Recent advances in our understanding of the structure and function of the signalosome and eukaryotic translation initiation factor 3) contain protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) that regulate protein degradation. Three protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) contain protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) that regulate protein degradation. Three protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) contain protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) that regulate protein degradation. Three protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) contain protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) that regulate protein degradation.

The PCI complexes are a recently discovered family of multisubunit protein complexes that regulate development and signal transduction. There are three known PCI complexes: the regulatory lid of the 26S proteasome ("P"), the COP9 signalosome (CSN) ("C") and the translation initiation factor eIF 3 ("I")

Three protein complexes (the proteasome regulatory lid, the COP9 signalosome and eukaryotic translation initiation factor 3) contain protein subunits with a well defined protein domain, the PCI domain. At least two (the COP9 signalosome and the lid) appear to share a common evolutionary origin. Recent advances in our understanding of the structure and function of the three complexes point to intriguing and unanticipated connections between the cellular functions performed by these three protein assemblies, especially between translation initiation and proteolytic protein degradation.

PCI complexes: pretty complex interactions in diverse signaling pathways

Tae-Houn Kim, Kay Hofmann, Albrecht G. von Amim and Daniel A. Chamovitz
Table 1. MPN and PCI-containing proteins encoded in the Arabidopsis genome

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*The predicted proteins from the Arabidopsis genome (ftp://ftpmpis.gsf.de/cress/arabiprot/) were searched for MPN and PCI-containing motifs. Only obvious matches are listed. Several of the proteins result from duplicated genes.

§AMSH, associates molecule with the SH3 domain of STAM.

The MPN domain is more easily identified and forms an α/β fold. The significance of these domains is not understood, although the PCI domain might stabilize protein–protein contacts within the complex5–7 and the MPN domain might have a more general role in transient protein–protein interactions.

The similarities between the CSN, elf3 and the proteasome regulatory lid suggest that the three complexes, or their subunits, share a common evolutionary ancestor. Although both the proteasome lid and the CSN contain eight subunits, six of which contain the PCI domain, and two MPN proteins2, elf3 is more distantly related (Table 1). Only five of the elf3 subunits have the PCI or MPN signature domains. It is clear that, at least for the MPN-containing subunits, the proteasome and CSN subunits are more closely related to each other than they are to the elf3 proteins (Fig. 1). The phylogenetic relationship among the PCI proteins is less clear. For example, for some PCI proteins, the closest relative appears to be a subunit of another complex (such as CSN4 and Rpn5, which are CSN and lid subunits, respectively), whereas others might be closer to subunits within the same complex.

Proteasome lid complex

The eukaryotic proteasome is a ~2.5 MDa complex that specifically directs ubiquitin-mediated protein degradation8. The proteasome plays a crucial role in diverse processes including cell-cycle regulation, DNA repair, circadian rhythms and responses to extracellular signals. In plants, the proteasome has been implicated in light and hormone signaling6,10. The proteasome holoenzyme (also known as the 26S proteasome) can be divided into two major subcomplexes: the 20S core particle (CP), which contains the protease subunits, and the 19S regulatory particle (RP), which regulates the function of the CP. The RP can be further dissected into two multisubunit structures: a regulatory lid and an ATPase-containing base. In S. cerevisiae, the regulatory lid is composed of eight subunits that can be separated from the base as a distinct 500 kDa complex9. Putative orthologs for each of these proteins are encoded in the Arabidopsis genome (Table 1). To date, no distinct function or enzymatic activity has been shown for any of the lid subunits. Given that attachment of the lid is necessary for proteolysis of a ubiquitinated protein, the lid probably interacts with the polyubiquitin chain12. Alternatively, the lid might affect the cooperativity of the ATPase activity of the base13. The lid is part of the RP that is most exposed to the cytoplasm, and therefore is an ideal candidate to interact with other cellular factors that could fine tune its function or affect its subcellular localization.

