The Arabidopsis COP9 signalosome is a multisubunit repressor of photomorphogenesis that is conserved among eukaryotes. This complex may have a general role in development. As a step in dissecting the biochemical mode of action of the COP9 signalosome, we determined the sequence of proteins that copurify with this complex. Here we describe the association between components of the COP9 signalosome (CSN1, CSN7, and CSN8) and two subunits of eukaryotic translation initiation factor 3 (eIF3), eIF3e (p48, known also as INT-6) and eIF3c (p105). To obtain a biochemical marker for Arabidopsis eIF3, we cloned the Arabidopsis ortholog of the eIF3 subunit eIF3b (PRT1), eIF3e coimmunoprecipitated with CSN7, and eIF3c coimmunoprecipitated with eIF3e, eIF3b, CSN8, and CSN1. eIF3e directly interacted with CSN7 and eIF3c. However, eIF3e and eIF3b cofractionated by gel filtration chromatography in a complex that was larger than the COP9 signalosome. Whereas eIF3, as detected through eIF3b, localized solely to the cytoplasm, eIF3e, like CSN7, was also found in the nucleus. This suggests that eIF3e and eIF3c are probably components of multiple complexes and that eIF3e and eIF3c associate with subunits of the COP9 signalosome, even though they are not components of the COP9 signalosome core complex. This interaction may allow for translational control by the COP9 signalosome.

Light is the major environmental signal regulating plant development. Its effect on plant development is dramatically seen in seedlings of dicotyledonous plants such as Arabidopsis. Dark-grown seedlings, which undergo skotomorphogenesis, differ vastly from light-grown seedlings, which go through photomorphogenesis, both at the morphological and molecular levels. Illumination of dark-grown seedlings induces photomorphogenesis. The transition from skotomorphogenic growth to photomorphogenic growth involves changes in transcription, translation (1), and protein modifications such as ubiquitination and protein degradation (2).

Multisubunit protein complexes play central roles in the regulation of all three processes and thus in the light-controlled regulation of plant development. The COP9 signalosome (previously referred to as the COP9 complex (30)) is a recently discovered protein complex of at least eight subunits (renamed CSN1–CSN8 (4)) with a key role in regulating development in plants and animals (reviewed in Ref. 5). The COP9 signalosome has been found primarily in the nucleus and has been implicated in regulating protein kinase pathways (6–8).

Four Arabidopsis mutants that lack the COP9 signalosome (cop9, fus6, fus5, and cop8) exhibit a constitutive photomorphogenic (cop) phenotype, both at the morphological and molecular levels, in the absence of light. Therefore, the COP9 signalosome acts as negative regulator of photomorphogenic development in the dark. However, as in other eukaryotes, the biochemical activity of the COP9 signalosome remains to be elucidated. The pleiotropic nature of the mutants implies that the COP9 signalosome acts at the nexus between multiple photoreceptors and a variety of downstream regulatory events controlling specific aspects of cellular differentiation, presumably via transcription, translation, and/or post-translational modification. However, given that all mutations known to disrupt the COP9 signalosome are seedling-lethal and begin to show defects during late embryogenesis, i.e. before the decision between the photomorphogenic and skotomorphogenic pathways is made, it is likely that the COP9 signalosome is involved in more than just the dark repression of photomorphogenesis (5, 9).

The COP9 signalosome is similar to two other multisubunit protein complexes, eukaryotic translation initiation factor 3 (eIF3) and the regulatory lid of the 19 S component of the proteasome (9, 10). Multiple subunits of all three complexes share a common motif, termed the proteasome-COP9 signalosome-initiation factor 3 (11) or proteasome-Int6-Nip1-Trip15 (12) domain. The conservation between the COP9 signalosome and proteasome lid is especially striking, where all eight subunits of each complex have a corresponding similar protein (~20% amino acid identity) in the other complex. Therefore, the three complexes may share an overall architecture and a common evolutionary ancestor.

