Development 128, 4277-4288 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV0375

A role of *Arabidopsis* COP9 signalosome in multifaceted developmental processes revealed by the characterization of its subunit 3

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Accepted 19 July 2001

SUMMARY

The COP9 signalosome is a highly conserved eight-subunit protein complex initially defined as a repressor of photomorphogenic development in *Arabidopsis*. It has recently been suggested that the COP9 signalosome directly interacts and regulates SCF type E3 ligases, implying a key role in ubiquitin-proteasome mediated protein degradation. We report that *Arabidopsis FUS11* gene encodes the subunit 3 of the COP9 signalosome (CSN3). The *fus11* mutant is defective in the COP9 signalosome and accumulates significant amount of multi-ubiquitinated proteins. The same mutant is specifically impaired in the 26S proteasome-mediated degradation of HY5 but not PHYA, indicating a selective involvement in protein degradation. Reduction-of-function transgenic lines of CSN3 produced through gene co-suppression also accumulate multi-ubiquitinated proteins and exhibit diverse developmental defects. This result substantiates a hypothesis that the COP9 signalosome is involved in multifaceted developmental processes through regulating proteasome-mediated protein degradation.

Key words: COP9 signalosome, Ubiquitin, Proteasome, Protein degradation, *Arabidopsis*, Development regulator

INTRODUCTION

The COP9 signalosome was originally identified as a repressor of light mediated development in *Arabidopsis* (Wei and Deng, 1992; Wei et al., 1994a; Wei et al., 1994b; Chamovitz et al., 1996). Plant development is highly plastic and optimized according to environmental cues, of which light plays a dominant role (Kendrick and Kronnberg, 1994). In response to light, *Arabidopsis* seedlings undergo photomorphogenic development characterized by a short hypocotyl, expanded cotyledons and straightened apices. By contrast, dark-grown seedlings follow a skotomorphogenic developmental program and display an elongated hypocotyl, closed cotyledons and apical hooks. Genetic screens for constitutive photomorphogenic seedling development in darkness identified 11 loci in *Arabidopsis* collectively known as the *COP/DET/FUS* genes (Chory et al., 1989; Deng et al., 1991; Miséra et al., 1994; Kwok et al., 1996). Dark-grown mutant seedlings of this group display a pleiotropic phenotype including short hypocotyl, open and expanded cotyledons, accumulation of anthocyanin, and constitutive expression of light regulated genes. The recessive nature of these mutations suggests that their encoded proteins are negative regulators of photomorphogenic development.

Of the 11 *cop/det/fus* loci, eight are required for the biogenesis of the COP9 signalosome. Mutations in any of these eight loci result in severely retarded seedling development and lethality after the seedling stage (Kwok et al., 1998). So far, at least four genes from this group, *COP9*, *FUS6/COP11*, *FUS5* and *COP8/FUS4*, have been found to encode distinct subunits of the COP9 signalosome respectively (Chamovitz et al., 1996; Staub et al., 1996; Karniol et al., 1999; Serino et al., 1999). CSN5, in contrast, is encoded by two redundant genes in the genome (Kwok et al., 1998). Thus no mutation has been identified for CSN5.

Biochemical studies in both plant and animal systems have demonstrated that the COP9 signalosome is a highly conserved, nuclear enriched protein complex with 8 distinct subunits, designated CSN1 to CSN8 respectively (Deng et al., 2000). Both subunit composition and subunit amino acid sequences are conserved among diverged organisms implying a conserved cellular function of the COP9 signalosome. Interestingly, a remarkable subunit-to-subunit similarity between the COP9 signalosome and the lid subcomplex of the 26S proteasome has been observed (Glickman et al., 1998; Seeger et al., 1998; Wei et al., 1998). In mammals, the COP9 signalosome and/or its subunits have been implied in a variety of cellular pathways (Wei and Deng, 1999; Tsuge et al., 2000; Tomoda et al., 1999; Li et al., 2000; Bech-Otschir et al., 2001). In *Arabidopsis*, most of our understanding of its role in plant development is limited to the seedling stage because all mutants are lethal after the seedling stage (Castle and Meinke, 1994; Miséra et al., 1994; Kwok et al., 1996). Similarly, *Drosophila* mutations in the COP9 signalosome subunits result in lethality at the late larval or pupal stage (Freilich et al., 1999). The lethality is probably a result of the essential role of

the COP9 signalosome in late developmental processes (Wei and Deng, 1999).

Recently, the viable transgenic lines exhibiting a reduced level of the COP9 signalosome using subunit 5 gene co-suppression and antisense approaches, provided an opportunity to examine the role of COP9 signalosome in other developmental processes (Schwechheimer et al., 2001). These transgenic lines had auxin-response defects similar to the loss-of-function mutants of the E3 ubiquitin ligase SCF^{TIR1}. Furthermore, the COP9 signalosome directly interacts with the SCF^{TIR1} E3 ligase complex in vivo and was required for efficient degradation of PSIAA6, a candidate substrate of SCF^{TIR1}. A possible mechanism for the COP9 signalosome-mediated regulation of the SCF E3 ligases might be through the promotion of the RUB1 de-conjugation from AtCUL1 subunit of SCF complex (Lyapina et al., 2001). Indeed, the COP9 signalosome mutants over accumulate RUB1, a conjugated form of AtCUL1 in vivo (Schwechheimer et al., 2001). It is interesting to note that reduction of the COP9 signalosome resulted in phenotypes similar to those of mutants in AXR1, a component of an enzyme cascade that conjugates the ubiquitin-related protein RUB1 to the AtCUL1 subunit of SCF^{TIR1} (del Pozo et al., 1999). Thus, RUB1 conjugation and de-conjugation cycles must work together to promote this SCF^{TIR1} E3 ligase-mediated auxin response and degradation of SCF^{TIR1} substrates.

In both mammals and plants, the interaction between the COP9 signalosome and SCF complexes is mediated through CUL1 and RBX1, the SCF core subunits (Lyapina et al., 2001; Schwechheimer et al., 2001). It is well accepted that distinct SCF-type E3 ubiquitin ligases share common core subunits such as CUL1 and RBX1 but differ in their F-box domain subunit, which is responsible for their substrate specificity (Deshaies, 1999). In *Arabidopsis*, there are over 300 F-box proteins in the sequenced genome. Several characterized F-box proteins, including UFO (Samach et al., 1999), COI1 (Xie, et al., 1998), EID1 (Dieterle et al., 2001), ZTL (Somers et al., 2000), and FKF1 (Nelson et al., 2000) have been shown to play important roles in distinct developmental pathways. By interacting with the core subunits, it is therefore possible that the COP9 signalosome has the capacity to interact with many different SCF-type E3 ubiquitin ligases and therefore be involved in the respective developmental processes that these SCF E3 ligases regulate.

