

COP9 signalosome revisited: a novel mediator of protein degradation

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The COP9 signalosome is an evolutionarily conserved multiprotein complex that was first identified as an essential complex that represses light-regulated development in *Arabidopsis*. The COP9 signalosome has similarity to the lid of the 19S regulatory particle of the 26S proteasome and has recently been shown to interact with SCF-type E3 ubiquitin ligases. Although its precise role in the process of protein degradation remains to be established, the COP9 signalosome is a positive regulator of E3 ubiquitin ligases that functions at least in part by mediating the deconjugation of the NEDD8/RUB-modification from the cullin subunit of SCF-type E3 complexes. Here, we discuss these recent findings, which add an additional component to the biology of substrate-specific protein degradation.

The COP9 signalosome is a nuclear-enriched multiprotein complex that has been found in plants, mammals, *Drosophila* and the fission yeast *Schizosaccharomyces pombe*¹⁻⁷. Initially, the COP9 signalosome was identified from *Arabidopsis* mutants that mimic light-induced seedling development when grown in the dark^{2,8} (Fig. 1). Loss-of-function mutants have been identified from *Arabidopsis* that carry defects in at least four COP9 signalosome subunits^{1,8-12}. In these mutants, the loss of one subunit typically results in loss of the entire protein complex, and this can be used to explain the pleiotropic but identical phenotype of these mutants. Subsequent to its isolation from plants, the COP9 signalosome was also identified from other species, in which it was found to be important for processes such as cell-cycle control in fission yeast and proper development in *Drosophila*^{5,6}. Interestingly, the COP9 signalosome seems to be absent from the budding yeast *Saccharomyces cerevisiae* as all but one COP9 signalosome subunit (CSN5) is missing from the genome of this organism¹³⁻¹⁵.

The eight subunits of the COP9 signalosome are each paralogous to one of the eight subunits that form the lid of the 26S proteasome¹³ (Table 1). The 26S proteasome carries out the bulk of non-lysosomal protein degradation in eukaryotic cells. It consists of a 20S core cylinder and is capped on either side by a 19S regulatory particle that can be further subdivided into a base and a lid¹⁶ (Fig. 2). The lid is thought to recognize ubiquitylated substrates that are then unfolded by the base and funneled into the proteolytic 20S core complex for degradation¹⁶ (Fig. 2). Based on the homology between the COP9 signalosome and the 26S proteasome, it has been speculated that the COP9 signalosome plays a role in protein degradation, possibly as the lid of a new kind of COP9 proteasome^{12,15}.

Six of the eight COP9 signalosome subunits have a PCI domain, a 200-amino-acid domain that is conserved in subunits of the 26S proteasome, the COP9 signalosome and the eukaryotic initiation factor eIF3 (Table 1)^{14,17}. The two remaining subunits have a 140-residue domain known as the MPN (Mpr1p and Pad1p N-terminal) domain, which is also present in subunits of the 26S proteasome and eIF3 (Table 1)^{14,18}. The biochemical function of these domains is not known but, because they are only found in multiprotein complexes, it has been postulated that they mediate the interaction between individual subunits of the complex^{14,15}. Although, in most cases, the homology between proteins with a PCI or an MPN domain is restricted to these domains, the homology between the COP9 signalosome and the 26S proteasome subunits extends over their entire length, suggesting a common evolutionary origin for these two complexes¹³⁻¹⁵.

Recently, new insights have been gained into the biochemical role of the COP9 signalosome that link the COP9 signalosome closely to the function of E3 ubiquitin ligases^{19,20}. At least in part, the COP9 signalosome might function by removing the NEDD8/RUB modification from the cullin subunit of SCF-type E3 ubiquitin ligases. In this article, we summarize these recent findings and try to place previous observations on the COP9 signalosome in a meaningful context.

COP9 signalosome interacts with SCF-type E3 ubiquitin ligases

New insights into COP9 signalosome function have come from work on E3 ubiquitin ligases. E3 ubiquitin

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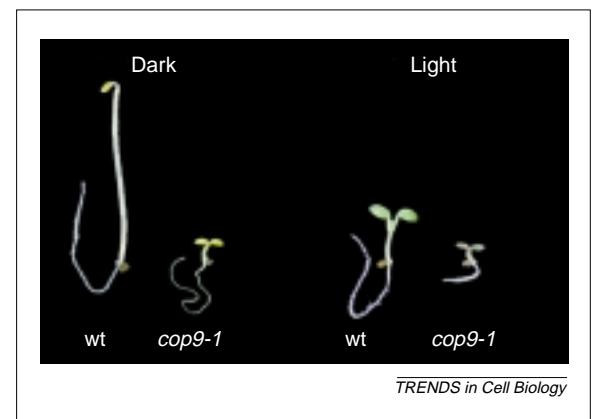


Fig. 1. The COP9 signalosome is required for the repression of photomorphogenesis in the dark. COP9-signalosome-null mutants from *Arabidopsis* (here, *cop9-1*) mimic the phenotype of a light-grown wild-type plant when grown in the dark.

