag is important during cell division, inflammation, transcription of genetic information, and development of the eye.

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The articles about the proteasome and signalosome were published on August 15, 2002, in *ScienceExpress*, which provides rapid electronic publication of select articles that will later appear in the journal *Science*. Howard Hughes Medical Institute (HHMI) investigator Raymond J. Deshaies and colleagues at the California Institute of Technology led the research team, which also included scientists from the National Institutes of Health (NIH), The Scripps Research Institute and the University of California, Los Angeles (UCLA).

Deshaies and his colleagues began their work by trying to figure out how the signalosome performs its pruning task. Bioinformaticians Eugene V. Koonin and L. Aravind at the NIH, together with Deshaies' team, hypothesized that a metal-containing catalytic center called JAMM — present in the Csn5 component of signalosome — was central to the action of the signalosome in clipping conjugates that contain the ubiquitin-like molecule Nedd8 attached to another molecule known as cullin.

Gregory A. Cope in Deshaies's group found that metal-binding chemicals inhibited the snipping activity of signalosome. Then, by altering JAMM in

yeast to knock out its activity, he confirmed that the JAMM element of Csn5 was needed for the cleavage action of signalosome.

To see whether JAMM was physiologically important, Deshaies's group teamed up with HHMI investigator Lawrence Zipursky at UCLA to test whether the Csn5-dependent development of the eye in fruit flies depended on JAMM's function. They found that when they specifically mutated the JAMM element in Csn5, there were massive defects in eye development and the flies did not survive beyond the larval stage.

"What is significant beyond our findings of the biochemistry of JAMM is that this is a completely new class of metalloproteases that had never been discovered before," said Deshaies. "And now we can say that it exists from bacteria to humans. In addition, the Csn5 enzyme has been linked to a broad variety of processes, and it was conceptually difficult before to understand how it could possibly be affecting all of these different processes."

As the work on Csn5 was proceeding, HHMI research associate Rati Verma in Deshaies's group was trying to understand how the proteasome strips off ubiquitin, which marks proteins for degradation. "You can think of a protein as a ball of yarn, and in order to get the protein into the proteasome for destruction, it has to unwind and thread through a small hole in the proteasome," said Deshaies. "But if ubiquitin is attached (to the protein), the proteasome somehow has to cut it off, because otherwise, it would jam up the process." The twin mysteries, said Deshaies, were the identity of the enzyme that snips off ubiquitin and how important that cleavage was to proteasome function.

A major obstacle facing Deshaies and his colleagues was that researchers had not been able to block the cleavage process by using chemicals that commonly inhibit isopeptidases, the types of enzymes that were thought to snip off ubiquitin.

However, the proteasome contains a component, called Rpn11, which is similar to Csn5 and likewise contains the JAMM element. This suggested that like the signalosome, the proteasome might have a metallo-isopeptidase activity.

In initial studies, the scientists treated the proteasome with chemicals that bind to metals, discovering that they inhibited the ubiquitin-snipping process. To test whether Rpn11 was the key ubiquitin-cleaving enzyme, they then produced strains of yeast in which the JAMM element of Rpn11 was made non-functional. They found that not only was Rpn11 the ubiquitin-cleaving enzyme, but that protein degradation in the proteasome ceased without Rpn11 activity.

"It was a surprise to us that the Rpn11-catalyzed process was vital to protein turnover," said Deshaies. "Most people in the field seemed to think that if the cell couldn't cut ubiquitin off the protein as it was unraveling, somehow the attached ubiquitin would just unravel along with the protein and get dragged into the proteasome, too. And then everything would be destroyed inside the

proteasome together."

Finding that Rpn11 is essential for degradation could have clinical implications, said Deshaies. Studies have shown that cancer cells appear to be particularly sensitive to chemicals that inhibit the proteasome, and anti-cancer drugs are being developed that shut down the activity of the proteasome, he noted.