The *Arabidopsis* homologue of an eIF3 complex subunit associates with the COP9 complex

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Abstract The *Arabidopsis* COP9 complex is a multi-subunit repressor of photomorphogenesis which is conserved among multicellular organisms. Approximately 12 proteins copurify with the COP9 complex. Seven of these proteins are orthologues of subunits of the recently published mammalian COP9 complex. Four of the proteins show amino acid similarity to various subunits of the COP9 complex, eIF3 complex and 19S cap of the proteasome. We have studied one of these proteins in order to determine if it is a component of the COP9 complex. *Arabidopsis* p105 is highly similar to the p110 subunit of the human eIF3. The p105 gene is induced during photomorphogenesis, and RNA and protein analysis reveal different tissue accumulation patterns. p105 is found in a large protein complex. p105 interacts in yeast with both COP9 and FUS6, two known components of the COP9 complex. Our results indicate that p105 is not a component of the COP9 core complex, though it may interact with components of the complex.

Key words: Protein complex; Photomorphogenesis; Developmental regulation; Proteasome; Protein-protein interaction

1. Introduction

Signal transduction pathways respond to numerous signals to regulate development through differential transcription, translation and/or post-translational processes. While the signals are diverse, and the responses specific, these pathways contain common components and are integrated through sophisticated, though largely unknown mechanisms. Maintaining the proper equilibrium between transcription, translation and post-translational mechanisms is essential for normal development.

Large, multi-protein complexes have been found to have central roles in the regulation of transcription (e.g. TFIID [1]), translation (e.g. eIF3 [2]) and post-translational processes (e.g. proteasome [3]). An additional multi-subunit regulatory protein complex, the COP9 complex, is an essential nuclear-localized protein complex which has a central role in regulating development in plants, and most likely in animals as well [4]. *Arabidopsis* mutants lacking the COP9 complex have a variety of pleiotropic phenotypes most evident in the loss of light regulation of seedling development. Various genetic studies showed that the complex functions as a downstream repressor of photomorphogenic growth patterns in darkness [5,6]. However, the complex has a larger role beyond mediating the light control of plant development: mutations in the COP9 complex are lethal following the seedling stage [5,7]; transcription of several other groups of genes may be misregulated in these mutants [8]; and the complex is conserved between plant and animal systems [9–11].

In spite of the central role for the COP9 complex in developmental regulation, its biochemical activity remains elusive. A major breakthrough in the study of the COP9 complex has been its purification from cauliflower [12]. Through an empirically derived purification strategy based on an immuno-assay for COP9 protein, we showed that the COP9 complex is negatively charged, binds heparin and has multiple subunits. The purification of the COP9 complex identified FUS6, which is encoded by an additional photomorphogenic locus in *Arabidopsis*, as a component of the complex.

Recent reports have highlighted the similarities between the COP9 complex, eIF3 complex and the 19S regulatory complex of the proteasome. All three complexes are multi-subunit and similar in size and subunits of all three complexes share a similar motif, termed the PCI (Proteasome-COP9 complex-Initiation, [13]) or PINT (Proteasome-Int6-Nip1-Trip15 [14]) domain. A protein family based on one of the subunits of the COP9 complex, the FUS6 family of proteins [9], includes components of all three protein complexes. While the biological significance of the PCI/PINT domain is still unknown, the domain, together with the other similarities, suggests that there may be some form of interaction between these complexes, or shared subunits between the complexes.

We report here the identification, based on peptide sequence data, of the proteins which copurify with the COP9 complex and the cloning of the gene for one of these proteins. Our data suggest that this protein associates with the COP9 complex, though not as a member of the core complex, and that it may have a role as a member of the eIF3 complex.

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2. Materials and methods

2.1. Peptide sequencing

Proteins copurifying with the COP9 in the COP9 complex were isolated and peptide sequenced as described [12].

2.2. Plant materials and growth conditions

The *cop*9-1 and *fus*6 mutants are in Wassilewskija (Ws) background. Plant germination and growth conditions in darkness and white light were as described [5,15]. Light/dark cycle conditions of 16 h of white light at 75 μmol m^{-2} s^{-1} and 8 h of darkness was used for light-grown seedlings.