Table 1. COP9 signalosome subunits, their 19S proteasome lid paralogs and their former designations in *Arabidopsis* and human^a

COP signalosome subunit	Lid paralog	Homology	Domain ^b	<i>Arabidopsis</i>	Human
CSN1	RPN7p	22%	PCI	COP11/FUS6	GPS1
CSN2	RPN6p	21%	PCI		TRIP15
CSN3	RPN3p	20%	PCI		SGN3
CSN4	RPN5p	19%	PCI	COP8/FUS4	
CSN5	RPN11p	28%	MPN	AJH1/AJH2	JAP1
CSN6	RPN8p	22%	MPN		VIP
CSN7	RPN9p	15%	PCI	FUS5	
CSN8	RPN12p	18%	PCI	COP9/FUS7	COP9

^aPrepared, with modifications, from Refs 7 and 15.

^bAbbreviations: MPN, a conserved domain in Mpr1p and Pad1p N-terminal; PCI, a conserved domain in the 26S proteasome, COP9 signalosome and eukaryotic initiation factor eIF3.

ligases recognize protein substrates for degradation and bring them into the vicinity of an E2 ubiquitin-conjugating enzyme that mediates substrate ubiquitylation^{20–22} (Table 2; Fig. 3). The ubiquitylated substrates are then recognized by the lid of the 26S proteasome and degraded in its 20S proteolytic core cylinder¹⁶ (Figs 2 and 3).

SCF-type E3 ubiquitin ligases are one of several types of proteins or protein complexes with E3 activity^{21,22}. SCF-type E3 complexes are found in all eukaryotes and typically consist of four subunits: a SKP1-like subunit, a CDC53/cullin subunit (hereafter referred to as 'cullin'), an F-box-domain-containing subunit and a RBX1/HRT1/ROC1 subunit (hereafter referred to as 'RBX1'; Fig. 3)^{21,22}. The substrate specificity of these complexes is mediated by a protein–protein interaction domain of the F-box-domain subunit²¹. During experiments to identify interacting partners for human cullin 1 (hCUL1), a physical interaction was discovered between SCF-type E3 ubiquitin ligases and the COP9 signalosome²⁰. Affinity-purified hCUL1 from mammalian cells bound the expected set of SCF-complex subunits (SKP1, F-box domain proteins and RBX1) as well as the entire COP9 signalosome²⁰ (Fig. 3). Other members of the cullin family (and their respective E3 complexes) from mammals, fission yeast and *Arabidopsis* also associate with the COP9 signalosome, suggesting that the interaction with the COP9 signalosome is a conserved feature of E3 ubiquitin ligase function in these organisms^{19,20}.

Two-hybrid analysis between the individual subunits of the COP9 signalosome and SCF complexes showed a direct physical interaction between the two protein complexes^{19,20}. For SCF-type E3 ubiquitin ligases, it is known that cullin and RBX1 form a core complex that recruits distinct combinations of a SKP1-like and an F-box-domain-containing subunit²¹. The COP9 signalosome binds specifically to the two core subunits of the SCF complex, and so the COP9 signalosome might interact with many SCF-type E3 ubiquitin ligases that differ in their SKP1-like and F-box-domain

subunits, and consequently in the substrates and the pathways that they control.

COP9 signalosome is required for the activity of SCF-type E3 ubiquitin ligases

The physiological relevance of the interaction between E3 ubiquitin ligases and the COP9 signalosome was revealed through studies in plants. The phytohormone auxin is believed to play a role in almost every aspect of plant growth and development²³. The transcription of a family of transcriptional repressor proteins from *Arabidopsis*, the AUX/IAA proteins, is rapidly induced upon exposure to auxin²⁴. It is believed that AUX/IAA proteins repress their own transcription through a negative-feedback loop in the absence of auxin and that this transcriptional repression is relieved in the presence of auxin through degradation of the AUX/IAA repressor protein(s)^{24–30} (Fig. 3). Genetic data suggest that at least some AUX/IAA proteins are degraded by the E3 ubiquitin ligase SCF^{TIR1} (Refs 27–31).

SCF^{TIR1} interacts with the COP9 signalosome, and, interestingly, SCF^{TIR1} loss-of-function mutants have several phenotypes that are also observed in plants with reduced COP9 signalosome function, such as reduced lateral root development and reduced root hair elongation¹⁹. In addition, the auxin inducibility of the AUX/IAA genes and the rate of AUX/IAA protein degradation is reduced in plants with reduced COP9 signalosome function. Taken together, the physiological and molecular data suggest that the COP9 signalosome acts as a positive regulator of SCF^{TIR1} (Fig. 3)¹⁹.