The role of eIF3 includes stabilizing the ternary complex between eIF2, GTP, and trNA\(^{\text{Met}}\) and promoting mRNA binding to the 40 S ribosomal subunit (13, 14). Various reports have placed the size of eIF3 between 550 and 700 kDa (17–23). Its subunit composition has been a matter of debate, with the exact composition often being dependent on the method of purification. Mammalian eIF3 now appears to contain 11 sub-
units as follows: p170, p116 (PT1), p110, p66, p45 (INT-6), p47 (mov34), p44, p40, p36, p35, and p25, whose SDS-PAGE patterns fit reasonably well with the wheat eIF3 pattern (15–20). Recently, it has been reported that plant eIF3 closely resembles the subunit composition of mammalian eIF3 having 10 out of 11 subunits in common and also contains a novel subunit not present in either mammals or Saccharomyces cerevisiae (21). The S. cerevisiae eIF3 core complex is smaller and consists of only five subunits that are homologous to human, p170, p116, p110, p44, and p36 (22), although other purifications have identified additional proteins, which may bind the core complex with lower affinities (23–25). A unified eIF3 nomenclature has recently been proposed, with the largest subunit as eIF3a and the smallest eIF3k (21). Interestingly, two of the eIF3 subunits have been independently identified. The S. cerevisiae subunit corresponding to human eIF3c (p110) was originally isolated in a screen for mutants with defects in nuclear targeting (26), whereas the mammalian eIF3e (p48) subunit, also known as INT-6, was found in nuclear bodies and has been implicated in carcinogenesis (27).

Recent findings suggested interactions between the COP9 signalosome, the regulatory 19 S proteasome subcomplex, and eIF3. The CSN1 subunit of the Arabidopsis COP9 signalosome interacts with the AtS9 subunit of the proteasome lid in a yeast two-hybrid assay (28). Analysis of proteins that copurify with the COP9 signalosome from cauliflower identified another component of the proteasome, p43/RPN7, and two putative subunits of eIF3, eIF3c (p105) and eIF3e (p48). We have previously shown that Arabidopsis eIF3e is highly conserved with the human eIF3c subunit and that eIF3c interacts in yeast with the COP9 signalosome components CSN1 and CSN8, although probably not as a core component of the COP9 signalosome (29).

To clarify the relationship between the COP9 signalosome and eIF3, we have now analyzed the eIF3e protein that copurifies with the COP9 signalosome. We show that eIF3e is likely the Arabidopsis ortholog of the human eIF3e. Our data indicate that eIF3e and eIF3c are subunits of Arabidopsis eIF3 that also interact with components of the Arabidopsis COP9 signalosome.

**EXPERIMENTAL PROCEDURES**

**Plant Materials and Growth Conditions**—Wild-type plants are in the Arabidopsis thaliana Columbia background. The cop9-1 mutant is in the Wassilewskija background (30). Plant germination and growth conditions in darkness and white light were as described previously (31). Day/night cycle conditions consisted of 16 h of white light at 75 μmol m−2 s−1 and 8 h of darkness. Brassica oleracea (cauliflower) was purchased at the supermarket.

**Isolation and Cloning of Arabidopsis eIF3e and eIF3c**—Internal peptide sequences of the cauliflower COP9 signalosome-associated protein eIF3e were described previously (29). Based on amino acid sequence data from four peptides, a cDNA (EST number 142F10T7) for eIF3e was identified through the Arabidopsis genome sequencing effort, sequenced on both strands, and deposited under GenBank™ accession number AF255679.

A search of the Arabidopsis data base with the human eIF3b identified an expressed sequence tag (EST clone 195A21T7) of 578 base pairs. This EST was used as a probe in a screen of a ZAPIII size-fractionated cDNA library from Arabidopsis (32). The library was screened as described for eIF3c (29). Phages from positive clones were excised in vivo according to standard procedure. The longest cDNA clone found was sequenced, and the sequence was confirmed through comparison with the genomic eIF3b sequence (accession number AC005405) and deposited under GenBank™ accession number AF255680.