S. cerevisiae has been the most widely used system for studying the regulatory lid, but plants are serving as adequate higher eukaryotic systems. In plants, the base, not the lid, of RP has been studied in the
moss Physcomitrella patens\textsuperscript{14}. As previously shown in yeast, the P. patens base subunit MCB1 (Rpn10) has affinity for polyubiquitin chains in vitro, suggesting that it might contribute to the recognition of ubiquitinated substrates. The yeast ortholog of MCB1 also helps to tether the lid to the base of the RP (Ref. 3). Interestingly, in the moss, and unlike S. cerevisiae, disruption of the gene encoding MCB1 by homologous recombination caused developmental arrest, which could be partially rescued by exogenous application of auxin and cytokinin. This is an important result because it confirms for a multicellular plant that an RP subunit has a conditional role within the proteasome.

In Arabidopsis, a 500 kDa complex (PR500) with many attributes expected of the proteasome lid has been purified\textsuperscript{15}. PR500 contains at least three components: Rpn3, Rpn5 and Rpn6, in common with a larger, 800 kDa complex, which might be the RP. The lid-like PR500 complex can be isolated in a free form, independent of the proteasome base, in wild-type plants. However, in Arabidopsis mutants defective for the CSN, PR500 was absent, suggesting that its subunits had become incorporated into the 19S RP or the 26S holoenzyme. These data foreshadow interactions between the two PCI complexes that will be discussed in more detail below.

**CSN**

The CSN is a highly conserved complex that has a major role in regulating the development of eukaryotes. The CSN was first identified in Arabidopsis, and most research on CSN has been in Arabidopsis\textsuperscript{5,16}. Mutations in the Arabidopsis CSN result in dark-grown seedlings that mimic light-grown wild-type seedlings in almost all developmental parameters that have been monitored. Genetic studies indicated that the CSN acts to repress photomorphogenesis in the dark, and that light reverses this repression. Among the CSN subunits, mutations in CSN1, CSN4, CSN7 and CSN8 (originally described as FUS6, COP8, FUS5 and COP9, respectively) all lead to almost identical phenotypes, underscoring the close cooperation of these gene products. CSN5 is encoded by two genes that are presumably functionally overlapping\textsuperscript{17}. A specific direct role for the CSN in light signaling was highlighted by showing that overexpression of CSN1 or CSN8 causes mild gain-of-function phenotypes in the repression of light-regulated development\textsuperscript{18}.

Although it was originally described as a master repressor of photomorphogenic development in plants, the CSN has a more general role. Mutations in the CSN lead to lethality following the transition from embryo to seedling development, even under normal light conditions, indicating that the complex has an essential role in normal light-grown development. Furthermore, several non-light-regulated genes are also misregulated in one of the csn mutants, further indicating that the CSN is a pleiotropic regulator involved in numerous pathways\textsuperscript{19}. This pleiotropic nature implies that the CSN acts at the link between multiple signal inputs and a variety of downstream regulatory cascades controlling specific aspects of cellular differentiation. Thus, the CSN could be a general developmental regulator whose activity is modulated by other signals in addition to light, and whose targets extend beyond light regulation.

The hypothesis that the CSN is a general developmental regulator is further supported by the identification of the CSN in animal systems. Analysis of the CSN from mammals\textsuperscript{20,21} and Drosophila\textsuperscript{22}, as well as from cauliflower (Brassica oleracea)\textsuperscript{23} and Arabidopsis\textsuperscript{24}, indicate that this complex comprises eight core subunits. Although a readily recognizable CSN is apparently absent from S. cerevisiae\textsuperscript{24}, a complex containing several homologs of the CSN was identified in fission yeast Schizosaccharomyces pombe\textsuperscript{25}. Genetic analyses have also begun to elucidate the role of the CSN in animal systems. The CSN is essential for the developmental transition of Drosophila melanogaster from larva to adult\textsuperscript{7}. This phenotype is curiously reminiscent of the Arabidopsis CSN mutants because the lethality is revealed only after successful embryogenesis and several days of development. In fission yeast, although a CSN mutant was viable, it had cell-cycle defects and overexpression of the CSN5 subunit caused drug resistance\textsuperscript{26}, a phenotype also seen upon overexpression of the c-jun-unrelated transcription factor Pap1 (AP-1)\textsuperscript{27}.

