

When the Lights Went On for COP9

A protein's role in ubiquitin-mediated proteasomal degradation plants the seed for big ideas | By Eugene Russo

It doesn't take a green thumb to predict what happens to plants left in the dark: They wither. But in the late 1980s and early 1990s, researchers, including people in Xing-Wang Deng's Yale University lab, stumbled upon a group of intriguing *Arabidopsis* mutants that seemed to defy intuition. If provided the right nutrition, these plants could retain a shape, form, and cellular state similar to those grown in ample light for weeks, and even months, of sustained darkness. Some could even flower.

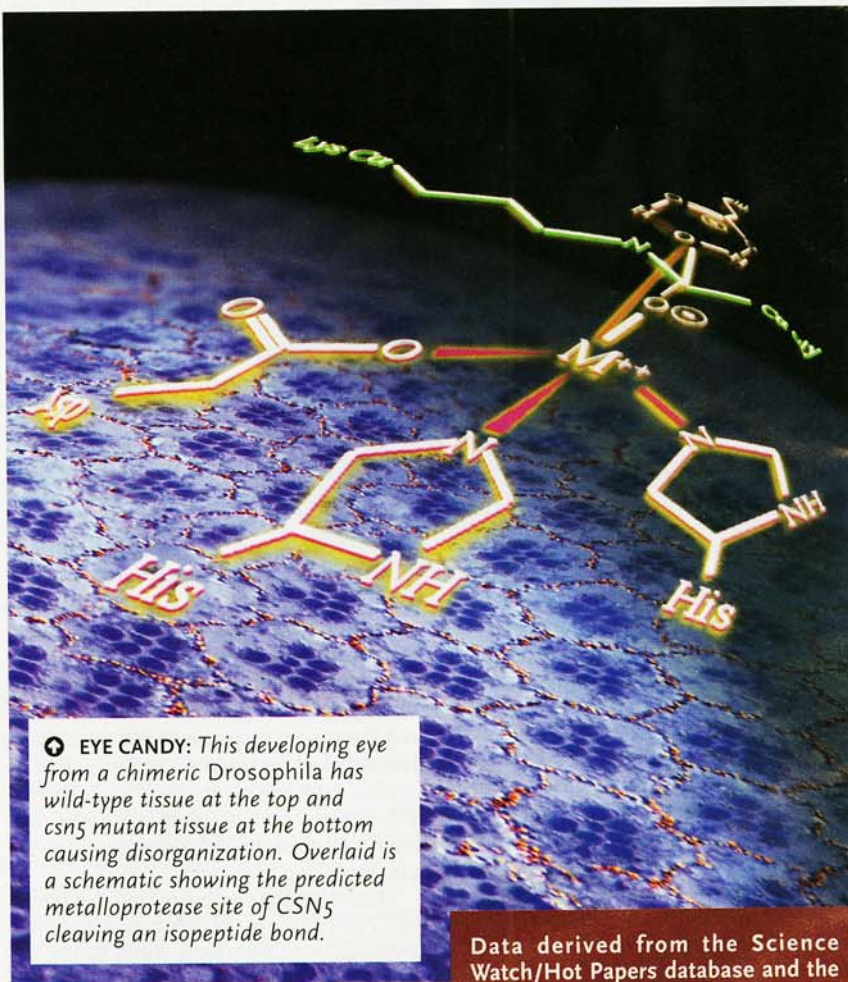
In 1994, Deng's group identified *COP9*, one of the genes responsible for this impressive feat.¹ After doing some bioinformatics digging and biochemistry work, they found that the *COP9* gene encoded a novel protein that was part of a larger protein complex later called the *COP9* signalosome (CSN).

As it turns out, the CSN does more than regulate plant responses to light; Deng's lab and others subsequently found signalosome homologs in mammals and other species. "There were a variety of different facts floating around and a lot of speculation," says Svetlana Lyapina, a Hot Paper first author and now a manager of strategy and corporate development at Amgen, Thousand Oaks, Calif. "But there was no sort of unified theory of what signalosome does and how it does it."