**Antibody Production and Affinity Purification**—The complete coding sequence of eIF3e was cloned into the pET29b vector (Novagen, Madison, WI) and a pGEX-3C vector (Amersham Pharmacia Biotech). Because of poor solubility of the His6-tagged eIF3e, the protein was solubilized with SDS sample buffer and run on an SDS-PAGE gel. The fusion protein was cut from the gel and used to immunize rabbits (AniLab, Rehovot, Israel). For affinity purification of the antibodies, the glutathione S-transferase (GST)-eIF3e fusion was immobilized on an N-hydroxysuccinimide Hi-Trap column (Amersham Pharmacia Biotech). Antibodies bound to the GST-eIF3e protein were eluted with low pH buffer (2 μM glycine, pH 2.5). The resulting affinity purified anti-eIF3e antibodies were neutralized by addition of 1 μM Tris-HCL, pH 8.8.

The sequence encoding amino acids 58–538 of eIF3b was cloned into pGEX-4T1 and pET28a. GST-eIF3b fusion protein was over-produced in Escherichia coli strain BL21 and was used to immunize rabbits (AniLab, Rehovot, Israel). The His6-eIF3b was purified on a nickel column, and eIF3b antibodies were affinity-purified over His6-eIF3b coupled to an N-hydroxysuccinimide Hi-Trap column (Amersham Pharmacia Biotech) as described above.

**Protein Extraction and Immunoblot Analysis—Arabidopsis or cauliflower,** as indicated, was grown in liquid nitrogen, and the powder was suspended in a phosphate buffer, pH 7.0, containing 10% glycerol, 10 mM NaCl, 10 mM MgCl2, and 5 mM EDTA with freshly added protease inhibitors including 0.5 μM phenylmethylsulfonyl fluoride and Complete Protease Inhibitor Mixture Tablets (Roche Molecular Biochemicals). Protein concentrations were determined by the BCA protein assay (Pierce). Proteins separated by SDS-PAGE were transferred to Immobilon-P membranes (Millipore, Bedford, MA) and probed with 1:5000 dilution of affinity-purified rabbit polyclonal antibodies. Bound antibody was detected with alkaline phosphatase-coupled secondary antibodies, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate as substrates.

**Yeast Two-hybrid Assay**—The complete coding region of eIF3e was cloned into the EcoRI-Nol site of pG4-5 with partial EcoRI digestion to make an in-frame fusion with the transcription activation domain. The complete coding sequence of eIF3b was cloned into the EcoRI site of pEG202 to make an in-frame fusion with LexA. CSN1, CSN7, CSN8, and eIF3c yeast constructs have been described previously (8, 29). The plasmids were transferred into yeast EGY48. Selection for interaction was as described (33). β-Galactosidase quantitation was done as described (29).

**Gel Filtration Chromatography—**Total homogenates were prepared as described above. The homogenate was spun in a microcentrifuge for 30 min at 22,000 × g at 4 °C, and the supernatant was filtered through a 0.45-μm filter (Sartorius A2, Gottingen, Germany). Approximately 100 μg of total soluble protein was fractionated at 4 °C through a Superose 6 HR column (Amersham Pharmacia Biotech), with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.4 mM KH2PO4) containing 5 mM MgCl2 and 10% glycerol, at a flow rate of 0.3 ml/min. Fractions of 0.5 ml each were collected and concentrated by Strata Clean Resin Beads (Strata, La Jolla, CA). The protein standards for the gel fractionation were as follows: thyroglobulin (669 kDa), apoferritin (443 kDa), catalase (232 kDa), aldolase (158 kDa), and bovine serum albumin (66 kDa).