We report the molecular characterization of the COP9 signalosome subunit 3 (CSN3), which is encoded by the *Arabidopsis FUS 11* locus. The *fus11* mutant is defective in the COP9 signalosome and accumulates significant amount of multi-ubiquitinated proteins. Partial loss-of-function plants of the COP9 signalosome, generated using co-suppression approach, exhibited defects in multiple aspects of plant development, including pattern formation, phyllotaxy and organ identity. These results support the notion that the COP9 signalosome interacts with multiple SCF E3 ligases and is involved in multifaceted developmental processes.

MATERIALS AND METHODS

Plant materials and *Arabidopsis* strains

The *fus11-U203, fus6-T236,* and *cop10-1* mutants have been described previously (Miséra et al., 1994; Wei et al., 1994a; Wei et al., 1994b). The *cop1-6* mutant was described by McNellis et al. (McNellis et al., 1994). The seeds were planted on agar plates containing GM medium plus 1% sucrose (Wei et al., 1994), and were cold treated at 4°C for 5-12 days before being transferred to the indicated growth chambers. The light intensity used was 156 and 111 μmole/m^2/second for white and red light respectively.

Isolation and analysis of *CSN3* cDNAs from *Arabidopsis*

The peptide sequences of cauliflower CSN3 (Serino et al., 1999) were used to search the NCBI gene bank and the *Arabidopsis* EST database. A partial cDNA clone of tomato (*Lycopersicon chilense*, U19099) was found to share high homology with two peptide sequences from cauliflower. This cDNA clone was obtained by RT-PCR from tomato. The tomato cDNA obtained was then used as a probe to screen a λ ZAP II cDNA library obtained from the *Arabidopsis* Biological Resource Center (Columbus, Ohio). Among the 60 positive plaques, 10 were chosen for further screening and *in vivo* excision. An in frame stop codon at the 5′ untranslated region indicated that two clones obtained are full-length cDNA clones.

Mapping the *CSN3* gene

To map the *CSN3* gene, the full-length *CSN3* cDNA was used as a probe to hybridize the CIC YAC filter obtained from the *Arabidopsis* Biological Resource Center (Columbus, Ohio) according to the instructions provided. Among the 5 positive YACs, two of them (CIC9B8 and CIC5H3) have been physically mapped and were found to be close to the *FUS11* locus on the chromosome V.

Plant transformation and analyses of the transgenic plants

The full-length cDNA of *Arabidopsis CSN3* gene (ecotype Columbia) was cloned into the binary vector pPZPY122 at the *Bam*HI and *Kpn*I sites in sense orientation under the control of the 35S promoter (Yamamoto et al., 1998). The constructs were transformed into wild-type *Arabidopsis* in both Columbia and Landsberg ecotypes, and the *fus11-U203* heterozygous plants via vacuum infiltration. 26 independent transgenic lines in the heterozygous *fus11-U203* background were examined for possible complementation. For the transgenic plants in wild-type background, 30 independent lines were used for phenotype observation in both the Columbia and Landsberg background respectively. To facilitate gentamycin selection, the seeds were germinated on gentamycin plates (100 mg/l) for 3 days in darkness and then transferred to continuous white light. The greening process of the sensitive plants was completely blocked while the transgenic plants turned green normally.

Protein extraction and gel filtration chromatography

Arabidopsis seedlings were homogenized in a Tris buffer (TB) containing 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 5 mM EDTA, 5 mM DTT, 10% glycerol, with freshly added protease inhibitor, phenylmethylsulfonylfluoride (PMSF; 2 mM) and protease inhibitor-cocktail™ (Boehringer Mannheim) as instructed by the manufacturer. The homogenate was microcentrifuged for 15 minutes and the supernatant was filtered through a 0.2 μm filter (Gelman Sciences, Ann Arbor, MI) before loading onto a Superose 6 (HR 10/30) gel filtration column (Pharmacia). The column was equilibrated with a buffer containing 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 5 mM EDTA, 2 mM DTT, and 10% of glycerol. After loading the sample, proteins were eluted in the same buffer at a flow rate of 0.2 ml/minute. Fractions of 0.5 ml each were collected, starting from the onset of the column void volume (7.5 ml) and were concentrated using StrataClean Resin (Stratagene) as described by Kwok et al. (Kwok et al., 1998). All manipulations were carried out at 4°C. Equal volumes of each fraction were used for SDS-PAGE and then subjected to immunoblot analysis.

Antibody production

A CSN3 recombinant protein was generated by fusing the full-length CSN3 protein to the His tag at the *Bam*HI and *Eco*RI sites in the vector pRSET C (Invitrogen). The overexpressed proteins were purified by Ni-NTA superflow resin (QIAGEN) and used to immunize rabbits. Rabbit polyclonal antibodies to CSN1, CSN5, CSN7 and CSN8 were described previously (Kwok et al., 1999: Karniol et al., 1999; Staub et al., 1996; Chamovitze et al., 1996). The polyclonal antibodies against ubiquitin were purchased from Sigma.

Protein blot analysis and immunoprecipitation

All western blot and immunoprecipitation analyses were carried out as previous described (Staub et al., 1996) with minor modifications. The antibodies coupled with protein A beads were mixed with the protein extracts for 3 hours instead of 6 hours and the beads were washed with PBSM buffer supplemented with 0.4 M NaCl and 0.2% SDS. The immunoprecipitated proteins were released from the protein A beads and analyzed with SDS-PAGE. To detect the ubiquitinated proteins, the filter was autoclaved for 10 minutes to completely denature ubiquitin after the proteins blotted to the filter.

Northern blot analysis

12-day-old transgenic seedlings without true leaves or leaf buds were collected. The roots and hypercotyls were removed. Total RNAs were extracted with Qiagen RNAeasy plant kit. Wild-type plants treated in the same way were used as control. Northern blots were carried out as previously described (Peng et al., 1996) and detected with a phosphoimage system.

RESULTS

Molecular cloning and characterization of the *Arabidopsis CSN3* gene

To clone the gene encoding CSN3 of the *Arabidopsis* COP9 signalosome, we used the CSN3 peptide sequences obtained from the purified cauliflower COP9 signalosome (Serino et al., 1999) to search the NCBI and the *Arabidopsis* databases. At the time, no *Arabidopsis* EST clone or genomic clones corresponding to the peptide sequences were found; however, a highly homologous partial cDNA clone of tomato (U19099) was identified and used to screen an *Arabidopsis* cDNA library (Kwok et al., 1998). Ten *Arabidopsis* cDNA clones were recovered and further analyzed. A near full-length cDNA sequence was deduced that encoded for a 429 amino acid protein (Fig. 1A). The predicted protein sequence contains all three regions corresponding to the peptide sequences from cauliflower (Fig. 1B). The CSN3 protein contains an imperfect leucine zipper domain in the N-terminal region (amino acid 18 to 46) and a PCI domain in the C-terminal region (amino acids 194 to 362) (Fig. 1A). The *Arabidopsis* CSN3 shares 42% and 31% identities with its human and *Drosophila* counterparts respectively (Fig. 1A). Genomic Southern blot analysis and database searches of the completed *Arabidopsis* genome sequence revealed that the CSN3 is encoded by a single copy gene in *Arabidopsis* (data not shown).