This issue's Hot Papers^{2,3} link CSN function to ubiquitin ligases, a family that includes hundreds of known key regulators of inflammation and the cell cycle. Approaching signalosome function from different fields (biochemistry and plant genetics) and with different agendas, the two groups found that in plants,² yeast, and mammalian cells,³ the CSN directly interacts with an ubiquitin ligase complex that mediates proteasomal degradation of proteins involved in cell cycle and development.

"Basically, this provided a biochemical mechanism, a biochemical connection for how *COP9* signalosome is involved in protein degradation mediated by the proteasome," says Deng. The complexes are now known to be major signaling processors in the cell, and they may be relevant in treating diseases such as cancer. Thus, with the shade drawn, a new research window had burst open.

TWO APPROACHES Deng and his lab were working explicitly on the signalosome,



EYE CANDY: This developing eye from a chimeric *Drosophila* has wild-type tissue at the top and *csn5* mutant tissue at the bottom causing disorganization. Overlaid is a schematic showing the predicted metalloprotease site of *CSN5* cleaving an isopeptide bond.

while Raymond Deshaies, a Howard Hughes Medical Institute assistant investigator at California Institute of Technology, was interested in identifying ubiquitin ligases and understanding their regulation in mammals and yeast. Deng says that he had immediately suspected that the *COP9* signalosome he'd discovered was of some importance. But the enigmatic mutant phenotype offered few clues as to its function.

The first hint came in 1998. A German group⁴ and Deng's group⁵ both confirmed that mammals shared the same protein complex that had been discovered in plants. In the same year, another group, led by Harvard cell biologist Daniel Finley,⁶ discovered that the eight subunits of the CSN closely resemble a

Data derived from the Science Watch/Hot Papers database and the Web of Science (ISI, Philadelphia) show that Hot Papers are cited 50 to 100 times more often than the average paper of the same type and age.

C. Schwechheimer et al., "Interactions of the *COP9* signalosome with the E3 ubiquitin ligase SCFTIR1 in mediating auxin response," *Science*, 292:1379–82, 2001. (Cited in 128 papers)

S. Lyapina et al., "Promotion of NEDD8-CUL1 conjugate cleavage by *COP9* signalosome," *Science*, 292:1382–5, 2001. (Cited in 128 papers)

Courtesy of Greg Suh, University of California Los Angeles; Andrew Moore, InfransMoore Group

subcomplex of the proteasome, a veritable cellular trashcan that assists with protein degradation. This subcomplex, the so-called lid of the proteasome, appeared to play a key role in recognizing ubiquitin-tagged substrate proteins and channeling them for proteasomal degradation.

Deng and colleagues provided more definitive details in 2000.⁷ One of Deng's students, Mark Osterlund, showed that the COP9 signalosome helps degrade the transcription factor HY5, a player in the light-regulated development of *Arabidopsis*, which explains in part the COP9 mutant's response to darkness. "The discovery of the lid influenced that [signalosome] field tremendously, because it said that the signalosome must be part of the ubiquitin system in some way," explains Finley. But although the 2000 work suggested that the CSN must be important for proteasome-mediated degradation, only the 2001 work would show that this was through direct protein contact.

Meanwhile, Deshaies, who was known primarily for his work on ubiquitin ligases in yeast, and erstwhile graduate student Lyapina sought to understand ubiquitin-ligase regulation in mammalian cells. They hunted proteins that copurified with the ubiquitin E3 ligase complexes, a specific type labeled SCF, to elucidate how the complex is regulated through its protein partners. To their surprise, they discovered that the SCF interacts with the signalosome.