**Immunoprecipitation and Pull-down Assay—**Total plant extract was cleared of aggregates by centrifugation for 30 min at 4 °C. The supernatant was filtered through a 0.45-μm filter (Sartorius A2, Gottingen, Germany). Following incubation on an Orbitron rotator (Boekel Industries Inc.), the samples were centrifuged for 15 min at 22,000 × g, and the supernatant was transferred to new tubes containing 20 μl of protein A-agarose beads (Sigma). The tubes were incubated for an additional hour at 4 °C. The tubes were spun at 22,000 × g for 1 min, and protein A beads were washed 5 times with 1 ml of RIPA buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS). Proteins were eluted by boiling for 5 min in SDS sample buffer, separated by SDS-PAGE, and blotted for antibody detection.

For the pull-down assay with CSN7, constant amounts (140 μg) of total soluble proteins from E. coli expressing GST-eIF3e were incubated for 1 h at room temperature in PBS buffer containing 1% Triton X-100, with increasing amounts of total soluble proteins from E. coli expressing either His6-CSN7 or control empty pET28a vector (4 μg/ml each). Following incubation on a rotator, the tubes were centrifuged for 15 min at 22,000 × g, and the supernatant was transferred to new tubes containing 20 μl of Ni-NTA-agarose (Qiagen, Germany). The tubes were incubated for an additional hour. The tubes were spun at 22,000 × g for 1 min. As the Ni-NTA agarose absorbed many nonspecific proteins, the NTA beads were washed with PBS containing 1% Triton X-100 and once with buffer containing 8 M urea, 0.1 M sodium phosphate, and 0.01% Triton X-100. For pull-down assay with eIF3c, constant amounts of total soluble protein (16 μg) from E. coli expressing His6-eIF3c were incubated for 1 h at room temperature in PBS buffer containing 1% Triton X-100 and 5 μg of bovine serum albumin, with increasing amounts of total soluble proteins from E. coli expressing (AniLab, Rehovot, Israel).
either GST-eIF3e or control empty pEGX-3C vector (1 μg/μl each). Following incubation and centrifugation as above, the supernatant was transferred to new tubes containing 20 μl of 50% glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) for additional hour. The tubes were spun at 22,000 × g for 1 min and washed 5 times with 1% Triton X-100 in PBS. Proteins were eluted from both Ni-NTA and glutathione beads by boiling for 5 min in SDS sample buffer, run on an SDS-PAGE gel, and blotted for antibody detection.

**Table I**

Comparison of Arabidopsis eIF3e and eIF3c from human, Drosophila, C. elegans, and S. pombe

| % amino acid identity | Amino acid similarity |
|-----------------------|-----------------------|
| Arabidopsis           | Human                 |
| 48                    | 62                    |
| Drosophila            | 41                    |
| C. elegans            | 38                    |
| S. pombe              | 34                    |

**Table II**

Comparison of Arabidopsis eIF3b and eIF3c from human, Drosophila, S. pombe, and S. cerevisiae

| % amino acid identity | Amino acid similarity |
|-----------------------|-----------------------|
| Arabidopsis           | Human                 |
| 39                    | 59                    |
| Drosophila            | 35                    |
| S. pombe              | 35                    |
| S. cerevisiae         | 28                    |

**RESULTS**

**p48 Associates with Both eIF3-p105 and COP9 Signalosome-CSN7**

Alignments and percent identity and similarity were calculated using ClustalX. GenBank™ accession numbers for eIF3–eIF3e are from human, T09582; Drosophila, AAFl9421.1; S. pombe, Q10425; S. cerevisiae, P06103.

**Fig. 1. eIF3e and eIF3c coimmunoprecipitate with each other and with components of the COP9 signalosome**

Total soluble protein extracts from Arabidopsis wild type (wt) and cop9 mutants were immunoprecipitated with the antibodies as indicated above each lane. Following washes with RIPA buffer the proteins bound to the protein A beads were separated on an 8% SDS gel (A) or a 12.5% SDS gel (B), transferred to PVDF membrane, and probed with anti-eIF3c antibodies (A) or anti-CSN7 antibodies (B). The band seen in B around 100 kDa is nonspecific.