2.3. Isolation of Arabidopsis p105 cDNA clones

The cauliflower peptide sequence from P71 matches 8 amino acids in the *Arabidopsis* EST clone 210I18T7 (accession number N37787). This partial cDNA clone was used to obtain the full-length cDNA clone from λZAPII *Arabidopsis* size fractionated cDNA library [16]. A DIG labeled (Boehringer Mannheim) DNA probe was generated using the EST as template. For cDNA screening, 5×10^5 phages displayed on duplicate sets of filters (positively charged nylon membranes, Boehringer Mannheim). Prehybridization, hybridization and filter washings were according to standard DIG procedure at 68°C (Boehringer Mannheim). Positive clones were excised in vivo according to standard procedure. PCR analysis was performed on the first round plaque picks in order to select the longest cDNA clone. A specific primer from the EST coding region and the T7 promoter were used. Among the plaques screened, 12 positives were identified. Two clones were chosen for further analysis, pDC121.1, which contains a 1600-bp cDNA, corresponding to the 2/3 carboxyl region of p105, and the longest clone named pDCBK5, which contains the full coding region.

2.4. Antibody production

The insert in pDC121.1 was cloned into pGEX5x-2 (Pharmacia). The *E. coli* overexpressed GST-fusion protein was insoluble. The 2k centrifugation pellet containing the fusion protein in inclusion bodies was separated through an 8% PAGE gel, and the fusion protein excised and used to immunize rabbits (AniLab, Rehovoth, Israel). For affinity purification of the resulting serum, a fragment of p105 was expressed in pET29b (Novagen). The expressed protein was solubilized from inclusion bodies by 1% *N*-lauroylsarcosine and immobilized on an NHS-activated column (Pharmacia). Anti-p105 anitbodies were purified as described [6].

2.5. Protein extraction, gel filtration chromatography, and immunoblot analysis

Plant tissues were homogenized and proteins analyzed as described [12] except that proteins were separated on 8% SDS-PAGE gels. The protein gels were transferred onto polyvinylidene difluoride (PVDF) membranes, and probed with affinity-purified polyclonal antibodies against p105. Protein extraction and conditions for gel filtration chromatography were as described [6,12] with the following modifications: 100 μg of total soluble protein was fractionated through a Superose 6 column (Pharmacia), with extraction buffer at a flow rate of 0.3 ml/min; fractions of 0.5 ml each were collected and concentrated by binding to Strataclean resin (Stratagene). An equal volume of individual fractions was used for immuno-blot analysis.

2.6. RNA analysis

Total RNA was isolated using Trizol (Gibco-BRL). Equal amounts of total RNA (2.5 μg per lane) were electrophoresed on a 1% agarose/formaldehyde gel for 3.5 h at 50 V. RNA was blotted to positively charged nylon membrane (Boehringer Mannheim) and UV cross-linked. Hybridization was conducted using the insert in pDC121.1 as a riboprobe in 50% formamide, 2% blocking reagent (Genius; Boehringer Mannheim), 5× SSC, 0.02% SDS, 0.1% *N*-lauroylsarcosine at 68°C. An antisense riboprobe corresponding to the pDCBK5 clone was generated using SP6 RNA polymerase with non-radioactive digoxigenin dUTP and detected with chemiluminescent substrate (CSPD; Boehringer Mannheim). For size standards, an RNA ladder was used (Boehringer Mannheim). The blot was reprobed with 18S rRNA which served as a control for equal loading.

2.7. Yeast two-hybrid assay

The insert in pDC121.1 was cloned into *Eco*RI site of pEG202 to make in-frame fusion with LexA. The generated plasmid was designated pEG-P105. Yeast strain EGY48-0 [17] was transformed with a