COP9 signalosome might also be required for the activity of non-SCF-type E3 ubiquitin ligases. COP1 is required for the repression of photomorphogenesis in dark-grown *Arabidopsis* seedlings^{32,33}. The *cop1* mutants were identified in the same mutant screens that led to the *Arabidopsis* COP9 signalosome mutants⁸. Genetic and molecular

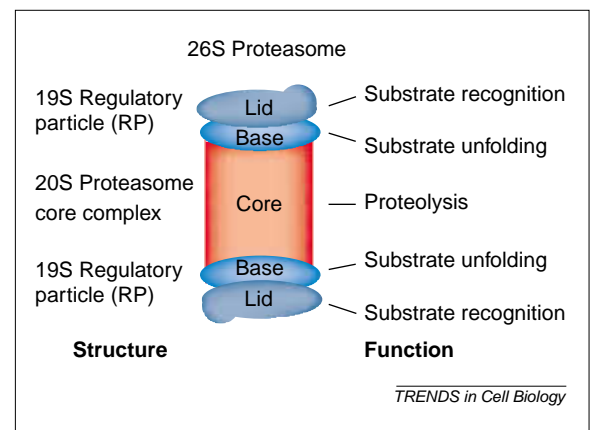


Fig. 2. Schematic representation of the 26S proteasome. The 26S proteasome consists of a 19S regulatory particle and a 20S proteolytic core. The 19S regulatory particle can be divided into lid and base subcomplexes. The COP9 signalosome is paralogous to the lid of the 19S regulatory particle (Table 1).

Table 2. Enzymes involved in substrate conjugation of ubiquitin and ubiquitin-like modifying proteins^a

Ubiquitin	E1 activating enzyme	E2 conjugating enzyme	E3 ligase	Substrates
Ubiquitin	Uba1	Ubc1 (+isozymes)	Many	Many
NEDD8/RUB1	Uba3–Ula1	Ubc12	Cullin/RBX1?	Cullins
SUMO/SMT3	Uba2–Aos1	Ubc9	Not required?	RanGAP1, p53, I-κB, septins and others

^aPrepared, with modifications, from Ref. 43.

studies have established that COP1 and the COP9 signalosome are required for the dark-dependent degradation of the transcription factor HY5, a positive regulator of photomorphogenesis^{34,35}. COP1 is closely related to c-Cbl, an E3 ubiquitin ligase of the RING-finger type, and this, together with the role of

COP1 in HY5 degradation, suggests that COP1 functions also as an E3 ubiquitin ligase^{35,36}. Although a direct physical interaction between COP1 and the COP9 signalosome remains to be established, the genetic data point at a close interplay between the COP9 signalosome and COP1 (see also the section below on nucleocytoplasmic partitioning)^{34,35}. COP1 is therefore a good candidate for a non-SCF-type E3 ubiquitin ligase that requires COP9 signalosome function, and in turn this provides circumstantial evidence that the COP9 signalosome interacts with a diverse set of E3 ubiquitin ligases.

Protein degradation: the common theme?

Many regulatory proteins have been shown to interact with distinct COP9 signalosome subunits in protein–protein interaction screens (Table 3). It is noteworthy that most of the proteins that interact with the COP9 signalosome are either directly involved in protein degradation or known to be regulated by protein degradation, such as transcription factors and cell-cycle regulators (Table 3). In addition, biochemical studies suggest that the COP9 signalosome or an associated protein functions as a protein kinase of various COP9 signalosome-interacting proteins^{3,37,38}. In many cases, substrate phosphorylation is required for E3 ubiquitin-ligase-mediated ubiquitylation²¹. Therefore, it could be envisioned that the COP9 signalosome functions as a scaffold complex that brings together several components of the protein degradation process, ranging from substrate binding and phosphorylation to substrate ubiquitylation and possibly also degradation (Figs 3 and 4).

COP9 signalosome promotes deneddylation

NEDD8/RUB (hereafter referred to as 'NEDD8') and SUMO are two small ubiquitin-like modifier proteins from eukaryotes^{39,40}. Like ubiquitin, NEDD8 and SUMO are conjugated to their respective targets through a cascade of E1-, E2- and E3-related enzymes^{41–43} (Table 2). However, as opposed to the role of ubiquitin in protein degradation, the NEDD8 and SUMO modifications are thought to have regulatory roles. The cullin subunits of SCF-type E3 ubiquitin ligases are the only known proteins modified by NEDD8 ('neddylation')^{43,44}. Whereas wild-type cells typically contain a mixture of neddylated and un-neddylated cullins, COP9-signalosome-deficient cells accumulate exclusively neddylated cullins^{19,20}.