**Antibodies were generated against eIF3e and eIF3b, and affinity-purified as described under “Experimental Procedures.”**

**eIF3e and eIF3c Interact with COP9 Signalosome Subunits**

The similarity of Arabidopsis eIF3e, eIF3c, and eIF3b with their animal counterparts, which are components of eIF3, suggests that these three proteins also interact in Arabidopsis in the same complex. If the three proteins are components of the same complex, we reasoned that antibodies against one should immunoprecipitate the others. On the other hand, if one or more of the proteins interact with the COP9 signalosome or its subunits, then we expect that these antibodies will precipitate subunits of the COP9 signalosome. To test this hypothesis, total protein extracts from Arabidopsis were incubated with antibodies against eIF3c, eIF3b, CSN1, or CSN8, and the immunoprecipitate was analyzed by protein blot with antibodies against eIF3c or CSN7. As seen in Fig. 1A, eIF3c coimmunoprecipitated with eIF3e and eIF3b, indicating that these three proteins are components of the same protein complex, presumably eIF3. eIF3c also coimmunoprecipitated with CSN8 and humans (19, 24, 25). eIF3b is found exclusively in a high molecular weight complex (22), and in yeast mutants, eIF3b was unstable outside of the eIF3 complex (38). In addition, eIF3b did not copurify with the COP9 signalosome (29, 36). We identified a cDNA encoding a putative homolog of eIF3b in Arabidopsis. The cDNA encodes a protein with a predicted molecular mass of 82,125 Da that is highly similar over its entire length to the eIF3b subunit of mammalian and yeast eIF3 and its likely homologs (Table II). As for other eIF3b proteins, the Arabidopsis protein contains a domain common to a number of RNA-binding proteins between amino acids 75 and 132.

Antibodies were generated against eIF3e and eIF3b, and affinity-purified as described under “Experimental Procedures.”
CSN1 (Fig. 1A), confirming our earlier yeast two-hybrid analysis. Since CSN8 and CSN1 proteins are found solely as components of the COP9 signalosome and are unstable as monomers (30), these results suggest that the interaction is between eIF3c and these proteins in the COP9 signalosome. eIF3e coimmunoprecipitated a core component of the COP9 signalosome, CSN7, in extracts from both wild type and cop9 mutants, whereas antibodies against eIF3b did not coimmunoprecipitate CSN7 (Fig. 1B). These results suggest that eIF3e and eIF3c interact with both complexes, eIF3 and the COP9 signalosome, whereas eIF3b has no connection to the COP9 signalosome. It must be pointed out that all immunoprecipitates were washed with RIPA buffer, conditions that would not allow identification of weak interactions.

**eIF3e Interacts Directly with CSN7 and eIF3c—CSN7 exists both as a component of the COP9 signalosome and in a COP9 signalosome-independent form (8).** The coimmunoprecipitation assays suggested that CSN7 and eIF3e reside in the same protein complex. Because Arabidopsis cop9 mutants lack the COP9 signalosome, and CSN7 exists as a monomer or lower molecular weight form in this mutant, the interaction of eIF3e with CSN7 in cop9 mutants (Fig. 1B) suggests that eIF3e interacts directly with the CSN7 monomer. To test this hypothesis, we examined the interaction of eIF3e with CSN7 in a heterologous system in the absence of other Arabidopsis proteins or recognizable homologs of the COP9 signalosome. To this end, we have used the yeast two-hybrid assay (39). As shown in Fig. 2A, eIF3e did not interact with the LexA domain by itself. The interaction of eIF3e with CSN7 clearly activated β-galactosidase activity indicating a direct interaction between eIF3e and CSN7 and further supporting the immunoprecipitation data. eIF3e also interacted with eIF3c, suggesting that the immunoprecipitation of eIF3c and eIF3e is a result of direct interaction between these proteins.