Table 1
Analysis of proteins copurifying with COP9

Molecular weight (kDa)	Peptide sequence	Identity *Arabidopsis*	Similar to	
			Mammalian	Misc.
71	DHVMAATR	This work	eIF3-p110	Yeast NIP1
56	RYGDLFLRQIAK	–	–	–
52	ILYARHADQRNATFQK	FUS6	COPS1, GPS1	–
	ELEALLITDNQIQARIDSHNK	FUS6	COPS1, GPS1	
	SLYHTEDAPQDMVERRAEVVARLK	FUS6	COPS1, GPS1	
50	ASFLVNSSQNEVLNLQYK	EST T45438	COPS4	–
48	IDSESGTVIMEPTQPNVHEQLINHTK	EST T75664	INT6	Similar to COPS2
	VIQQEHYSYK	EST T76471	INT6	
	LVTQLLEHSQGQAAR		INT6	
	SLYHTEDAPQDMVERRAEVVARLK	EST T43721	INT6	
44	DGMVRFLEDPEQYK	–	COPS3	Tomato LC15
	EAEMHVLQMIQDGQIHALINQK	–	COPS3	Tomato LC15
	KNERLWFK	–	COPS3	Tomato LC15
43	LFEEGGDWERK	EST N95941		FUS6-like
	IADAEENLGESEVREAHLK	EST N95941		FUS6-like
42	SSLDSHLLDLLWNK	AJH1	COPS5, JAB1	
	VEQPDSSSSDGIFYYDEASQTK	AJH1	COPS5, JAB1	
41	FQYYYRNLSRQQAQQQAWLQK	EST N96623	–	MOV34-Protein
36	ALL(E/P)QVSVL	Putative protein g2982463	COPS6	
	KLGPLVIVM	g2982463	COPS6	
27	LFAHGTWGDYK	–	COPS7	AcoB
	QAEIIDQLVR	–	COPS7	AcoB
	KCNASRIPQLSPDQILK	–	COPS7	AcoB
	QLTVLTLAESNK	–	COPS7	AcoB
22	LWTRDYAGVYEAIRGFDWSQDAK	COP9	HCOP9	

For each protein, peptide sequences were determined as described [12]. These data are cumulative from three separate COP9 complex preparations. Some peptides were sequenced more than once. If a protein has been reported under more than one name, both are given. –, None detected. eIF3-p110 [22], NIP1 [21], FUS6 [7], COPS1-7 [11], GPS1 [41], INT6 [42], tomato LC15 (U19099), AJH1 [18], JAB1 [39], AcoB [43], COP9 [6], HCOP9 [9].

combination of 3 plasmids: a bait (pEG-P105), a prey (pJG-COP9 or pJG-FUS6, [18]), and a reporter (pSH18-34), according to [19]. Colonies were selected on synthetic complete media without histidine, tryptophan and uracil. A β-galactosidase activity assay of the transformants was performed using *o*-nitrophenyl-β-D-galactoside, according to [20]. Relative activity units were calculated according to [17].

2.8. Multiple sequence alignments

Amino acid sequences were aligned with ClustalX.

3. Results

3.1. Peptide sequencing of proteins copurifying with the COP9 complex

Towards our goal to define the biochemical activity and the subunit composition of the COP9 complex, a breakthrough in the analysis of the complex was its biochemical purification from cauliflower [12]. Putative subunits of the purified complex were separated by SDS-PAGE, and individual protein bands were excised and subjected to amino acid sequencing.

Over 30 peptides corresponding to the 10 proteins copurifying with COP9 and FUS6 in the COP9 complex were sequenced. As shown in Table 1, these peptide data have allowed us to identify the identity of the majority of these proteins. p22 and p52 were previously shown to be COP9 and FUS6, respectively [12]. Five additional proteins, p27, p36, p42, p44, and p50, are highly similar to five other subunits of the recently published mammalian COP9 complex [10,11]. In total we identified in our preparations seven of the eight subunits of the mammalian COP9 complex.

Four other proteins which copurify with the COP9 complex, p41, p43, p48 and p71, appear to be the cauliflower orthologues of four PCI/PINT motif containing proteins. p41 is a member of the MOV34 family, which is also similar to subunits of the COP9 complex, proteasome and eIF3; p43 is a member of the FUS6 family of proteins; p48 is highly conserved with INT6, which is proposed to be a member of the eIF3 complex, but is also very similar to the mammalian COP9 complex subunit 2; p71 is the cauliflower orthologue of the human p110 subunit of eIF3. No proteins were found to be similar to the peptide sequence of p56.

The presence of these four proteins in our COP9 complex preparations may be a result of non-specific copurification. On the other hand, the recent discovery of the PCI/PINT family of proteins suggests that there may also be a functional relationship between subunits of the COP9 complex, eIF3 and the 19S regulatory subunit of the proteasome. In order to clarify this matter, we have concentrated this study on defining the relationship between p71 and the COP9 complex.

