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Life Is Degrading—Thanks to Some Zomes

Meeting Review

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Three structurally related protein complexes, the COP9 signalosome, the proteasome lid, and the eukaryotic translation initiation factor 3, are revealing new insights into developmental processes and into cell cycle control in healthy cells and cells exposed to genotoxic stress. Newly discovered cullin-RING E3 ubiquitin ligases assembled on the CUL4 platform may provide links between DNA replication, chromatin, and proteolysis.

"Life is degrading—thanks to some zomes." This quote from Alfred Goldberg (Harvard University, Cambridge, Massachusetts) encapsulates one aspect of the conference "Zomes IV—The 4th International Symposium on the COP9 Signalosome, Proteasome, and eIF3—at the Interface between Signaling and Proteolysis" held on the Yale University campus in New Haven, Connecticut, June 18–21, 2006. Since the inaugural Zomes meeting (Deng et al., 2000), this biannual conference has thrived by bringing together more than 100 investigators from diverse fields who focus on three formerly eclectic protein complexes that have since moved straight into the cell biological mainstream: the COP9 signalosome (CSN), the 26S proteasome lid complex, and the eukaryotic translation initiation factor 3 (eIF3). These three complexes share a unique molecular architecture characterized by up to six subunits carrying the *p*roteasome-*C*OP9-eukaryotic *i*nitiation factor (PCI) domain and up to two subunits harboring the *M*pr1-*P*ad1 *N* terminus (MPN) domain (Figure 1; Scheel and Hofmann, 2005). By popular demand, the meeting roster of these three so-called PCI complexes has been expanded to include also the cullin-RING E3 ubiquitin ligases (CRLs), which interact with CSN and steer the polyubiquitylation of proteins destined for proteasomal degradation (Figure 2). While the CRLs, CSN, and the proteasome control protein degradation, the eIF3 complex functions in cytosolic translation initiation and thus provided a subtle counterpoint to the melody of protein degradation played out at the meeting.

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Biochemical Studies of CRLs and the CSN

CRLs make up the largest class of E3 ubiquitin ligases. Best understood among various classes of CRLs are the SCF (*S*kp1-*c*ullin-*F* box) complexes. A large scaffold protein, CUL1, binds the RING finger protein Rbx1, which guides the transfer of ubiquitin from an E2 conjugating enzyme to a degradation substrate (Figure 2). The substrate protein is bound via a CUL1-interacting heterodimer that consists of the SKP1 adaptor protein and one of up to several hundred substrate-specific F box proteins (hereafter referred to as substrate receptors). Binding of a substrate to the F box protein triggers polyubiquitylation via Rbx1, which is typically followed by its proteolysis in the 26S proteasome. The overall architecture of the SCF E3 complexes is repeated in the already established CUL2/5- and CUL3-containing CRLs. The composition of the more recently discovered CUL4 complexes, however, is currently hotly debated (Figure 2).

Many if not all cullins are subject to an idiosyncratic—and, in higher eukaryotes, essential—posttranslational modification, the attachment of a small ubiquitin-like protein termed Nedd8 to a lysine residue near the carboxyl terminus of the cullin (Figure 2; Bostick et al., 2004; Pan et al., 2004). How cullin neddylation controls the ubiquitylation process is not fully understood, however. At Zomes IV, Zhen-Qiang Pan (Mount Sinai School of Medicine, New York City, New York) addressed this point by presenting a detailed in vitro analysis of site-directed CUL1 mutations. His studies with the $SCF^{\beta TrCP2}$ complex, which targets IκBα, suggested that CUL1 neddylation releases an autoinhibitory effect by the extreme carboxyl terminus of CUL1 on its interaction with Rbx1. Thus, neddylation may boost Rbx1's ability to recruit an E2 to the $SCF^{\beta TrCP2}$ CRL, leading to an up to 20-fold enhanced polyubiquitylation of the IκBα substrate.

The COP9 signalosome, which is composed of the eight subunits CSN1–CSN8, interacts with CRLs and deconjugates Nedd8 from the cullin subunit. To date, cullin deneddyation—an essential activity in higher eukaryotes mediated by CSN5—is the only known enzymatic activity of CSN per se (Lyapina et al., 2001; Pan et al., 2004). Hence, the biochemistry and biology of CSN is tightly interwoven with that of the CRLs. Just like cullin neddylation, genetic studies suggest that cullin deneddylation is required for efficient CRL activity in vivo. This apparent paradox has been reconciled by the hypothesis that cycles of neddylation and deneddylation are critical for CRL function (Lyapina et al., 2001; Schwechheimer et al., 2001). One prominent model postulates that CSN-mediated deneddylation controls the signal-dependent exchange of substrate receptors, such as F box proteins (Cope and Deshaies, 2003). A second function of the CSN in CRL maintenance may reside in the reversal of accidental autoubiquitylation, a function attributed to the CSN-associated Ubp12/USP15 deubiquitylating enzyme identified in fission yeast and mammalian cells (Zhou et al., 2003; Hetfeld et al., 2005). At Zomes IV, several investigators reported data consistent with this resolution of the "CSN