The mammalian CSN is thought to associate with a kinase that phosphorylates the transcription factor c-jun, implying a role for the CSN in regulating the activity of c-jun, possibly by stabilizing c-jun against proteasome-mediated degradation\textsuperscript{20}. Moreover, overexpression of the CSN2 subunit increased the level of c-jun-unrelated gene expression\textsuperscript{28}. These results mirror the findings in fission yeast of a genetic interaction between the human CSN5 subunit TAP1 and the c-jun-unrelated Pap1 protein\textsuperscript{29}. Additional evidence supports a role for the CSN in regulating signaling pathways mediated by mitogen-activated-protein kinase (MAP kinase) in mammalian cells, because several of the CSN subunits have been independently identified as putative regulators of MAP kinase signaling, cell-cycle control and hormone responses\textsuperscript{29}. The direct connection between these pathways and the CSN is unknown.

Although some of the CSN subunits are found exclusively in the complex (e.g. CSN1 and CSN8), studies in Drosophila and Arabidopsis have shown that at least three subunits (CSN4, CSN5 and CSN7) are also found in other forms\textsuperscript{7,17,23}. This raises the possibility that some of the subunits might have roles independent of the complex. In particular, in Arabidopsis, the CSN5 subunit exists in an uncomplexed, and possibly cytoplasmic, form\textsuperscript{6,17}, whereas the assembled plant CSN was found in the nucleus\textsuperscript{22}. Mammalian CSN5 interacts with many
cellular proteins in both the cytoplasm and the nucleus (Fig. 2). CSN5 might well function as an adapter or a recruitment device for the CSN. How CSN5 might exert its regulatory role is brought into focus most clearly in connection with p27Kip1, a repressor of the G1–S phase cell-cycle transition. The interaction between CSN5 and p27Kip1 results in the relocalization of nuclear p27Kip1 to the cytoplasm, suggesting a direct or indirect role for CSN5 in regulating protein relocalization.

Taken together, the biochemical and genetic data from diverse eukaryotic model organisms are converging on a consensus for the role of the CSN as a key regulator involved in numerous signaling pathways and developmental transitions. Some of these interactions appear to have been conserved through evolution along with the protein sequences of the CSN subunits.

eIF3
Only one other PCI complex is currently known: eIF3. In eukaryotes, translation initiation is a multistep process. Briefly, when the 40S ribosomal subunit is associated with tRNA–methionine, it is brought into contact with the 5′ end of the mRNA, followed by 5′-to-3′ scanning to select the AUG start codon. The 60S ribosomal subunit then joins it, which permits binding of the second tRNA and progression into the translation elongation phase. Among the numerous eukaryotic translation initiation factors (eIFs) involved, four play key roles: eIF4F, eIF2, eIF5 and eIF3.

Structurally, eIF3 is the most complex of the eIFs because of its three fundamental roles:

- It prevents association of the 40S and 60S ribosomal subunits in the absence of mRNA.
- It facilitates loading of the 40S subunit with the ternary eIF2–tRNA–Met–GTP complex.
- It interacts with eIF4B and the eIF4F mRNA

http://plants.trends.com
cap-binding complex, thus arranging the contact between the mRNA and the 40S subunit. After recognition of the start codon, which might involve the small protein eIF1, GTP is hydrolysed by eIF2 and eIF3 dissociates from the preinitiation complex, which in turn permits the 60S subunit to join the 40S subunit. The three activities of eIF3 are mediated by its interaction with other eIFs. A labile supercomplex has been found recently that consists of eIF1, eIF2, eIF3, eIF5 and tRNA-Met, which is consistent with eIF3 having a central role.

The subunit composition of eIF3 has been a matter of debate, with the composition often depending on the method of purification. Mammalian eIF3 now appears to contain 11 subunits: eIF3a–eIF3k, in descending size order from 170 kDa to 25 kDa. Plant eIF3 closely resembles mammalian eIF3, having 10 of its 11 subunits in common, but it also contains a novel subunit, eIF3l, that is not present in mammals. The S. cerevisiae eIF3 core complex is smaller and consists of only five subunits, which are homologous to human eIF3a, eIF3b, eIF3c, eIF3g and eIF3l (Ref. 40), although other proteins remain associated with the core complex when different purification conditions are used. This has given rise to the concept that eIF3 consists of a core of five subunits, conserved among all eukaryotes, and the other eIFs (Fig. 2). Exacerbated by the lack of mutants in higher eukaryotes, the role of the non-core subunits, eIF3g, which is lethal, and eIF3e of S. pombe, has remained speculative. They might have regulatory functions or provide structural support (e.g. eIF3e)44. In wheat, the expression profiles of the eIF3 subunits are not coordinated stoichiometrically, and this includes both core and non-core subunits, which is suggestive of regulatory or accessory roles for at least some of the subunits45.