3.2. Cloning and characterization of p105 cDNA

The 8 amino acid peptide sequence of p71, DHVMAATR, was used to search the *Arabidopsis* cDNA sequence database. The predicted gene product of one expressed sequence tag (EST) cDNA clone was found to contain the exact same 8 amino acid sequence. A DIG labeled RNA probe derived from the EST clone was generated and used in a Northern analysis on total *Arabidopsis* RNA. A single RNA band migrating at 3.1 kb hybridized with the probe, indicating that the full length cDNA is longer than the EST. To obtain the full length cDNA sequence, an *Arabidopsis* cDNA library was screened with a DNA probe derived from the same EST, and

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1    MTSRFFTQVGSESEDESDYEVEVNEVQNDDVNNRYLQSGSEDDDDTDTKRVVKPAKDKRF
61   EEMTNTVDQMKNAMKINDWVSLQENFDKVNKQLEKVMRITEAVKPPTLYIKTLVMLEDFL
121  NEALANKEAKKKMSTSNSKALNSMKQKLKKNNKLYEDDINKYREAPEVEEEKQPEDDDDD
181  DDDDDEVEDDDDSSIDGPTVDPGSDVDEPTDNLTWEKMLSKKDKLLEKLMNKDPKEITWD
241  WVNKKFKEIVAAREKKGTARFELVDQLTHLTKIANTPAQKLEILFSVISAQFDVNPGLSG
301  HMPINVWKKCVLNMLTILDILVKYSNIVVDDTVEPDENETSKPTDYDGKIRVWGNLVAFL
361  ERVDTEFFKSLQCIDPHTREYVERLRDEPMFLALAQNIQDYFERMGDFKAAAKVALRRVE
421  AIYYKPQEVYDAMRKLAELVEEEEETEEAKEESGPPTSFIVVPEVVPRKPTFPESSRAMM
481  DILVSLIYRNGDERTKARAMLCDINHHALMDNFVTARDLLLMSHLQDNIQHMDISTQILF
541  NRTMAQLGLCAFRAGMITESHSCLSELYFGQRVRELLAQGVSQSRYHEKTPEQERMERRR
601  QMPYHMHLNLELLEAVHLICAMLLEVPNMAANSHDAKRRVISKNFRRLLEISERQAFTAP
661  PENVRDHVMAATRALTKGDFQKAFEVLNSLEVWRLLKNRDSILDMVKDRVQEEAVRTYLF
721  TYSSSYESLSLDQLAKMFDVSEPQVHSIVSKMMINEELHASWDQPTRCIVFHEVQHSRLQ
781  SLAFQLTEKLSILAESNERAMESRTGGGGLDLSSRRRDNNQDYAGAASGGGGYWQDKANY
841  GQGRQGNRSGYGGGRSSGQNGQWSGQNRGGGYAGRVGSGNRGMQMDGSSRMVSLNRGVRT
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B.

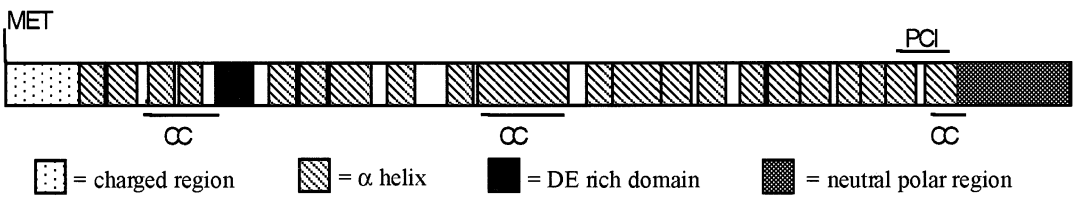

Fig. 1. A: Amino acid sequence of p105. The three predicted coiled-coil regions are underlined. Charged residues in the amino terminus are shown in white on black background. Uncharged, polar amino acid residues in the carboxyl terminus are shown with a light gray background. The domain conserved among a number of RNA binding proteins is boxed. B: Schematic structure of p105. The first methionine is noted (MET) as are the coiled-coil regions (CC) and PCI domain. Secondary structures were predicted using the PHD server [40].

the longest cDNA clone found was sequenced on both strands as described in Section 2.

pDCBK5 contains a 2925-bp insert with an open reading frame that encodes a hydrophilic protein of 900 amino acids with a calculated mass of 102 787 Da and a p*I* of 5.01. The amino acid sequence derived from the open reading frame is shown in Fig. 1. A BLAST search for proteins similar to p105 indicates that it shares a 31% amino acid identity with human p110, which is a subunit of the eIF3 complex, and 28% identity with the yeast NIP1 protein, as well as predicted proteins from *C. elegans* and *S. pombe* (Table 2). Although NIP1 was identified initially as a protein involved in nuclear import [21], more recent evidence suggests that it may have a role in translation, though its connection to eIF3 is controversial [22,23]. The identity of p105 with p110 and NIP1 is highest in the second half of the protein. While the amino half of the protein is more divergent, the hydrophilic nature of the protein is highly conserved.