CSN3 is only present in the COP9 signalosome

While *Arabidopsis* CSN1 and CSN8 are exclusively present in the COP9 signalosome (Wei et al., 1994; Staub et al., 1996), CSN5 and CSN7 exists both in complex and monomeric forms (Kwok et al., 1998; Karniol et al., 1999). To examine whether CSN3 cofractionates with the COP9 signalosome and whether it also exists in any other form, we raised polyclonal antibodies against CSN3 and carried out gel filtration fractionation analysis of the total protein extracts of light-grown seedlings. The elution profiles of CSN3 and other representative subunits of the COP9 signalosome are shown in Fig. 2A. Consistent with previous observations, CSN1 is only present in the complex form and CSN5 existed in both monomeric and complex forms. The CSN3 protein had exactly the same elution profile as CSN1, indicating that CSN3 is only present in the COP9 signalosome.

To further substantiate that CSN3 is part of the COP9 signalosome, we carried out co-immunoprecipitation experiments using antibodies against CSN3 and CSN5 respectively. The immunoprecipitated proteins from crude cell extracts were then examined by protein blot analyses for the presence of CSN1, CSN3 and CSN5. As shown in Fig. 2B, CSN1, CSN5 and CSN3 were clearly precipitated by antibodies against either CSN5 or CSN3, but not by the corresponding pre-immunoserum. This result indicates that CSN3 is physically associated with the COP9 signalosome.

The *CSN3* gene corresponds to *FUS11* locus

Among the 11 pleiotropic *COP/DET/FUS* loci, mutations in eight of them result in apparent absence of the COP9 signalosome (Kwok et al., 1998; Wei and Deng, 1999). Molecular characterization of the COP9 signalosome has demonstrated that *COP9, FUS6/COP11, FUS5* and *COP8/FUS4* encode subunits of the COP9 signalosome (Wei et al., 1994; Kaniol et al., 1999; Staub et al., 1996; Serino et al., 1999). To investigate whether CSN3 is also encoded by one of the eight loci, the full-length *CSN3* cDNA was used as a probe to hybridize the CIC YAC filter, set at high stringency conditions. Two over-lapping YAC clones, CIC9B8 and CIC5H3, were found to contain the CNS3 gene. These two clones have been mapped to a region close to the *FUS11* locus between markers nga 151 and nga 106, indicating that the *FUS11* locus is a good candidate for the *CSN3* gene.

To verify this prediction, the genomic *CSN3* gene was obtained by PCR from both wild-type and *fus11-U203* mutant seedlings. Sequence analysis revealed that the 2.9 kb transcribed genomic region of the wild-type *CSN3* gene contains 11 exons and 10 introns (Fig. 3A). In the *fus11-U203* mutant, there is a G to A mutation at the 3′ splicing junction site of the ninth intron (Fig. 3B). RT-PCR analysis of cDNAs from the *fus11-U203* mutant indicated that this mutation resulted in an aberrant splicing to a cryptic receptor site within the ninth intron, introducing two novel amino acids followed by a stop codon. This stop codon results in early termination of the reading frame and a possible production of a mutant protein missing the C-terminal 44 amino acids (Fig. 3C). Protein gel filtration analyses revealed that CSN3, CSN1 and CSN5 were all absent in the *fus11-U203* mutant, while CSN5 could be found exclusively in the monomeric form (Fig. 2A). This indicates that the last 44 amino acids of CSN3 play an essential role in either the assembly or stability of the COP9 signalosome.

Overexpressed CSN3 rescues the phenotype of the *fus11* mutant

To confirm that CSN3 is encoded by the *FUS11* gene, the *CSN3* cDNA under the control of the cauliflower mosaic virus 35S