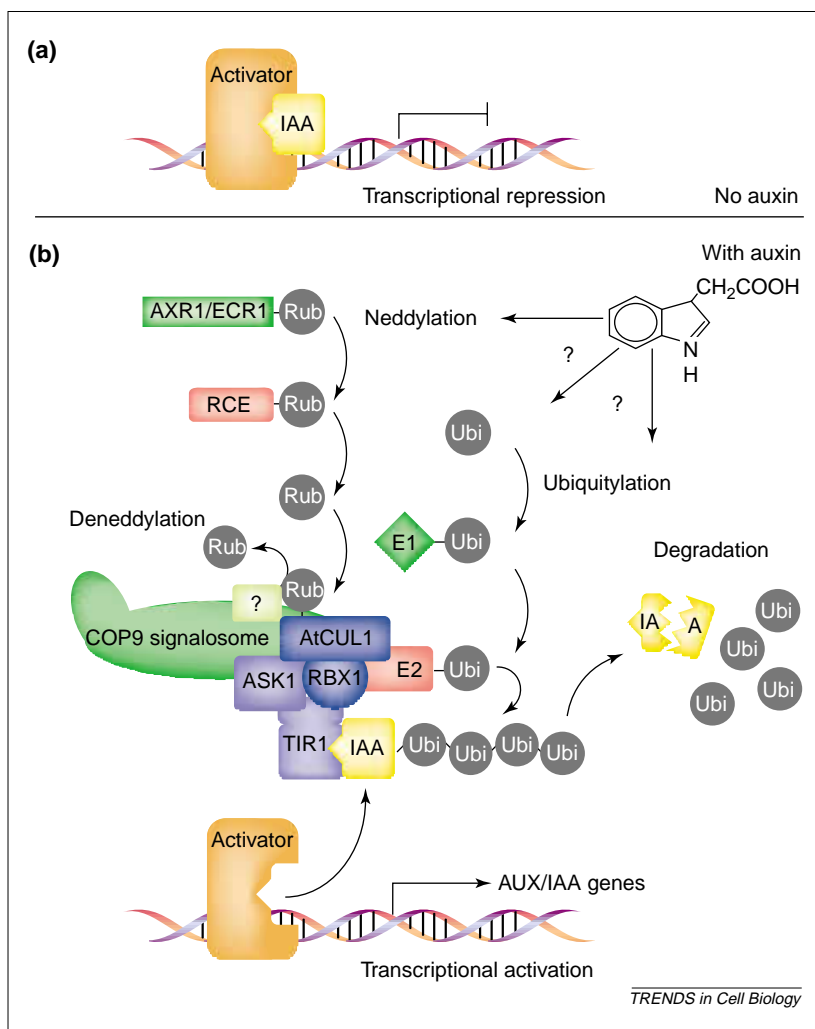


Fig. 3. Model of the molecular mechanisms that mediate the response to the phytohormone auxin in plants. (a) AUX/IAA proteins (IAA) are thought to act as repressors of their own transcription in the absence of auxin. (b) In the presence of auxin, the transcriptional repression is relieved through the degradation of AUX/IAA proteins possibly by the E3 ubiquitin ligase SCF^{TIR1} (blue). The COP9 signalosome interacts physically with SCF^{TIR1} and is also required for AUX/IAA protein degradation. TIR1, the F-box-domain subunit of SCF^{TIR1}, is thought to recognize the AUX/IAA proteins as degradation substrates. The cullin subunit of SCF^{TIR1} (AtCUL1) is modified with the ubiquitin-like protein RUB1, the *Arabidopsis* NEDD8 homolog ('neddylation'). The RUB1 modification can be removed by the COP9 signalosome or by an unidentified associated protein ('deneddylation'). Abbreviations: ASK1, SKP1-like subunit; AXR1/ECR1, E1-like heterodimeric RUB-activating enzyme; E1, E1 ubiquitin-activating enzyme; E2, E2 ubiquitin-conjugating enzyme; RCE, E2-like RUB-conjugating enzyme; Rub, NEDD8/RUB1 modification; Ubi, ubiquitin modification.