The primary sequence of p105 indicates that, excluding the amino and carboxyl termini, it is comprised almost entirely of α-helical structures, with three putative coiled-coil domains which may be involved in protein-protein interactions. Twenty-four of the first 60 amino acids are charged, and a search of the available databases with this region identifies a number of transcription factors and nuclear RNA binding proteins. The carboxyl terminal region is comprised of uncharged, polar amino acids. This region identifies in the databases a number of glycine rich RNA binding proteins. p105 also contains a PCI domain and is a member of the FUS6 family of proteins (Fig. 1B).

In order to determine if p105 is a member of the COP9 core complex, we employed several approaches. Antibodies were raised against the COOH-terminal portion of the cDNA carrying about 66% of the coding region, and affinity purified as described in Section 2. The affinity-purified anti-p105 antibodies recognize a protein of 105 kDa in total *Arabidopsis* proteins and proteins of 105 and 70 kDa in samples of the partially purified COP9 complex from cauliflower (not shown). It is not clear if p71 is a specific or non-specific degradation product of P105.

3.3. p105 is present in mutants lacking the COP9 complex

Known subunits of the COP9 complex have different accumulation patterns in mutants lacking the complex. COP9 and FUS6 proteins are absent in mutants lacking the COP9 complex [6,24] while p42 (= AJH proteins) is present in these mutants [18]. As shown in Fig. 2A, p105 is present in *cop9* mutants which lack the COP9 complex. However, the amount of p105 in *cop9* and *fus6* is reduced relative to the wild type. This result suggests that while p105 may be influenced by the COP9 complex, it also has a role beyond the complex.

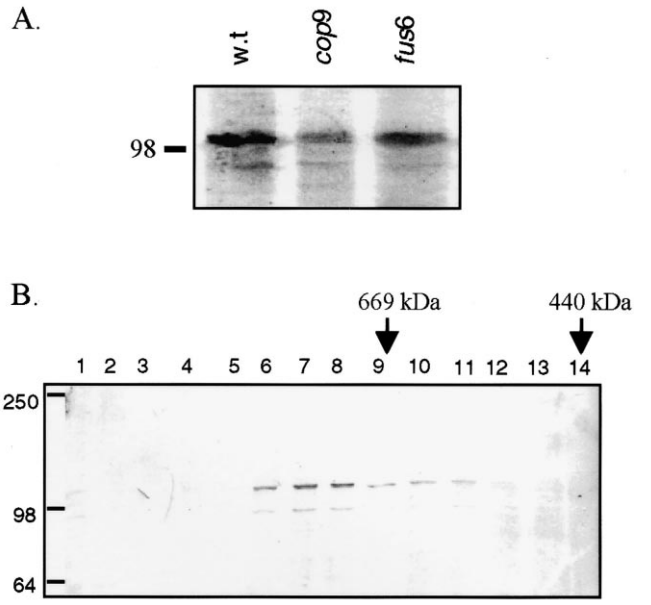

Fig. 2. A: p105 in wild-type and mutant strains. Strataclean beads (Stratagene) were added to 280 μg of total protein, and the pelleted protein/bead mixture was separated on an 8% polyacrylamide gel. B: Gel filtration analysis of p105. Equal volumes of protein for each fraction were separated on an 8% polyacrylamide gel, transferred to a PVDF membrane and reacted with anti p105 antibodies. No p105 monomer was detected (not shown).

3.4. p105 is found in a large protein complex

In order to determine if p105 is found as a large molecular weight species, total soluble proteins from *Arabidopsis* were separated by gel filtration chromatography, and the fractions subjected to Western blot analysis with anti-p105 antibodies. As shown in Fig. 2B, p105 is found in a large molecular weight species of about 750 kDa, but it is also present in the 500-kDa fractions. In partially purified complex fractions from cauliflower, the elution profile of p105 overlaps that of other COP9 complex components (not shown).