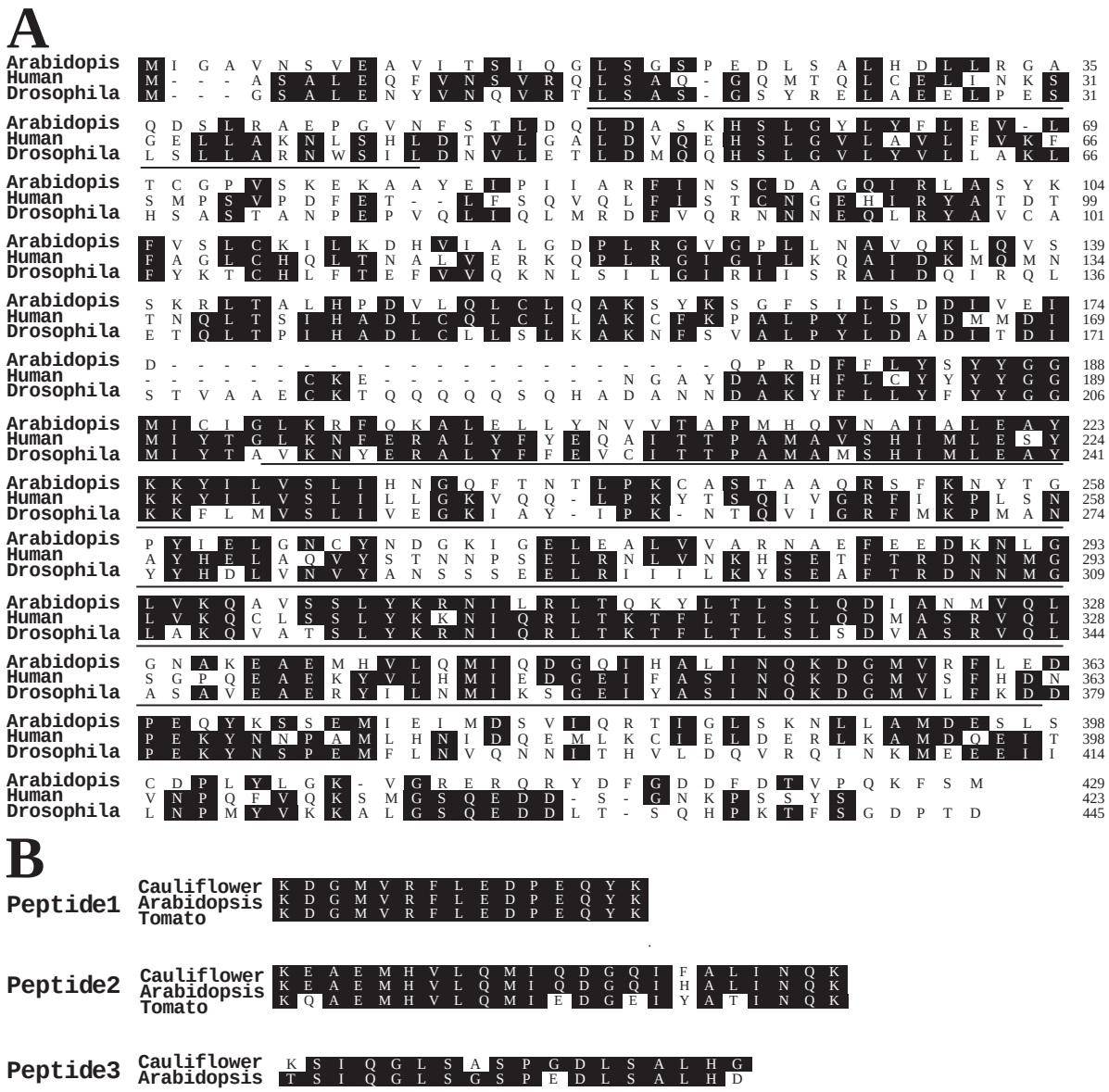

Fig. 1. Sequence comparison of *Arabidopsis* CSN3 with its homologs from other organisms. (A) Protein sequence comparison of *Arabidopsis* CSN3 and its homologs from human and *Drosophila*. The underlined sequences are the N-terminal leucine zipper domain and (second) the C-terminal PCI domain. Numbers on the right indicate the positions of the amino acid residues. The GenBank Accession Numbers are AF361759 for *Arabidopsis*, AF098109 for human (*Homo sapiens*) and AF071313 for *Drosophila*. (B) Comparison of the cauliflower (*Brassica oleracea* var. Botrytis) peptide sequences with the deduced CSN3 protein sequences from *Arabidopsis* and tomato (*Lycopersicon chilense*).

promoter was introduced into the *fus11* mutant background to test functional complementation. Since the homozygous *fus11* mutant is lethal, the transgene was stably transformed into heterozygous plants. Both the phenotype and the transgene segregation were analyzed in the T_2 generation to reveal the number of the T-DNA insertion loci and possible functional complementation. In the absence of phenotype rescue, the wild type to mutant ratio in the T_2 generation should be 3:1. With functional complementation by a single locus of the transgene unlinked to the *FUS11* locus, the wild type to mutant ratio should be 15:1. If multiple transgene loci are involved, a higher wild type to mutant ratio is expected. The segregation ratios of five representative transgenic lines are summarized in Table 1. For the 4 lines with single insertion, the ratios of wild type to mutant were 12.3:1, 13.6:1, 14.2:1 and 17.0:1 respectively. For the line with two possible transgene loci, a ratio of 53 to 1 was observed. These results clearly indicated that the overexpressed *CSN3* cDNA functionally complements the *fus11* mutation.

The *fus11* and other COP9 signalosome mutants exhibit altered patterns of cellular ubiquitinated proteins

As an initial step to extend our hypothesis that the COP9 signalosome may regulate multiple E3 ligase-mediated protein degradation, we examined the effect of the COP9 signalosome defect on the overall profile of cellular ubiquitinated proteins. To this end, total soluble proteins from wild-type and COP9 signalosome mutants were separated by gel filtration according

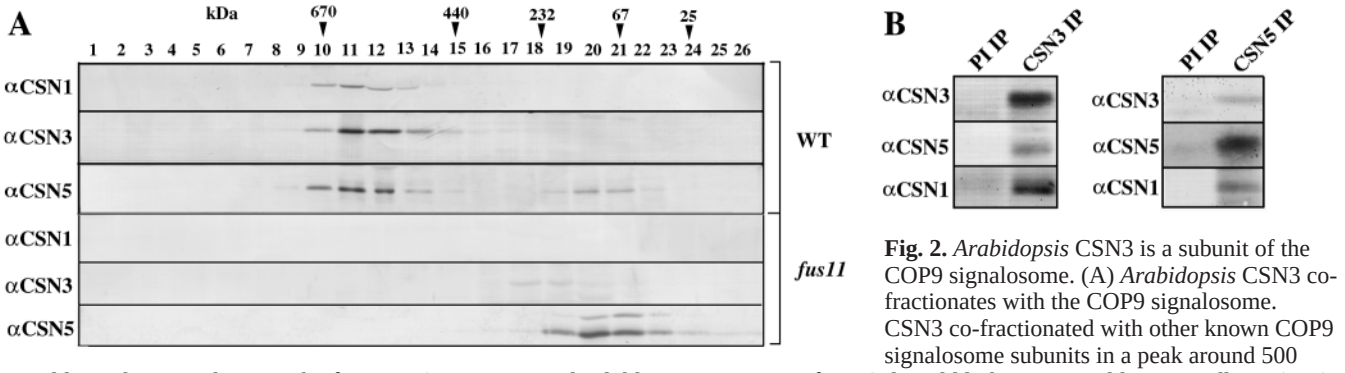

Fig. 2. *Arabidopsis* CSN3 is a subunit of the COP9 signalosome. (A) *Arabidopsis* CSN3 co-fractionates with the COP9 signalosome. CSN3 co-fractionated with other known COP9 signalosome subunits in a peak around 500 kDa in wild type but was absent in the *fus11-U203* mutant. Total soluble protein extracts from 6-day-old light-grown wild-type seedlings (WT) and 8-day-old light-grown *fus11-U203* seedlings were separated using a Superose 6 HR gel filtration column, followed by SDS-PAGE and protein blot analysis with antibodies against CSN1, CSN3 and CSN5 as indicated on the left. The plant materials used are indicated on the right. (B) *Arabidopsis* CSN3 co-immunoprecipitates with other known COP9 signalosome subunits. The antibodies used for immunoprecipitation from the total soluble proteins are indicated on the top. The antibodies used in the immunoblots are labeled on the right. 'PI IP' is the preimmune serum from the same rabbit before immunization and was used as negative control. The immunoprecipitates were separated using 10% SDS-PAGE and probed with antibodies against CSN1, CSN3 and CSN5.

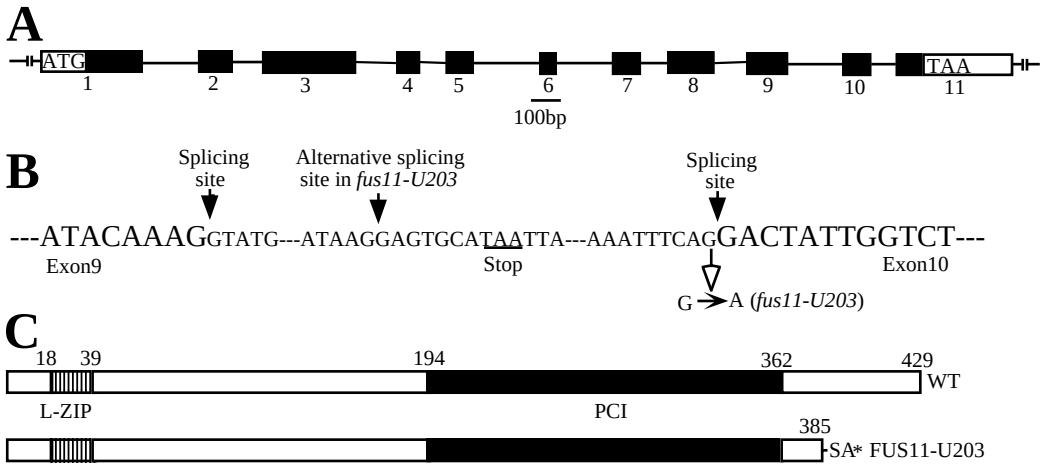

Fig. 3. *CSN3* gene structure and the molecular lesion in the *fus11-U203* mutant. (A) The genomic structure of the *CSN3* gene. The 11 exons are numbered and the protein coding regions of the exons are black. (B) The molecular lesion of the CSN3 gene in the *fus11-U203* mutant. The G to A mutation at the end of the intron 9 is indicated. This mutation results in utilization of a cryptic splicing site within intron 9, which results in in-frame termination of the reading frame after two novel amino acids. (C) Diagrams of the wild-type (WT) and mutant version of the CSN3 protein. The two inserted amino acids and the stop codon (asterisk) in the FUS11-U203 are indicated. The leucine zipper and the PCI domains are represented by striped and black boxes respectively. The amino acid positions are labeled on the top.