Table 3. Identity and function of COP9 signalosome-interacting proteins

Protein	Refs	Function	Interacting COP9 signalosome subunit	Link to protein degradation	Refs
Cullin/RBX1	19,20	E3 ubiquitin ligase	CSN2, CSN6	Neddylated Core subunits of SCF-complexes RBX is required for neddylation	19,20,44 21 65
COP1	34,35	E3 ubiquitin ligase?	Unknown	Genetic link	34
AtS9	54	19S proteasome subunit (lid)	CSN1	26S proteasome subunit	54
Bcl-3	55	Modulator of NF- κ B activity	CSN5		
c-JUN, JUN-D	56	Transcription factor	CSN5	Sumoylated Degraded by 26S proteasome	66 67
ID3	68	Inhibitor of DNA binding	CSN5	Degraded by 26S proteasome	68
Interferon consensus sequence binding protein	57	Transcription factor	CSN2		
p53	38	Transcription factor, cell-cycle regulator	CSN5	Sumoylated Nucleocytoplasmic partitioning Degraded by 26S proteasome	66 52 38
Progesterone receptor	58	Hormone receptor	CSN5	Sumoylated?	69
Retinoic acid receptor	59	Hormone-dependent transcription factor	CSN2	Degraded by 26S proteasome	70
Steroid receptor coactivator	58	Transcriptional coactivator	CSN5		
Thyroid hormone receptor	59	Hormone-dependent transcription factor	CSN2	Degraded by 26S proteasome	71
p27 ^{KIP1}	60	Cell-cycle regulator	CSN5	Nucleocytoplasmic partitioning Degraded by 26S proteasome	60 72
Macrophage migration inhibitory factor	61	Cytokine	CSN5		
Integrin LFA-1	62	Cell adhesion receptor	CSN5	Nucleocytoplasmic partitioning LFA-1 engagement linked to c-JUN activation	62
Lutropin/choriogonadotropin receptor (precursor)	63	Hormone receptor	CSN5	CSN5 promotes degradation of the precursor in the endoplasmic reticulum	63
HIV-1 Vpr	64		CSN6	Nuclear import	64

Because purified COP9 signalosome has the ability to deneddylate neddylated cullins *in vitro*, it is thought that the COP9 signalosome itself (or a tightly associated protein) has deneddylating activity²⁰.

CSN5 is a COP9 signalosome subunit that could be (part of) the deneddylating enzyme. CSN5 contains a Cys-box, a small protein domain that is characteristic for deubiquitinating enzymes^{20,45}. Thus, it is possible that proteins with a Cys-box like CSN5 might have a biochemically related function such as deneddylation. Interestingly, an *S. cerevisiae* strain with a deletion in the gene *YDL216C*, which encodes the yeast homolog of CSN5, is deneddylation deficient²⁰. This is particularly noteworthy because *S. cerevisiae* does not have a recognizable COP9 signalosome but does have the neddylation machinery. This would therefore indicate that *YDL216C*, and possibly also its homologs from other organisms, are essential for cullin deneddylation. Whether *YDL216C* acts alone or in concert with a yet-to-be-identified partner remains to be shown.

Findings from plant studies, however, indicate that things are not quite that simple. Wild-type *Arabidopsis* plants and COP9 signalosome mutants that have lost the COP9 signalosome because of a mutation in a subunit other than CSN5 have been found to lose the COP9 signalosome but still to

contain a monomeric form of CSN5 (Ref. 46). Despite the presence of monomeric CSN5, these plants have lost deneddylation activity and accumulate exclusively neddylated cullins¹⁹. In *Arabidopsis*, the monomeric form of CSN5 is found in both nucleus and cytosol, and so it can be ruled out that different compartmentalization is the cause for monomeric CSN5 failing to deneddylate *Arabidopsis* cullins⁴⁶. Further data on the deneddylation activity of CSN5 from yeast and on the role of the monomeric CSN5 from higher eukaryotes is required before conclusions can be drawn on this issue.

Neddylation and deneddylation: how does it work?

Until recently, neddylation has primarily been thought to regulate the activity or the localization of SCF-type E3 ubiquitin ligases^{40,44,47,48}. This hypothesis would also suggest that neddylation and deneddylation have antagonistic functions. However, the recent physiological data from plants unequivocally demonstrate that both processes act together to mediate protein degradation¹⁹. Therefore, the role of NEDD8 in protein degradation needs to be reconsidered.

One possibility is that neddylation and deneddylation regulate the interaction between

accumulate in the nucleus^{35,53} (Fig. 4). The mechanism that regulates COP1 distribution is not known, but, in COP9 signalosome mutants, COP1 is found in the cytoplasm independent of the presence or absence of light². Therefore, the COP9 signalosome is essential for the correct distribution of COP1, and it is intriguing to speculate that deneddylation of COP1 might be the signal that causes its redistribution from the nucleus to the cytoplasm.

The question of whether neddylation affects the nucleocytoplasmic distribution of SCF-type E3 ubiquitin ligases has been investigated in fission yeast. Loss of deneddylation in COP9 signalosome mutants of *S. pombe* causes a minor shift in the distribution of cullins from the cytoplasm to the nucleus, suggesting that nucleocytoplasmic partitioning is part of, but possibly not a major response to, deneddylation of SCF-type E3 ubiquitin ligases²⁰.