As the immunoprecipitation results suggested that eIF3b interacts with eIF3e, but not the COP9 signalosome, we monitored eIF3b further for direct protein-protein interactions in yeast. As seen in Fig. 2B, eIF3b results in variable levels of reporter gene expression by itself. Despite this, the results clearly show a direct interaction between eIF3b and eIF3c. eIF3b did not interact with either CSN1 or CSN8. eIF3b also appears not to interact with eIF3e and CSN7, although the high variability of the eIF3b-LexA-carrying strains is problematic in this analysis. In summary, the yeast two-hybrid assay has substantiated the hypothesis that interaction of the signalosome subunits is specific for the e subunit but not the b subunit of eIF3.

To substantiate further the direct interaction between eIF3e and CSN7 and eIF3c, a *in vitro* pull-down assay was used to determine whether these proteins interact in the absence of other plant or yeast proteins. As shown in Fig. 3A the amount of GST-eIF3e protein that was “pulled down” increased as the amount of His6-CSN7 protein increased, whereas the control failed to pull down GST-eIF3e. Similar results were obtained in a pull-down experiment using equal amounts of His6-eIF3c and increasing amounts of GST-eIF3e on glutathione-Sepharose beads (Fig. 3B).

The two-hybrid results are consistent with the immunoprecipitation analysis. Taken together with the *in vitro* pull-down assay, these results strongly indicate that eIF3e, but not eIF3b, interacts directly with CSN7 and eIF3c.
Subcellular Localization of eIF3e and eIF3b—To address the potential function of the COP9 signalosome interactive protein eIF3e, we examined its subcellular localization in relation to that of eIF3b and CSN7. If eIF3e and eIF3b both functioned solely as subunits of eIF3, then we expect that they will show identical localization patterns, most likely exclusively cytoplasmic localization. As a first test, eIF3e was expressed transiently as a green fluorescent protein (GFP) fusion protein in onion epidermal cells, where it was found in both the cytoplasm and the nucleus (Fig. 4, B and F). The distribution of eIF3e-GFP was similar to that of three core subunits of the COP9-signalosome, namely CSN1, CSN8 (34), and CSN7 (data not shown). GFP-eIF3e had a higher level of nuclear localization than a GFP dimer (Fig. 4, A and E), even though its molecular mass (80 kDa) is larger than that of the GFP dimer (55 kDa). Therefore, even though the nuclear level did not approach that of a nuclear control protein, GFP-Nla (Fig. 4, D and H), eIF3e appears to have an intrinsic propensity for nuclear uptake. In contrast, a GFP-eIF3b fusion showed exclusively cytoplasmic localization (Fig. 4, C and G), as expected for a subunit of eIF3.

Subsequently, an immunocytochemical assay was employed to determine the localization of endogenous eIF3e as well as of eIF3b and CSN7 in Arabidopsis protoplasts that were counterstained with DAPI. Endogenous eIF3e was primarily cytoplasmic in protoplasts derived from seedling leaf tissue (Fig. 5, D and H). However, protoplasts from roots often revealed both cytoplasmic as well as nuclear and/or perinuclear staining of eIF3e (Fig. 5, C and G). By comparison, as expected, β-glucuronidase was always cytoplasmic in protoplasts from roots (Fig. 5, A and E). On the other hand, the majority of Arabidopsis eIF3b was cytoplasmic in protoplasts isolated from seedling roots and leaves (Fig. 5, I and M, and J and N). The localization of eIF3e overlapped that of the CSN7 protein, which was found in both nucleus and cytoplasm of root and leaf protoplasts (Fig. 5, K and O, and L and P).