to their native sizes. The collected fractions were then concentrated, further separated on SDS-PAGE, and subjected to protein blot analysis with anti-ubiquitin antibodies. Consistent results were observed regardless of which batch of ubiquitin antibodies were used and an example of this profiling of ubiquitinated proteins in wild type, *fus11-U203*, *fus 6-T236* and *cop1-6* mutant seedlings is shown in Fig. 4A. Two general changes were observed in the overall profiles of ubiquitinated proteins in the COP9 signalosome mutants. First, absence of the COP9 signalosome lead to an overall increase in multi-ubiquitinated proteins, especially in the high molecular mass range (the smearing high molecular mass bands in fractions 5-15). Second, dramatic changes in the levels of several relatively abundant ubiquitin conjugated proteins were evident (see arrows). Similar changes in the profile of cellular ubiquitinated proteins were observed in several other COP9 signalosome mutants, but neither the *cop1* nor the *cop10* mutants examined exhibited such a change compared to wild type (Fig. 4A and data not shown). Thus, the COP9 signalosome has a distinct function from other COP/DET/FUS protein in the ubiquitin/proteasome pathway.

Table 1. Phenotype complementation of the *fus11* mutation by 35S promoter driven *CSN3* cDNA

Line	Resistant	Sensitive	R/S ratio	Wild type	Mutant	WT/Mu
B1	93	27	3.4	111	9	12.3
D49	108	38	2.8	136	10	13.6
E1	119	48	2.5	156	11	14.2
E45	165	51	3.2	204	12	17.0
C3	157	11	14.3	154	3	51.3

Shown are the segregation ratios of the T_2 seedlings

The effect of the COP9 signalosome on protein degradation exhibits substrate specificity

To test if the COP9 signalosome-promoted protein degradation is substrate specific, we examined the degradation of both HY5 and phytochrome A (phyA) proteins in the COP9 signalosome-deficient *fus11-U203* mutant. HY5 and phyA are two light regulatory components whose light regulated degradation has previously been shown to be mediated by the 26S proteasome (Vierstra and Callis, 1999; Osterlund et al., 2000). While HY5 is degraded rapidly in darkness, phyA degradation depends on

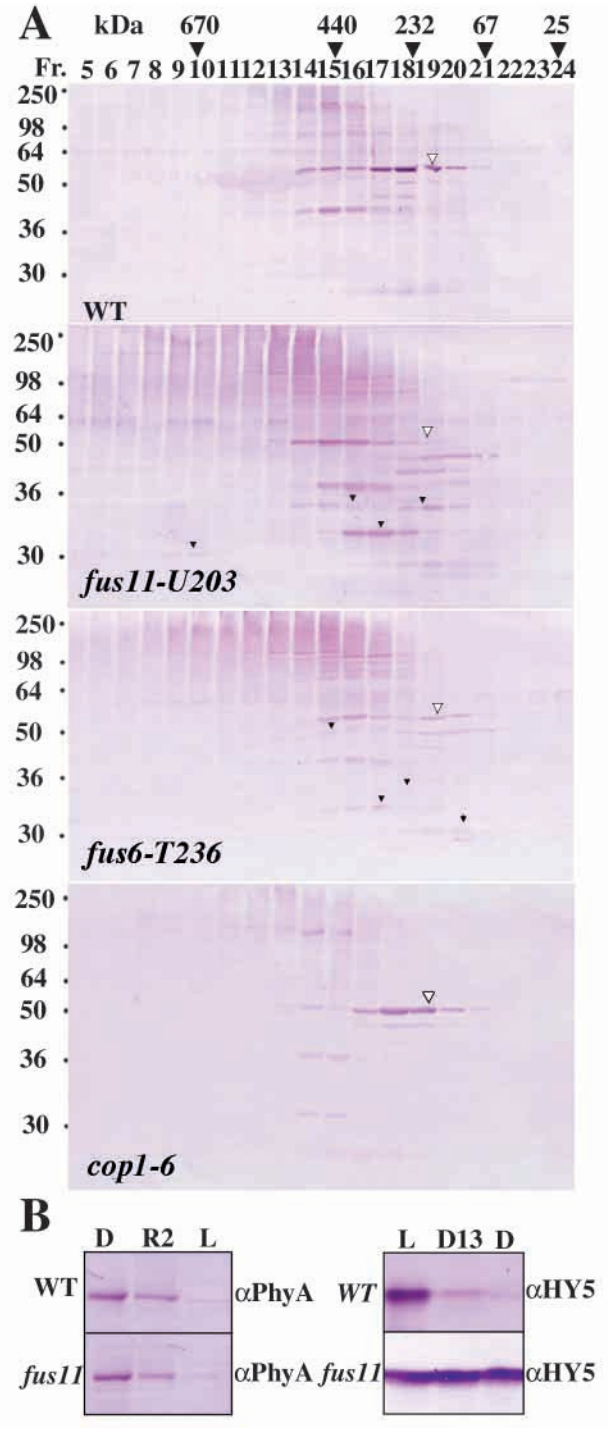

Fig. 4. Effect of COP9 signalosome deficiency on the profiles of cellular ubiquitinated proteins and on the degradation of HY5 and phyA. (A) Comparison of the profiles of cellular ubiquitinated proteins of the wild type, *fus11-U203*, *fus6-T236* and *cop1-6* mutant. The total protein extracts were size fractionated using a Superose 6 HR gel filtration column with TB buffer. The collected fractions were concentrated with StrataClean beads (Stratagene) and further separated on 10% SDS-PAGE. Antibodies against ubiquitin were used in the western blots to detect the ubiquitinated proteins. The gel filtration fraction numbers corresponding to each lane and the molecular size markers are indicated on the top. The molecular size markers on the SDS-PAGE are indicated on the left side. 8-day-old seedlings were used in the experiment. The smearing high molecular mass bands in fractions 5-15 are the multi-ubiquitinated proteins. White triangles indicate bands reduced in mutants, while black triangles indicate bands increased in mutants. (B) The effect of the COP9 signalosome deficiency on proteasome-mediated protein degradation. The seedlings were 4.5 days old. The antibodies used for western blot are indicated on the right side and the sample identity is indicated on the left side. Left: phyA degradation profile in both wild-type and *fus11-U203* seedlings. The three samples used were continuous dark-grown seedlings (lane D), continuous light-grown seedlings (lane L), and dark-grown seedlings shifted to red light for 2 hours (R2). Right: HY5 degradation profiles in both wild-type and *fus11-U203* seedlings. The samples shown were continuous light grown seedlings (lane L), continuous dark-grown seedlings (lane D), and continuous light-grown seedlings shifted to the dark for 13 hours (lane D13).