Concluding remarks

It is clear that further studies are required to elucidate the role of the COP9 signalosome in protein degradation. Based on the similarity of the COP9 signalosome to the lid of the 19S regulatory particle of the 26S proteasome, we currently favor the hypothesis that the COP9 signalosome acts as part of a proteasome in which the COP9 signalosome replaces the lid of the bona fide 26S proteasome^{12,15} (Fig. 5). Although biochemical and biological evidence for such an assembly is currently still lacking, this is an intriguing hypothesis because such a structure would allow ubiquitylated proteins to be funneled directly into a proteasome, guaranteeing rapid and efficient protein degradation. Future work has to try to understand the interplay between the COP9 signalosome and the 26S proteasome as well as between the COP9 signalosome, E3 ubiquitin ligases and their substrates.

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References

- Wei, N. *et al.* (1994) *Arabidopsis* COP9 is a component of a novel signaling complex mediating light control of development. *Cell* 78, 117–124
- Chamovitz, D.A. *et al.* (1996) The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* 86, 115–121
- Seeger, M. *et al.* (1998) A novel protein complex involved in signal transduction possessing similarities to 26S proteasome subunits. *FASEB J.* 12, 469–478
- Wei, N. *et al.* (1998) The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. *Curr. Biol.* 8, 919–922
- Freilich, S. *et al.* (1999) The COP9 signalosome is essential for development of *Drosophila melanogaster*. *Curr. Biol.* 9, 1187–1190
- Mundt, K.E. *et al.* (1999) The COP9/signalosome complex is conserved in fission yeast and has a role in S phase. *Curr. Biol.* 9, 1427–1430
- Deng, X-W. *et al.* (2000) Unified nomenclature for the COP9 signalosome and its subunits: an essential regulator of development. *Trends Genet.* 16, 202–203
- Kwok, S.F. *et al.* (1996) A complement of ten essential and pleiotropic *Arabidopsis* COP/DET/FUS genes is necessary for repression of photomorphogenesis in darkness. *Plant Physiol.* 110, 731–742
- Staub, J.M. *et al.* (1996) Evidence for FUS6 as a component of the nuclear-localized COP9 complex in *Arabidopsis*. *Plant Cell* 8, 2047–2056
- Serino, G. *et al.* (1999) *Arabidopsis cop8* and *fus4* mutations define the same gene that encodes subunit 4 of the COP9 signalosome. *Plant Cell* 11, 1967–1979
- Karniol, B. *et al.* (1999) *Arabidopsis FUSCA5* encodes a novel phosphoprotein that is a component of the COP9 complex. *Plant Cell* 11, 839–848
- Schwechheimer, C. and Deng, X.W. (2000) The COP/DET/FUS proteins – regulators of eukaryotic growth and development. *Semin. Cell Dev. Biol.* 11, 495–503
- Glickman, M.H. *et al.* (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. *Cell* 94, 615–623
- Kim, T-H. *et al.* (2001) PCI complexes: pretty complex interactions in diverse signaling pathways. *Trends Plant Sci.* 6, 379–386
- Wei, N. and Deng, X-W. (1999) Making sense of the COP9 signalosome. *Trends Genet.* 15, 98–103
- Baumeister, W. *et al.* (1998) The proteasome: paradigm of a self-compartmentalizing protease. *Cell* 92, 367–380
- Hofmann, K. and Bucher, P. (1998) The PCI domain: a common theme in three multiprotein complexes. *Trends Biochem. Sci.* 23, 204–205
- Aravind, L. and Ponting, C.P. (1998) Homologues of the 26S proteasome are regulators of transcription and translation. *Protein Sci.* 7, 1250–1254
- Schwechheimer, C. *et al.* (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} in mediating auxin response. *Science* 292, 1379–1382
- Lyapina, S. *et al.* (2001) COP9 signalosome promotes cleavage of NEDD8–CUL1 conjugates. *Science* 292, 1382–1385
- Deshaias, R.J. (1999) SCF and Cullin/RING H2-based ubiquitin ligases. *Annu. Rev. Cell Dev. Biol.* 15, 435–467
- Tyers, M. and Willems, A.R. (1999) One Ring to rule a superfamily of E3 ubiquitin ligases. *Science* 284, 601–604
- Estelle, M. and Klee, H.J. (1994) Auxin and cytokinin in *Arabidopsis*. In *Arabidopsis* (Meyerowitz, E.M. and Somerville, C.R., eds), pp. 555–578, Cold Spring Harbor Laboratory Press
- Abel, S. *et al.* (1994) Early auxin-induced genes encode short-lived nuclear proteins. *Proc. Natl. Acad. Sci. U. S. A.* 91, 326–330
- Abel, S. *et al.* (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *J. Mol. Biol.* 251, 533–549
- Ulmasov, T. *et al.* (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9, 1963–1971
- Leyser, H.M.O. *et al.* (1996) Mutations in the *AXR3* gene of *Arabidopsis* result in altered auxin response including ectopic expression from the *SAUR-AC1* promoter. *Plant J.* 10, 403–413
- Rouse, D. *et al.* (1998) Changes in auxin response from mutations in an *AUX/IAA* gene. *Science* 279, 1371–1373
- Worley, C.K. *et al.* (2000) Degradation of Aux/IAA proteins is essential for normal auxin signaling. *Plant J.* 21, 553–562
- Gray, W.M. and Estelle, M. (2000) Function of the ubiquitin–proteasome pathway in auxin response. *Trends Biochem. Sci.* 25, 133–138
- Gray, W.M. *et al.* (1999) Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev.* 13, 1678–1687
- Deng, X-W. *et al.* (1991) *cop1*: a regulatory locus involved in light-controlled development and gene expression. *Genes Dev.* 5, 1172–1182
- Deng, X-W. *et al.* (1992) COP1, an *Arabidopsis* regulatory gene encodes a protein with both a zinc-binding motif and a Gβ homologous domain. *Cell* 71, 791–801
- Ang, L-H. *et al.* (1998) Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol. Cell* 1, 213–222
- Osterlund, M.T. *et al.* (2000) Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 405, 462–465
- Joazeiro, C.A.P. *et al.* (1999) The tyrosine kinase negative regulator c-Cbl as a RING-type E2-dependent ubiquitin–protein ligase. *Science* 286, 309–312
- Naumann, M. *et al.* (1999) COP9 signalosome-directed c-JUN activation/stabilization is independent of JNK. *J. Biol. Chem.* 274, 35297–35300
- Bech-Otschir, D. *et al.* (2001) COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. *EMBO J.* 20, 1630–1639
- Hay, T.R. (2001) Protein modification by SUMO. *Trends Biochem. Sci.* 26, 332–333
- Hochstrasser, M. (1998) There's the Rub: a novel ubiquitin-like modification linked to cell cycle regulation. *Genes Dev.* 12, 901–907
- del Pozo, J.C. *et al.* (1998) The ubiquitin-related protein RUB1 and auxin response in *Arabidopsis*. *Science* 280, 1760–1763
- del Pozo, J.C. and Estelle, M. (1999) The *Arabidopsis* cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15342–15347
- Hochstrasser, M. (2000) All in the ubiquitin family. *Science* 289, 563–564
- Hori, T. *et al.* (1999) Covalent modification of all members of human cullin family proteins by NEDD8. *Oncogene* 18, 6829–6834