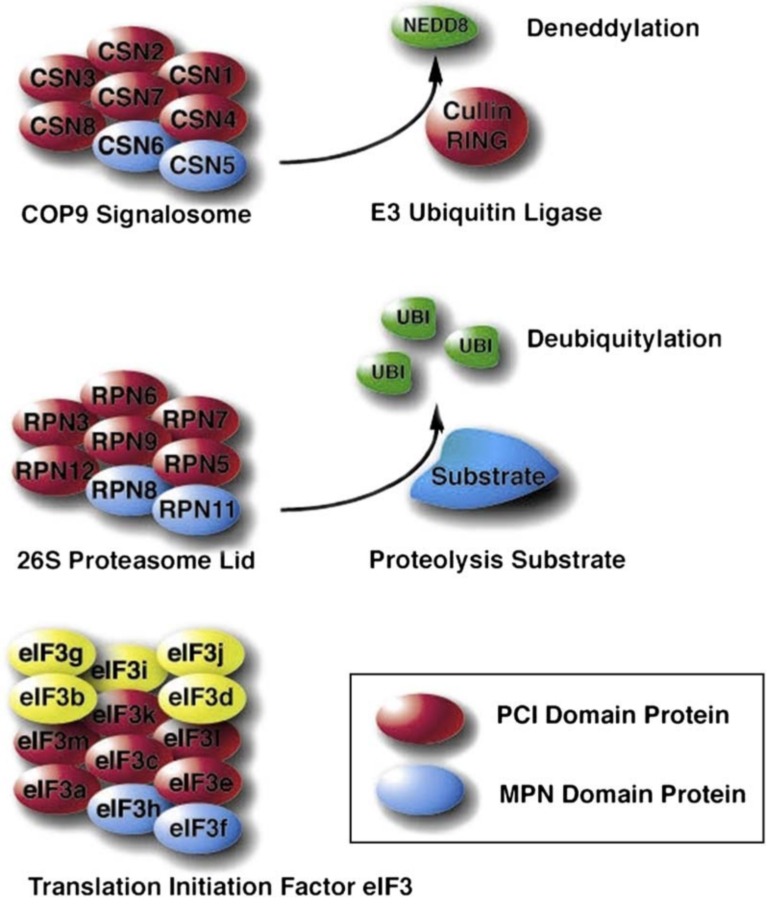

Figure 1. COP9 Signalosome, the Proteasome Lid, and eIF3 Each Contain Six PCI and Two MPN Domain Proteins

The JAMM (*Ja*b1/*M*PN domain *m*etalloenzyme) metalloprotease motif within CSN5's MPN domain confers deneddylation activity toward cullins. The proteasome lid has a homologous composition. Its RPN11 subunit is endowed with the JAMM motif, which in this case confers the ability to cleave ubiquitin from polyubiquitylated degradation substrates. MPN domains with the JAMM motif are also referred to as MPN+. Neither of eIF3's MPN proteins contains the JAMM motif. The five easily recognizable PCI proteins in eIF3 are now known to be accompanied by a sixth PCI protein, eIF3m, which is present in eIF3 complexes from mammals (Unbehaun et al., 2004), fission yeast (Zhou et al., 2005), and plants (Burks et al., 2001; Karen Browning, personal communication). Of the up to 13 eIF3 subunits, only five are present in the budding yeast eIF3 core complex.

paradox." Yi Liu (University of Texas at Dallas, Dallas, Texas) noticed that Neurospora circadian clock-associated SCF subunits were destabilized in *csn2* mutants (He et al., 2005), and, likewise, Elisabetta Bianchi (Institut Pasteur, Paris, France) reported that a knockdown of CSN4 or CSN5 in mammalian cells destabilized the F box protein Skp2 while its cognate target, the cell cycle regulator $p27^{KIP1}$, was stabilized. In turn, overexpression of SKP2 was able to restore the proliferation defects caused by the CSN knockdown. Detailed observations were made by the lab of Dieter Wolf (Harvard University, Cambridge, Massachusetts) who presented data for fission yeast substrate receptor proteins. The stabilization of BTB proteins relied more on the CSN-associated Ubp12, whereas F box protein stabilization relied more on the CSN subunit CSN5. Wolf identified a conserved proline residue in the F box domain, which makes direct contact with CUL1. The stability of five such F box proteins appeared to be CSN dependent, whereas three other F box proteins that lack the conserved proline did not interact with CUL1, were more stable, and were independent of CSN.

Proteasome Biochemistry

The recognition of a protein by a subunit of the 26S proteasome lid is generally considered as the point of no return for proteins targeted for degradation by polyubiquitylation (Figure 1). Like the CSN, the lid comprises eight subunits, and its CSN5-related subunit Rpn11 has the ability to cleave ubiquitin from the polyubiquitylated degradation substrate, presumably a form of ubiquitin recycling. Once deubiquitylated, the substrate is unfolded by AAA-ATPases in the 26S base complex and degraded by up to three different proteolytic activities built into the 20S core particle of the proteasome.

Biochemically, the 26S proteasome is undeniably the best understood of the zomes. Still, many aspects of its function remain enigmatic, and the idea of a passive shredder is challenged by data suggesting that the 26S actively discriminates between ubiquitylated substrates, orchestrates delivery to the 26S, and even pardons certain substrates prior to execution by deubiquitylating them. Mark Hochstrasser (Yale University, New Haven, Connecticut) generated yeast strains in which assembly of the 20S proteasome core is slowed down, which in turn permitted the identification of a series of assembly intermediates as well as discrete assembly factors including Ump1 and several novel proteins. Alfred Goldberg (Harvard University, Cambridge, Massachusetts) reviewed mechanisms whereby AAA-ATPases of the archaeal PAN complex trigger gate opening of the 20S core of the proteasome (Smith et al., 2005).

Homing in on the problem of substrate delivery, Dan Finley (Harvard, MA) introduced ubiquitylating and deubiquitylating enzymes that are peripherally associated with the 26S. One of these, the deubiquitylating enzyme Ubp6, unexpectedly delayed the degradation of ubiquitylated proteins by a noncatalytic mechanism. During degradation delay, the catalytic activity of Ubp6 progressively deubiquitylated the target protein,