light stimulation. As shown in Fig. 4B, the COP9 signalosome deficiency completely inhibited degradation of HY5 in both dark-grown seedlings and in light grown seedlings subjected to dark adaptation. However, the COP9 signalosome deficiency had no detectable effect on light-dependent degradation of phytochrome A. Both light-grown seedlings and red light-treated dark-grown *fus11* mutant seedlings had normal patterns of phyA degradation. Thus, the involvement of the COP9 signalosome in ubiquitin-proteasome mediated proteolysis appears to be substrate specific. Therefore alteration of the protein ubiquitination pattern in the COP9 signalosome mutants are likely limited to those proteins that are subjected to the COP9 signalosome regulation.

Reduction of CSN3 and the COP9 signalosome results in multifaceted developmental defects

Since all the *cop* or *fus* mutants for genes encoding the COP9 signalosome are lethal after the seedling stage, the role of the COP9 signalosome in late developmental processes remains unclear. The recent examination of CSN5 partial loss-of-function transgenic lines revealed a role of the COP9 signalosome in auxin response (Schwechheimer et al., 2001). To further define the developmental role of the COP9 signalosome, we obtained over 30 transgenic lines in each of the Columbia and Landsberg ecotypes by stable transforming the 35S driven *CSN3* cDNA transgene construct. The majority of the transgenic lines exhibited a variety of phenotypes in the multiple generations examined (Table 2). Western blot analyses with antibodies specifically against *Arabidopsis* CSN3 and other COP9 signalosome subunits revealed that all the plants with observed phenotypic defects from all lines examined had reduced amounts of the COP9 signalosome (Fig. 5A and data not shown). This indicates that a co-suppression event occurred in those transgenic lines, resulting in a reduction of CSN3 and the COP9 signalosome abundance. This reduction of the COP9 signalosome is likely responsible for the observed phenotypes.

A striking feature of these transgenic lines is that each independent line segregates a variety of different phenotypes regardless of the specific phenotype of the parental plants (with the exception of early seedling defects, see below). Although the phenotype of a given progeny varies, the overall spectra of phenotypes within a given line are quite consistent. In fact, the same phenotypic spectra have been inherited through at least four tested generations so far. It was also common to have an individual transgenic plant exhibiting multiple phenotypes. To

Table 2. Summary of phenotype distribution among representative *CSN3* co-suppression transgenic lines

Line	Total number of plants	Juvenile leaves	Adult leaves	Inflorescence defects	Flower defects	Seedling defects	Lethal	Others	Normal
S3-C-A	190	32	21	9	6	30	37	48	82
S3-C-C	210	37	28	7	12	41	54	46	69
S3-C-K	165	31	16	4	0	37	50	23	70
S3-C-S	180	18	19	10	2	25	60	39	59
S3-C-T	120	14	15	0	0	23	20	39	61
S3-L-D	171	22	31	20	9	30	67	22	38
S3-L-O	151	24	18	7	3	51	69	20	24
S3-L-T	191	15	27	4	1	90	87	15	23

The plants were transformed with full-length *CSN3* cDNA under the control of 35S promoter. The transgenic lines of 'S3-C-X' series were of T_2 generation in the Columbia background and the 'S3-L-X' series were of T_3 generation in the Landsberg background. The transgenic plants were germinated in darkness for 3 days and then transferred to white light. The total plants number only included the gentamycin resistant (transgenic) plants. Because many plants have more two or multiple phenotypes, the combined number of plants in all phenotype categories is larger than the total number of plants examined. Plant phenotypes are classified into general categories (see text for more details). Plants without true leaves, with cotyledon number change, or without root were considered as 'Seedling defect'. 'Others' group includes plants with phenotypes not categorized in the table, such as loss of apical dominance, fused organs, asymmetric organs, curled stems and silique phenotypes etc. 'Normal' group includes those plants that appeared to be normal both developmentally and morphologically.

better describe the observed phenotype in our partial loss-of-function COP9 signalosome transgenic plants, selected phenotypes are presented according to the organ and the developmental stage affected.

Leaf development

The vegetative growth of *Arabidopsis* consists of two consecutive phases, the juvenile stage and the adult stage. The first pair of true leaves at the juvenile phase are small, round and positioned in opposite phyllotaxy, whereas later adult leaves are spatulate, serrate and positioned in spiral phyllotaxy (Medford et al., 1992). In the *CSN3* co-suppression plants, a variety of leaf shape defects have been observed at both the juvenile and adult phases (Fig. 6A). The selected leaf shapes include strip (panel a of Fig. 6A), needle (panel b of Fig. 6A), and horn (panel c of Fig. 6A). In addition, phyllotaxy changes have also been observed at the juvenile stage in 5 of the 30 transgenic lines in the Landsberg background. For example, panel d of Fig. 6A shows phyllotaxy pattern of three instead of two symmetric leaves in rosette pattern. Western blot analyses revealed that all these defective leaves have reduced levels of CSN3 and other COP9 signalosome subunits (Fig. 5A and data not shown). At the same time, over accumulation of ubiquitinated proteins were also detectable in these defective leaves (Fig. 5B). Together, these results suggest that proteasome-mediated protein degradation defects are probably responsible for the leaf developmental defects.

Other vegetative phenotypes

In the progeny of the *CSN3* co-suppression lines, early seedling developmental defects have been constantly observed. As shown in Fig. 6B, the phenotypes included seedlings with single, triple and quadruple cotyledons (panels a, b and c of Fig. 6B), and seedlings without embryonic roots