- 45 Wilkinson, K.D. and Hochstrasser, M. (1998) The deubiquitinating enzymes. In *Ubiquitin and the Biology of the Cell* (Peters, J.-M. *et al.*, eds), pp. 99–126. Plenum Press
- 46 Kwok, S.F. *et al.* (1998) *Arabidopsis* homologs of a c-Jun coactivator are present both in monomeric form and in the COP9 complex, and their abundance is differentially affected by the pleiotropic *cop/det/fus* mutations. *Plant Cell* 10, 1779–1790
- 47 Wu, K. *et al.* (2000) Conjugation of Nedd8 to CUL1 enhances the ability of the ROC1–CUL1 complex to promote ubiquitin polymerization. *J. Biol. Chem.* 275, 32317–32324
- 48 Read, M. *et al.* (2000) Nedd8 modification of CUL-1 activates SCF^{TrCP1}-dependent ubiquitination of IκBα. *Mol. Cell Biol.* 20, 2326–2333
- 49 Osaka, F. *et al.* (2000) Covalent modifier NEDD8 is essential for SCF ubiquitin-ligase in fission yeast. *EMBO J.* 19, 3475–3484
- 50 Kawakami, T. *et al.* (2001) NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J.* 20, 1–10
- 51 Lee, S. *et al.* (1999) Transcription-dependent nuclear-cytoplasmic trafficking is required for the function of the von Hippel–Lindau tumor suppressor protein. *Mol. Cell Biol.* 19, 1486–1497
- 52 Roth, J. *et al.* (1998) Nucleo-cytoplasmic shuttling of the hdm2 oncoprotein regulates the levels of the p53 protein via a pathway used by the human immunodeficiency virus Rev protein. *EMBO J.* 17, 554–564
- 53 von Arnim, A.G. and Deng, X.-W. (1994) Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* 79, 1035–1045
- 54 Kwok, S.F. *et al.* (1999) Characterization of two subunits of *Arabidopsis* 19S proteasome regulatory complex and its possible interaction with the COP9 complex. *J. Mol. Biol.* 285, 85–95
- 55 Dechend, R. *et al.* (1999) The Bcl-3 oncoprotein acts as a bridging factor between NF-κB/Rel and nuclear co-regulators. *Oncogene* 18, 3316–3323
- 56 Claret, F.-X. *et al.* (1996) A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. *Nature* 383, 453–457
- 57 Cohen, H. *et al.* (2000) Interaction between interferon consensus sequence-binding protein and COP9/signalosome subunit CSN2 (TRIP15). *J. Biol. Chem.* 275, 39081–39089
- 58 Chachereau, A. *et al.* (2000) JAB1 interacts with both the progesterone receptor and SRC-1. *J. Biol. Chem.* 275, 8540–8548
- 59 Chen, J.D. and Evans, R.M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* 377, 454–457
- 60 Tomoda, K. *et al.* (1999) Degradation of the cyclin-dependent-kinase inhibitor p27^{Kip1} is instigated by JAB1. *Nature* 383, 453–457
- 61 Kleemann, R. *et al.* (2000) Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature* 408, 211–216
- 62 Bianchi, E. *et al.* (2000) Integrin LFA-1 interacts with the transcriptional co-activator JAB1 to modulate AP1 activity. *Nature* 404, 617–621
- 63 Li, S. *et al.* (2000) p38^{JAB1} binds to the intracellular precursor of the lutropin/choriogonadotropin receptor and promotes its degradation. *J. Biol. Chem.* 275, 13386–13393
- 64 Mahalingam, S. *et al.* (1998) HIV-1 Vpr interacts with a human 34-kDa mov34 homologue, a cellular factor linked to the G2/M phase transition of the mammalian cell cycle. *Proc. Natl. Acad. Sci. U. S. A.* 95, 3419–3424
- 65 Kamura, T. *et al.* (1999) The Rbx1 subunit of SCF and VHL E3 ubiquitin ligase activates Rub1 modification of cullins Cdc53 and Cul2. *Genes Dev.* 13, 2928–2933
- 66 Müller, S. *et al.* (2000) c-Jun and p53 activity is modulated by SUMO-1 modification. *J. Biol. Chem.* 275, 13321–13329
- 67 Hermida-Matsumoto, M.-L. *et al.* (1996) Ubiquitinylation of transcription factors c-Jun and c-Fos using reconstituted ubiquitinylation enzymes. *J. Biol. Chem.* 271, 4930–4936
- 68 Boumpheng, M.A. *et al.* (2000) Degradation of Id proteins by the ubiquitin–proteasome pathway. *FASEB J.* 13, 2257–2264
- 69 Poulla, H. *et al.* (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). *Proc. Natl. Acad. Sci. U. S. A.* 97, 14145–14150
- 70 Kopf, E. *et al.* (2000) Dimerization with retinoic X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors α and γ through the ubiquitin–proteasome pathway. *J. Biol. Chem.* 275, 33280–33288
- 71 Dace, A. *et al.* (2000) Hormone binding induces rapid proteasome-mediated degradation of thyroid hormone receptor. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8985–8990
- 72 Mamillapalli, R. *et al.* (2001) PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27^{KIP1} through the ubiquitin E3 ligase SCF^{SKP2}. *Curr. Biol.* 11, 263–267