Mutational analysis in fission yeast, which contains most of the higher eukaryotic subunits, is beginning to substantiate a regulatory role for at least one of the non-core subunits, eIF3e. A mutation in eIF3e of S. pombe causes a reduced polysome/monosome ratio and slow growth, but is far less detrimental than a mutation of one of the core subunits, eIF3g, which is lethal. Interestingly, the eIF3e mutation results in different accumulation of cellular proteins46. In addition, it has discrete cellular phenotypes, including sporulation and chromosome segregation defects, and sensitivity to osmotic stress and caffeine, suggesting that this subunit makes more than just a quantitative contribution to eIF3 activity. These results support the idea of a regulatory role for the eIF3e subunit. Additional leads are now fueling speculation as to what this regulatory role might be.

Overexpression of eIF3e in fission yeast causes drug resistance. Although the molecular basis of this phenotype is not understood, it is intriguing that overexpression of the proteasome lid subunit Rpn11(Pad1)49, human CSN5 (Ref. 26) and fission yeast pap1/AP-1 transcription factor homologs also cause drug resistance46. Given that overexpression of other eIF3 subunits tested did not cause drug resistance, these results are exciting because they suggest a functional connection between the three PCI complexes, eIF3 via eIF3e, the proteasome via Rpn11 and the CSN via CSN5.

Interactions among the PCI complexes and their subunits

Although the CSN and the lid resemble each other in terms of subunit composition, they each have unique structures. However, there is now substantial yet circumstantial evidence hinting at a functional relationship among the PCI complexes in regulating common pathways.

CSN and the proteasome

Although the CSN and the lid resemble each other in terms of subunit composition, they each have unique structures. However, there is now substantial yet circumstantial evidence hinting at a functional relationship among the PCI complexes in regulating common pathways.

The CSN interacts with the E3 ubiquitin ligase complex SCF in animals and plants, and affects SCF activity by regulating the removal of the ubiquitin-like protein NEDD8 (deneddylation) from the SCF Cul1 subunit53,54. This result is particularly exciting because it suggests a mechanism linking light...
signaling through the CSN and auxin responses through the SCF and proteasome.

**CSN and eIF3**

Even though eIF3 is more distantly related to the PCI complexes, there is also intriguing evidence that eIF3 and the CSN cooperate in some way. The first evidence was biochemical: three eIF3 subunits purified with the CSN from cauliflower – the core subunit eIF3c and the non-core subunits eIF3e and eIF3h (Refs 55,56). This result is probably significant because direct interactions between eIF3 and the CSN were detected in vitro, in yeast and in vivo, whereas no such interaction was detected for another eIF3 core subunit, eIF3b, and the CSN. Arabidopsis eIF3c interacts with CSN1 and CSN8, whereas eIF3e interacts with CSN7. Because eIF3e and eIF3c interact with each other57, it seems likely that eIF3c and eIF3e and presumably eIF3h form a module that can interact with either the rest of eIF3 or the CSN.

The biochemical association between subunits of the CSN and eIF3 seems to be well established, but the question of the biological role of this interaction then arises, especially considering the role of the CSN in repressing light responses in plants. Clearly, additional data are needed to address this point. However, it is clear that the light regulation of development occurs at both the transcriptional and the translational levels. For instance, the pea ferredoxin-1 (Fed-1) mRNA rapidly and specifically dissociates from the polysomal (i.e. translated) ribosome fraction within 20 min of shifting plants from light to darkness, followed by mRNA destabilization. This process is reversible58,59. Other light-regulated RNAs, such as those for tobacco Fed-1 and Cab, are affected similarly, but not all photosynthesis-related messages are. Polysome assembly is probably regulated at the level of translation initiation. From a physiological point of view, translational control of early genes in photomorphogenesis ensures the rapid accumulation of protein soon after illumination. It is tempting to speculate that one of the roles of the CSN in regulating photomorphogenesis is to alter eIF3 activity such that these transcripts remain untranslated in the absence of light signals, perhaps by inhibiting the assembly of non-core subunits into eIF3.