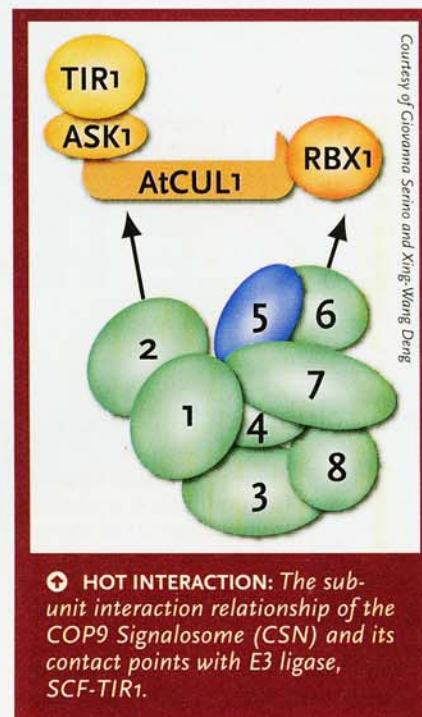
TWO HYPOTHESES Immediately there were two hypotheses, Lyapina explains: Either SCF ubiquitin ligase was somehow regulating the signalosome, or vice versa. In hopes of solving this puzzle, they contacted Deng to collaborate. Lyapina then set out to distinguish between these scenarios by attempting to downregulate the CSN and observe the consequences to SCF activity. She investigated the interaction in several model systems, but the breakthrough came in fission yeast, *Schizosaccharomyces pombe*, in which the signalosome had been discovered recently. Knocking out the CSN affected SCF function.

Both Deshaies and Deng suggest that the two papers complement each other. "Ours is at the level of the molecules and his is at the level of the organism," Deshaies explains. Deng and colleagues, studying *Arabidopsis*, showed that the CSN regulates the SCF E3 ligases by biochemical contact. It promotes degradation of the substrate involved in the plant's response to the developmental hormone auxin, long known for its critical role in plant development.

Deshaies's lab demonstrated that the interaction of SCF and CSN also occurs in

mammalian and yeast cells. Furthermore, they identified a particular biochemical modification: CSN regulates the ubiquitin ligase by cleaving a protein called NEDD-8 from a subunit of the SCF.

Deshaies emphasizes that signalosome knowledge, when Lyapina first started her work, was based largely on plant genetics. No biochemical assays were available, making targets hard to ascertain. But there was a wealth of biochemical knowledge about the SCF at the time of Lyapina's discovery, notes Deshaies. "So, she could kind of plug into that knowledge base to figure out what aspect of SCF was being modulated by signalosome."



MANY POSSIBILITIES Subsequent work from Deng's lab, Deshaies's lab, and others has illustrated the CSN's potential relevance for medical applications. Deshaies and graduate student Gregory Cope uncovered not only the ubiquitin connection but a metalloprotease correlation as well. Through a bioinformatics discovery that was later validated by a recently reported genetic analysis, Deshaies and Cope found that NEDD-8 cleavage occurred as a result of metalloprotease activity intrinsic to one of the signalosome subunits.⁸ The opportunities for drug development were clear, says Lyapina. An enzymatic signalosome mechanism that's relevant to protein degradation and the cell cycle could be an early step toward developing therapies for a variety of diseases.

Many illnesses, especially cancer, notes Deng, stem from some defect in the cell's mechanism of protein degradation. In the two-plus years since the Hot Papers' publication, Deng's colleague, Yale cell biologist Ning Wei, has shown that CSN affects tumor suppressors p53 and p21, as well as other cell-cycle regulators. Wei's work included generating a mouse knockout of the CSN.⁹

But the 2001 papers continue to have implications for basic plant cell biology as well. A few months after the papers appeared, Mark Estelle, a professor of plant biology at Indiana University in Bloomington, discovered that auxin response requires an ubiquitin ligase.¹⁰ "It's become clear that ubiquitin-mediated processes are really important," says Estelle, who occasionally collaborates with Deng. "That hadn't been clear at all 10 years ago ... it's been a huge revelation."

Estelle contends that the ubiquitin connection was "the first clearly defined function that everyone could agree on, that everyone believed." But it was just the beginning, says Lyapina: "We sort of answered one question. But it opened up many more questions, so that people now could form hypotheses to test." ☉

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References

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