Integrating stress-response and cell-cycle checkpoint pathways

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The DNA integrity checkpoint and stress kinase (SAPK/JNK and p38) pathways function to modulate cell-cycle, apoptotic and transcriptional responses to stress. Although initially considered to function independently, recent advances indicate a number of links between the stress-response and checkpoint pathways. Here, we consider the relationship between the stress-response and checkpoint pathways and how they interact to modulate cell-cycle control.

Considerable advances have been made in recent years in understanding how cells respond to DNA-damaging agents. The two central pathways – the DNA integrity checkpoints and the stress kinase pathways – have been extensively delineated and, owing to their significant medical relevance, their respective roles in the cellular response to stress are now well characterized. It is apparent that the stress kinase pathways can be activated through events at the cell membrane and cytoplasm, whereas, by contrast, the DNA-integrity checkpoints are activated as a result of

DNA damage. These findings support the notion that the stress kinase pathways and the DNA-integrity checkpoint pathways are involved in the cellular responses to cytotoxic and genotoxic stresses, respectively. Despite these apparently distinct mechanisms of activation, it is clear that a number of parallels can be drawn between these pathways. Both pathways can be rapidly activated by a number of common stress agents, including oxidative stress, ultraviolet radiation (UV), X-rays and γ-radiation. Activation of these pathways by genotoxic stress can lead to direct activation of multiple cellular targets through phosphorylation by signal-transduction kinases. Both pathways can additionally regulate the cellular response to stress indirectly by regulating the transcription factors that control stress-response genes. Moreover, both pathways function in a number of common processes, including development, cell

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