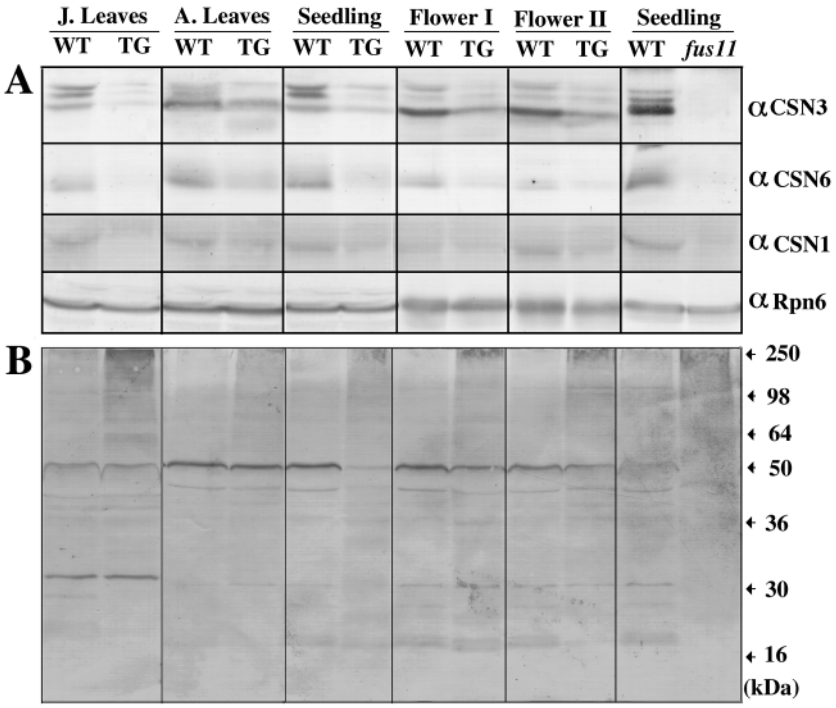

Fig. 5. Immunoblot analysis of the transgenic plants. (A) Levels of the COP9 signalosome subunits in the *CSN3* transgenic plants. Proteins were extracted from the defective organs (as indicated on top) from various transgenic lines (TG). Antibodies against CSN3, CSN6 and CSN1 were used to detect the COP9 signalosome level (marked on the right). The corresponding wild-type tissues (WT) were used as a control. Rpn6 was used to control equal loading. (B) Levels of ubiquitinated proteins in the transgenic plants and *fus11-U203* mutant. The total protein extracts were separated on 12% SDS page and detected with antibodies against ubiquitin. J. Leaves, juvenile leaves; A. Leaves, adult leaves. The juvenile leaves, adult leaves and flower I were from lines S3-C-A, S3-C-A and S3-C-C, respectively of the T_2 generation in the Columbia background. The transgenic seedling and flower II were from line S3-L-D of the T_3 generation in the Landsberg background. 8-day-old *fus11-U203* seedlings were used as a control. Many other transgenic lines were examined and similar results were observed.

(panel f, Fig. 6B). These seedling phenotypes are usually below 3% of the progeny of the given transgenic lines. These early seedling phenotypes are very similar to the auxin related mutants such as *PIN-FORMED* and *PINOID* in cotyledon number change (Goto et al., 1987; Bennett et al., 1995), confirming our observation that the COP9 signalosome indeed regulates $SCF^{TIR}1$ and the auxin responses (Schwechheimer et al., 2001). In addition, adult phenotypes of the CSN3 co-suppressing lines also supported this conclusion. One frequent adult phenotype in our transgenic progeny (between 5% and 30%) during vegetative growth is the loss of apical dominance (panels d and e of Fig. 6B). This type of plants often has wrinkled leaves (panel e of Fig. 6B). The appearance resembles the phenotype of auxin resistant mutants (Estelle and Somerville, 1987) and those CSN5 co-suppression lines recently reported (Schwechheimer et al., 2001).

Another common seedling defect is that the seedlings fail to produce true leaves and leaf buds (Fig. 6C). Northern blot analysis of such seedlings demonstrated that the Shoot Meristemless (STM) gene was expressed at a lower level compared with the wild type (Fig. 6C, lower panel). This type of plants occurred at relatively higher frequency compared to other seedling phenotypes shown in Fig. 6B (Table 2). It is important to note that the parental plants producing defective seedlings tend to display adult developmental defects and have reduced COP9 signalosome abundance (data not shown) while other later developmental defects usually have no correlation with their parental phenotypes.

Inflorescence development

The reproductive growth of *Arabidopsis* begins with the transition from an adult vegetative meristem to an inflorescence meristem, which will give rise to the floral inflorescence. A variety of phenotypes were observed in the inflorescence of *CSN3* transgenic plants as shown in Fig. 7A. The inflorescence in panel a has a phyllotaxy defect, so that four flowers were positioned on one side of the stem (see white arrow). In panel b, multiple poorly developed inflorescence branches radiated out from the same position, suggesting a possible defect in branching pattern. The inflorescence in panel c had a thick and expanded stem and the flowers originated without any particular phyllotaxy pattern – another malfunction in pattern formation. In addition to its big size, the inflorescence in panel d had no internode elongation at all, therefore the inflorescence somewhat resembles a sunflower head. While the plant shown in panel e had tightly packed siliques at the top end of inflorescence, and the plant shown in panel f has extremely short stalks. These developmental defects suggest that the COP9 signalosome is involved in multiple aspect of inflorescence development.

Flower development

Although the percentage of plants with floral defects was not high (Table 2), the diversity of the flower phenotypes in the *CSN3* transgenic plants was striking. Some selected examples are shown in Fig. 7B. In the flower in panel a, a gynoecium was developed within another gynoecium (see white arrow). While the stigmatic tissues of the later gynoecium were well developed (see green arrow), its two carpels failed to fuse at the top. Meanwhile, the adjacent flower only had two unfused carpels. The phenotype of this flower is similar to that of a weak AGAMOUS mutant (Sieburth et al., 1995). The gynoecium shown in panel b was developed within a partially fused sepal, while other floral organs were completely missing. Panel c presents an 'empty' flower, also described for the severe *ufo* mutants (Wilkinson and Haughn,