3.5. Expression patterns of p105

To examine the possible light regulation of p105 expression, total RNA or protein from 4-day-old dark and light-grown wild-type seedlings was isolated and subjected to gel blot analysis with the p105 riboprobe or p105-specific antibodies. The abundance of p105 transcript and protein, similar to FUS6, is

Table 2
Comparison of *Arabidopsis* p105, human eIF3-p110, yeast NIP1, and putative gene products from *C. elegans* and *S. pombe*

% Identity	% Similarity				
	Arabidopsis	Human	*S. cerevisiae*	*C. elegans*	*S. pombe*
Arabidopsis	–	45.3	30.9	45.1	36.4
Human	32.9	–	32.0	41.9	41.7
S. cerevisiae	28.1	20.2	–	32.0	39.8
C. elegans	32.3	37.0	20.0	–	37.3
S. pombe	25.5	28.6	26.8	23.3	–

Pairwise amino acid identity is shown in the lower left part of the table, while pairwise amino acid similarity is shown in the upper right half of the table. % identity and similarity were determined following multiple sequence alignment with ClustalX.

higher in light-grown than in dark-grown seedlings (Fig. 3A) [7]. p105 protein levels reach maximal levels within one hour of deetiolation, while the transcript levels increase up to two hours of deetiolation.

To determine the tissue expression pattern of p105 in *Arabidopsis*, we hybridized a p105 riboprobe to total RNA from organs from adult plants. The blot shown in Fig. 3B reveals that the p105 transcript is present in all tissues, however, as was previously found for COP9 and FUS6 [24,25], p105 transcript preferentially accumulates in floral and root tissues. The accumulation pattern of p105 protein in different tissues does not mimic that of the p105 transcript, as p105 protein accumulates at higher levels in green vegetative tissues such as leaves and stems.

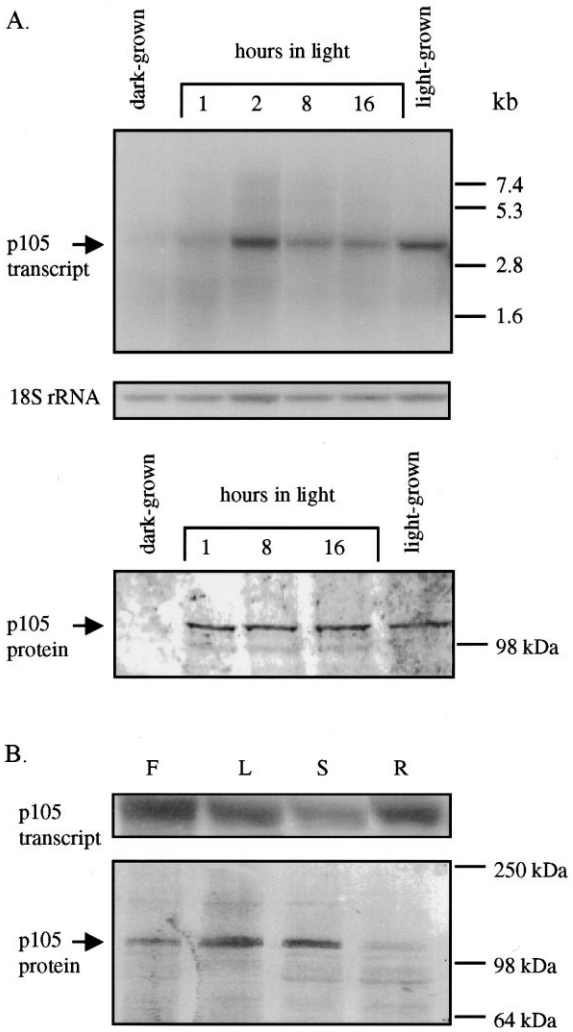

Fig. 3. Expression patterns on p105. A: p105 is induced during deetiolation. Total RNA or proteins were separated by gel electrophoresis and probed with either a p105 riboprobe (for RNA analysis) or anti-p105 antibodies (for protein analysis). The peak seen in p105 RNA after 2 h is due in part to unequal RNA loading as determined by the 18S rRNA amounts. B: Tissue specificity of p105. Equal amounts of RNA were loaded according to 18S rRNA (not shown). For protein analysis, Strataclean beads were added to 100 μg of soluble protein, concentrated and run on an 8% polyacrylamide gel. F = flower, L = leaf, S = stem, R = roots.