In summary, the subcellular localization of eIF3e was clearly distinct from that of eIF3b and similar to CSN7. These results are consistent with the interpretation that eIF3b functions only as a subunit of a cytoplasmic complex (eIF3), whereas eIF3e functions in multiple complexes both in the cytoplasm as well as in the nucleus. The subcellular localization data are also consistent with a direct interaction between eIF3e and the CSN7. Moreover, the nuclear localization of eIF3e may be regulated by cell type-specific factors.

The COP9 Signalosome—The high level of amino acid identity between eIF3e and eIF3b and their mammalian orthologs suggested that they are components of the eIF3 complex. On the other hand, the interactions presented above indicate that eIF3e is also part of the COP9 signalosome. To determine if eIF3e and eIF3b are found in a large molecular weight complex, total soluble proteins from cauliflower buds and Arabidopsis roots were separated by gel filtration chromatography, and the fractions were subjected to Western blot analysis with
α-eIF3e and α-eIF3b antibodies. In both tissues, the peak elutions of eIF3e and eIF3b were in a large molecular weight species, which is larger than the COP9 signalosome, as shown by the elution profile of CSN7 (Fig. 6). However, as we have shown for eIF3c, both eIF3e and eIF3b were also present in the 500-kDa fractions. In roots, the elution profile of CSN7 extended into the higher molecular weight fractions, overlapping the elution profile of eIF3e and eIF3b (Fig. 6). This gel filtration shoulder in the high molecular weight fractions is similar to the elution profile of the COP9 signalosome subunit CSN8 from dark-grown Arabidopsis seedlings (30), suggesting a heterogeneous pool of complexes containing the COP9 signalosome in roots. In summary, although eIF3e and eIF3b are primarily found in a complex larger than the COP9 signalosome, a larger COP9 signalosome overlapping eIF3e was detected in roots, the same tissue, where eIF3e colocalizes with the CSN.

**DISCUSSION**

A necessary step in elucidating the role of the COP9 signalosome in the control of plant development is determining its exact subunit composition. Approximately 10 individual proteins were identified in the initial purification of the COP9 signalosome (29, 36). Most of these proteins were subsequently shown to be orthologs of the subunits of the mammalian COP9 signalosome, although the p105- and p48-copurifying proteins showed similarities to components of eIF3, and p43 was similar to a subunit of the regulatory lid of the proteasome (29). Whereas eIF3, the lid of the proteasome, and the COP9 signalosome are similar in size and contain subunits that may be evolutionarily related, each complex has unique biochemical properties and different proposed biological functions. Therefore, the association of eIF3e (p48) and eIF3c (p105) with the COP9 signalosome raises some interesting possibilities, leading us to one of three hypotheses. 1) eIF3c and eIF3e are subunits of the COP9 signalosome. 2) They are subunits of eIF3 that also interact with the COP9 signalosome or its subunits, either through eIF3 or independent of eIF3. 3) Copurification of eIF3c and eIF3e with the COP9 signalosome may be due to similar fractionation properties of eIF3 and the COP9 signalosome.

Several lines of evidence indicate that eIF3e is a subunit of Arabidopsis eIF3. First, eIF3e is highly conserved with Int-6, the mammalian eIF3e (16). Second, like eIF3c, eIF3e is a component of a large complex, in the range of eIF3 in mammals. Third, eIF3e commounoprecipitates with eIF3c and interacts in yeast and in vitro with eIF3c, indicating that they are components of the same protein complex. Furthermore, eIF3e was recently identified in the purified Arabidopsis eIF3 (21). On the other hand, additional evidence suggests that eIF3e as well as eIF3c are associated with the COP9 signalosome. First, eIF3e and eIF3c copurified with the COP9 signalosome. Second, both eIF3e and eIF3c bound specific signalosome subunits by commounoprecipitation, and eIF3e interacted with the CSN7 subunit in vitro. These results are supported by the two-hybrid assay (see Ref. 29 and Fig. 2). Third, if eIF3e and eIF3c were simply components of a copurifying eIF3, one would expect to find most subunits of eIF3 in our COP9 signalosome preparation. However, after sequencing peptides from all copurifying proteins, eIF3e, eIF3c, and possibly an eIF3f ortholog are the only eIF3-related proteins that copurify with the COP9 signalosome (29, 36). Furthermore, eIF3c and eIF3e were present in equimolar amounts with other COP9 subunits (36). Fourth, whereas eIF3b was localized exclusively in the cytoplasm, as expected for an eIF3 subunit, eIF3e was also found in the nucleus in a fraction of cells, further suggesting that it plays a role outside of eIF3. Taken together these results suggest that in Arabidopsis, eIF3e and eIF3c interact with both eIF3 and the COP9 signalosome.