This model then predicts that the subcellular location of these eIF3 regulatory subunits should be dynamic, allowing them to interact with eIF3 in the cytoplasm or, alternatively, with the CSN in the nucleus. At least for eIF3e, this appears to be plausible. In Arabidopsis, immunofluorescence and green-fluorescent-protein fusion experiments indicated that eIF3e might associate with the cytoplasmic eIF3 and the nuclear CSN (Ref. 56) in a cell-type-specific fashion, whereas the eIF3 core subunit eIF3b was, as expected, consistently excluded from the nucleus. Because eIF3e was nuclear in roots, where the CSN is known to repress light-inducible gene expression, these data amount to a positive correlation between the level of activity of the CSN and the nuclear localization of eIF3e (Ref. 56).

Such a model is not too far fetched because, in fission yeast as well as in mammalian cells, evidence is slowly accumulating to support a regulatory, rather than housekeeping, role for the non-core eIF3 subunits in the translation of subsets of transcripts. A loss of polysome-associated mRNAs and their conversion into the monosome fraction is seen in fission yeast mutants defective for non-core eIF3 subunits67. In addition, eIF3e and another non-core subunit, eIF3d, were identified as contributors to a RAS signaling pathway in fission yeast60. These results set one precedent for cross-talk between a specific signaling pathway and eIF3. One caveat remains, in that no direct interaction has been detected between an eIF3 subunit and the CSN in fission yeast. However, although the cytoplasmic location of eIF3 subunits and the nuclear location of the CSN might argue against this, the subcellular localization of certain eIF3 subunits in S. pombe is dynamic. For example, nuclear mislocalization of eIF3d occurs in the absence of eIF3e and vice versa60, suggesting that the two have intrinsic nuclear localization potential yet tether each other in the cytoplasm. A regulatory role for eIF3e is also emerging from two lines of evidence in mammalian cells. Even before its identification as an eIF3 subunit, eIF3e had been identified as a preferred chromosomal integration site of mouse mammary tumor virus61. It was found in mammalian cells that overexpression of the interferon-inducible P56 protein represses translation via eIF3e (Ref. 62). These results suggest a model in which eIF3e, once inserted into the eIF3 holocomplex, functions as a receiver of cellular signals that impede or otherwise modulate eIF3 activity.

Although a regulatory role for eIF3e is now supported by solid data, how can we interpret the interaction between this and other eIF3 subunits, and the CSN? Especially, how could such an interaction occur in light of the partners’ presumably distinct cellular locations (cytoplasmic for eIF3 and nuclear for the CSN)? The subcellular distribution of eIF3e in animal systems has been controversial. Some investigators have only detected cytoplasmic localization46,47,63,64 but others showed nuclear targeting, which might be regulated by cytoplasmic localization64,65. Aside from eIF3a, eIF3e is the only plant eIF3 subunit with a predicted nuclear localization signal59. In addition, mammalian eIF3e has one or more nuclear export signals52 and the nuclear export-signal residues are conserved in the plant orthologs (T-H. Kim et al., unpublished). The presence of eIF3e in the
nucleus appears well established but its role within the nucleus, if any, remains to be confirmed. It remains to be determined whether the interaction allows translational regulation by the CSN or whether the activity of the CSN is modified by the eIF3 subunits.

What emerges from this diverse set of data is a complex and often confusing picture. However, if one considers that homeostasis and development require the coordinated efforts of diverse cellular processes, especially differential transcription and translation, and that regulated protein synthesis now appears to be a central mechanism in regulating these processes, it is logical that the coordination of these processes is dependent on sophisticated cross-talk. Perhaps this cross-talk is mediated through the PCl complexes and their interactions with central kinase pathways. However, this regulation does not end with these three complexes. In Arabidopsis, as in other organisms, there are a few ‘orphan’ PCl- and MPN-containing proteins that have yet to be characterized (Table 1). It will be interesting to determine whether these proteins form a fourth complex, can replace subunits of the known complexes or interact with the known PCl complexes.

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