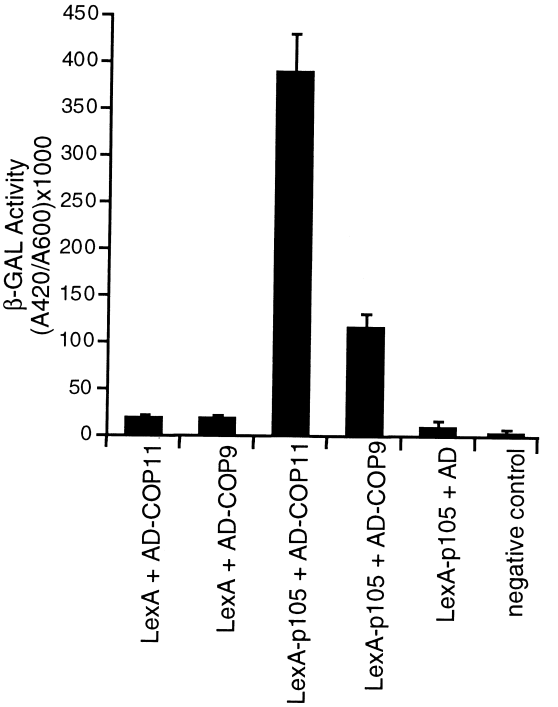

Fig. 4. P105 interacts with both COP9 and FUS6 in the yeast two-hybrid assay. The relative LacZ reporter gene activity in yeast cells for the different combinations of plasmids is shown. LacZ activity in the negative control represents the background levels in yeast cells. Ten individual transformants were used to measure relative LacZ activity for each pairwise combination. Error bars represent standard deviation. AD: activating domain.

3.6. p105 interacts directly with COP9 and FUS6 subunits of the COP9 complex

To substantiate the hypothesis that p105 specifically interacts with the COP9 complex, we examined the possibility that p105 interacts directly with one or more subunits of the complex in a heterologous system. To this end we have used the yeast two-hybrid assay. p105, FUS6 and COP9 were fused to either the LexA DNA binding domain (bait) or the yeast transcription activation domain (prey). If the bait and prey interact, the reporter lacZ gene which contains the LexA binding site in its promoter, will be activated. As shown in Fig. 4, p105 alone does not interact with the activation domain nor do the COP9 and the FUS6-AD fusions interact with LexA domain. However, the LexA-p105 fusion protein clearly interacts with both the COP9 and FUS6 proteins in yeast. The expression of the reporter genes was dependent upon growth on galactose containing medium, indicating that the expression of p105 is required for expression of the reporter genes.

4. Discussion

We describe here the identification of proteins which copurify with the COP9 complex, and the cloning of a new *Arabidopsis* gene which encodes one of these copurifying proteins. While our original goal was to elucidate the subunit structure of the COP9 complex, the results presented here indicate that the determination of this exact subunit composition of the COP9 complex may be more complicated.

We originally detected 10 proteins which copurified in equimolar ratios with COP9 and FUS6 [12]. Based on peptide

sequence data, seven of these proteins are likely orthologues of the subunits of the mouse COP9 complex. The identification of these proteins indicates that our initial preparation was highly enriched with COP9 complex and identifies a high level of conservation, both at the level of amino acid sequence, and at the level of complex composition, between the plant and animal COP9 complexes. While we have not identified a protein highly similar to the S2 subunit of the animal complex, this protein was identified in the affinity purified COP9 complex from *Arabidopsis* [11]. However, we have also identified several proteins which show similarities to components of eIF3 and the 19S cap of the proteasome. While eIF3, the 19S cap of the proteasome and the COP9 complex are similar in size and contain subunits which may be evolutionarily related, each complex has unique biochemical properties and different biological functions.

The p105 protein which copurifies with the COP9 complex is very similar to the p110 subunit of the human eIF3 complex. The proteins are similar in size and share 30% amino acid identity over the entire length of the protein. As the COP9 complex and eIF3 complex may be similar in size and in charge, it is plausible that p105 is found with the COP9 complex as a contaminant of copurifying eIF3. However, several lines of evidence suggest that p105 specifically interacts with the COP9 complex and that its purification is not simply a result of non-specific copurification:

1. p105 copurifies with the COP9 complex in an equimolar ratio with COP9 and FUS6 [12].
2. The expression pattern of p105 is similar to that of other components of the complex.
3. p105 contains a PCI/PINT motif common to COP9 complex components.
4. p105 interacts in yeast with both COP9 and FUS6, two known components of the COP9 complex. This interaction appears specific as (i) p105 by itself does not activate the yeast assay; (ii) while both COP9 and FUS6 are also PCI/PINT containing proteins, they do not interact with each other and share little if any amino acid similarity; (iii) p105 does not interact with additional components of the complex, such as p27 (Karniol and Chamovitz, unpublished). Taken together these results suggest that p105 is not simply a 'sticky' protein and that the interaction of p105 with COP9 and FUS6 in yeast is specific.
5. If p105 is a component of a copurifying eIF3, we would expect that other components of eIF3 would be found in our COP9 complex preparations. However, as shown in Table 1, only one other homologue of eIF3 copurifies with the COP9 complex, p48/INT6. Other known subunits, such as PRT1, clearly do not copurify with the COP9 complex (our own unpublished results). While INT6 is postulated to be a member of eIF3 [26], it also has an additional nuclear function [27], also contains a PCI/PINT domain [14], and is similar to the S2 subunit of the mammalian COP9 complex [11]. Thus it is plausible that p48/INT6 also interacts with the COP9 complex.

While the evidence presented above suggests that p105 specifically associates with components of the COP9 complex, other evidence indicates that p105 itself is not an integral component of the COP9 core complex. The presence of p105 in *cop9* mutants indicates that p105 has a function outside the complex. Furthermore, p105 is not present in the stringent affinity-purified preparation of the COP9 complex from *Arabidopsis* based on binding of the complex to anti-FUS6 [11], nor is its human orthologue, p110, present in the recently reported human COP9 complex [10,11]. However, affinity purification is much more stringent than biochemical purification as it includes multiple detergents in the procedure which may wash off loosely associated proteins. Indeed, in initial purifications of proteasome complexes, what were later identified as components of these complexes were lost due to over-stringent conditions [28]. Furthermore, we do not know the size of the immuno-purified complex, as it is released from the antibodies under denaturing conditions. It is thus possible that p105 is lost at this stage. In addition, the size of the human complex, 450 kDa, is less than that reported for the plant complex, 550 kDa, so the plant complex may contain additional subunits.

How then do we reconcile the presence of p105 in both eIF3 and the COP9 complex? While the p105 human homologue p110 has been shown to copurify with eIF3, the subunit composition of eIF3, and the size of the complex itself, is controversial in higher eukaryotes [22,29–31] and yeast [32–34]. Various reports have placed the size of eIF3 between 550 and 700 kDa [2,35–37]. The *Arabidopsis* p105 elutes from a gel filtration column as a large protein complex of 750 kDa. In yeast, NIP1 is the protein most similar to human eIF3 p110; it is therefore the most likely candidate for a yeast eIF3-p110 orthologue. However, a biochemically active yeast eIF3 preparation appears not to contain NIP1 [32], suggesting that NIP1 plays an alternative role. In lieu of an in vitro reconstitution of eIF3 activity, it is premature to determine the exact relationship of p110/NIP1 to eIF3 [22]. Whether the plant eIF3 contains p105 or another NIP1-like protein is not known.

Alternatively, p105 may participate in multiple protein complexes, such as eIF3 and the COP9 complex. One possibility may be that p105 fulfills a structural role in the complexes. The primary structure of p105 suggests that it may interact with multiple proteins through its different coiled-coil domains.

Several findings indicate that the COP9 complex or COP9 complex components may exist in different forms. First, at least two subunits of the mammalian COP9 complex, and one subunit of the human eIF3 complex are known to have roles outside of their respective complexes. The mammalian COP9 complex subunit S2/SGN2 is also known as TRIP15, a protein associated with the nuclear thyroid hormone receptor [38]. Subunit S5/SGN5 is JAB1, a protein originally isolated through its interaction with JUN [10,39]. The eIF3 subunit p48 is also known as INT-6, a protein associated with the PML protein in nuclear bodies [27]. So while eIF3 has a proposed cytoplasmic role in translational regulation, INT6 also has a nuclear function. Second, the gel filtration properties of the COP9 complex appear to be light dependent with a larger complex appearing in etiolated seedlings [6]. Third, non-denaturing gel electrophoresis indicated that the human COP9 complex is found in multiple forms ranging from 450 to 1000 kDa [10].

Considering these findings, a proposed role for p105 in both the COP9 complex and eIF3 is not too far fetched. An ap-

pealing, though as yet untested, model would have the COP9 complex interacting with both the proteasome and eIF3 to modulate development. p105 may mediate the interaction between eIF3 and the COP9 complex. Future studies should concentrate on underpinning the domains of p105 responsible for its interaction with the COP9 complex, identify components of eIF3 which interact with p105, and determine the subcellular localization of p105.

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