Subcellular localization data indicate that although eIF3b is cytoplasmic, eIF3e and CSN7 are both nuclear and cytoplasmic. When using protoplasts from green leaves, the immunolocalization results were identical to those with roots, with the
following exception; we were unable to detect nuclear-localized eIF3e protein. Therefore, it is possible that eIF3e is subject to a nuclear exclusion mechanism in photosynthetically active leaf cells. Onion cells may not show this, either because they lack chloroplasts, as do protoplasts from roots, or because the necessary partner protein for nuclear exclusion is present in insufficient amounts in the onion cell.

The possible cell type specificity for the subcellular localization of eIF3e is intriguing, although not understood. The nuclear localization of eIF3e was reminiscent of the predominantly nuclear localization of its ortholog Int-6 in certain mammalian cells (27, 40), although we have not observed an association of eIF3e with nuclear bodies. Coexpression of the human T-cell lymphotrophic virus, type I retroviral protein, Tax, with Int-6 causes the redistribution of Int-6 from the nuclear bodies to the cytoplasm (27), suggesting that the nucleocytoplasmic distribution of Int-6 may be regulated. Recent data have confirmed signals for both nuclear import and nuclear exclusion within mammalian Int-6 and have resulted in a reappraisal of the association of Int-6 with nuclear bodies (37). Given that the COP9 signalosome is thought to be nuclear-localized (36), even though a cytoplasmic or perinuclear localization has not been ruled out (7, 41), it seems most likely that eIF3e interacts with the COP9 signalosome in the nucleus rather than in the cytoplasm. However, the signalosome subunit CSN7 was detected in both nucleus and cytoplasm. It is conceivable that CSN7, by virtue of binding to eIF3e, may modulate the subcellular localization of eIF3e.

This hypothesis may be the key to understanding the significance of the CSN-eIF3e interaction. The role of eIF3e is unclear, and indeed eIF3e was absent from several mammalian eIF3 preparations (13, 42, 43), and eIF3e is not a subunit of eIF3 in S. cerevisiae (22). This suggests that eIF3e is not necessary for basic translation initiation but rather controls eIF3 activity, providing an additional translational control mechanism in higher organisms. We propose a model in which the COP9 signalosome, in addition to its role in transcriptional repression, also regulates translation by differentially sequestering eIF3 subunits (Fig. 7). As no COP9 signalosome was found during the isolation of plant eIF3 (21), this suggests that eIF3e and eIF3c interact with COP9 signalosome distinct from eIF3, probably in the nucleus.

The regulation of protein synthesis is an essential means of controlling gene expression, and the translational machinery is the target of global regulatory mechanisms (44). As initiation is the rate-limiting step in mRNA translation, regulatory mechanisms often target this event. In plants, translation initiation factors including eIF3 are differentially regulated during development and following heat shock (45). In wheat, specifically, initiation factors are expressed in a coordinated fashion during seed germination; however, during seed development or after heat shock treatment uncoupling of these coordinated expression patterns is observed (45). The activity of the initiation factors may be modulated by additional interacting proteins. Accordingly, eIF3 may be a protein complex consisting of core subunits and additional regulatory subunits that associate with the core as required during development. The eIF3e protein might be such a regulator of eIF3.