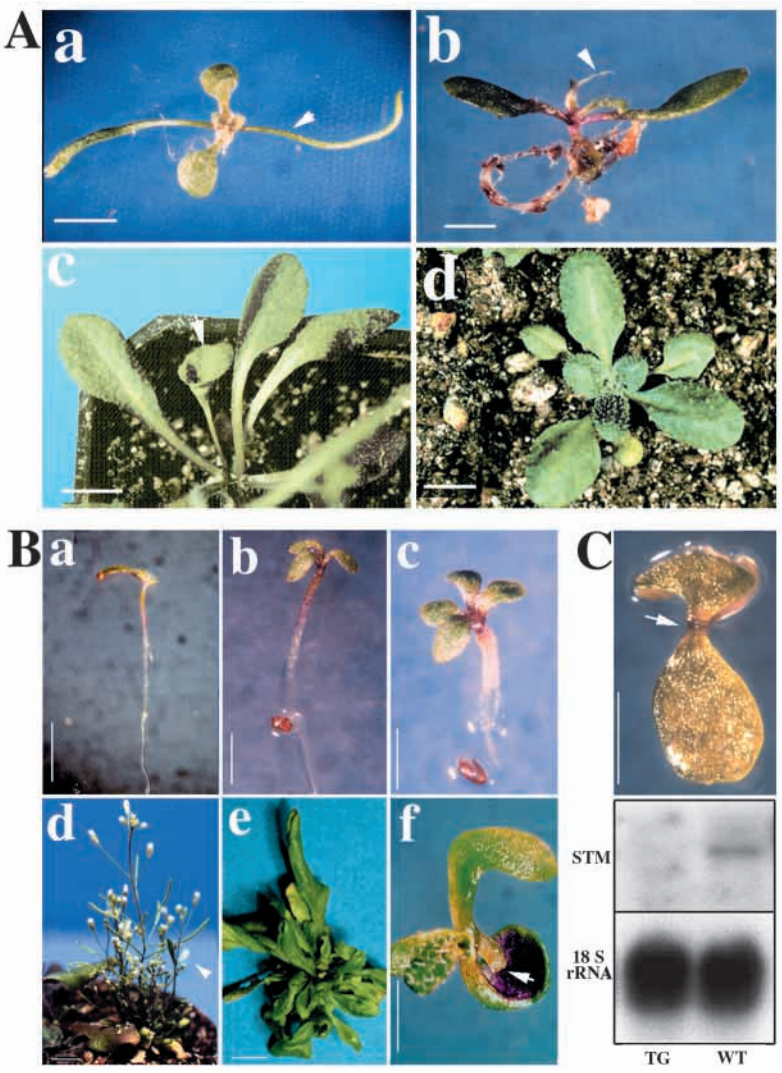

Fig. 6. Representative vegetative growth phenotypes of the CSN3 co-suppression plants. (A) Leaf developmental phenotypes of the *CSN3* transgenic plants. The plants in a, b and c were from the T_2 generation in the Columbia background. The plant in d was from the T_3 generation and in the Landsberg background. Scale bars: 5 mm. Arrows indicate defective leaves. (B) The developmental phenotypes of the *CSN3* transgenic plants with similarity to auxin related mutants. The plants were from the T_3 generation in the Columbia background except that the plant in d was from the T_2 generation. Scale bars: 5 mm. Arrows indicate defective organs. (C) Top: seedlings producing no true leaves. Bottom: northern blot analysis of the STM gene in transgenic and wild-type plants. The seedling shown was from the T_3 generation in the Columbia background.

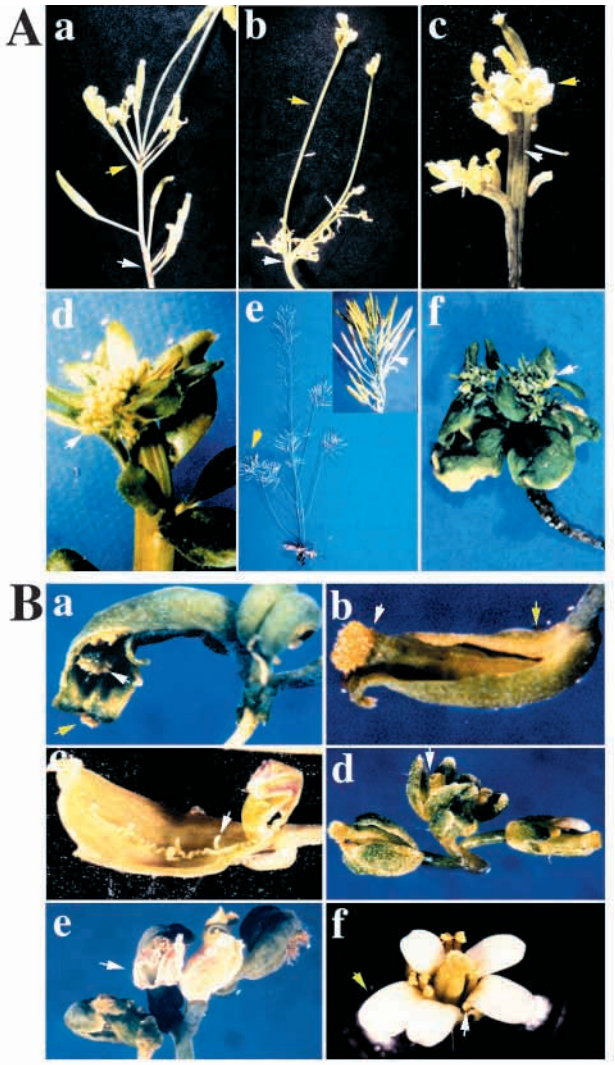

Fig. 7. Representative phenotypes of *CSN3* co-suppression plants during reproductive growth. (A) Representative inflorescence phenotypes of the *CSN3* transgenic plants. The inflorescences in a, b and c were from T_2 plants in the Columbia background. The inflorescences in d, e, and f were from the T_3 plants in the Landsberg background. The arrows indicate defective organs. (B) Representative flower phenotypes of the *CSN3* transgenic plants. The flowers in a, b, d and e were from T_3 plants in the Landsberg background. The flowers in c and f are from T_2 plants in the Columbia background. The arrows indicate organs with marked developmental abnormality.

1995). In this flower, one of the two sepal-like structures at the first whorl organ position had ovule-like structures along the middle axis (see white arrow), indicating a partial homeotic transformation. The flowers shown in panel d either completely missed or had reduced numbers in petals, stamen and sepals, suggesting that probably both A and B type flower identity genes were affected. This hypothesis gains support from the phenotypes shown in panel e. Here the flowers had no sepals and stamens but still produced structurally defected petals and carpels. In contrast to the above examples, the flower in panel f had an extra stamen and an extra petal. These few examples suggest that the floral defects include pattern formation, homeotic organ transformation and organ number modification. Western blots using flowers from two representative flower types, as shown in panels c and e, are presented in Fig. 5A. The results clearly indicate that those defective flowers had a reduced COP9 signalosome level. In addition, multi-ubiquitin-conjugated proteins were also over accumulated in these transgenic flowers (Fig. 5B and data not shown).

DISCUSSION

Arabidopsis *FUS11* encodes the subunit 3 of the COP9 signalosome

We have presented definitive evidence that subunit 3 of the COP9 signalosome is encoded by the *FUS11* gene in *Arabidopsis*. First, the *Arabidopsis* CSN3 protein cofractionated and co-immunoprecipitated with other known subunits of the COP9 signalosome. Thus CSN3 is a subunit of the COP9 signalosome. Second, the *CSN3* gene mapped to the same region on chromosome V as the *FUS11* locus. Further, a specific mutation in the *CSN3* gene, which results in an alternative splicing and early termination of the reading frame, has been identified in the *fus11-U203* mutant. Third, the *fus11-U203* mutant failed to accumulate CSN3 and several other subunits of the COP9 signalosome. And finally, overexpression of CSN3 functionally complemented the *fus11-U203* mutation. With this new identification of *FUS11* encoding subunit 3, it will be of interesting to determine the relationship between the three remaining *cop/det/fus* loci required for the COP9 signalosome accumulation and the two uncharacterized subunits of the COP9 signalosome (Kwok et al., 1998; Serino et al., 1999).

The absence of the COP9 signalosome in the *fus11-U203* mutant confirms our earlier observations that when any single subunit is defective, the complex becomes unstable and fails to accumulate. While some subunits, such as CSN5 and CSN7, are present also as monomers, other subunits, including CSN1, CSN3, CSN4 and CSN8, are degraded to undetectable levels in vivo. The fact that CSN3 is only present in the complex form indicates that CSN3 acts exclusively as a subunit of the COP9 signalosome. Thus, our manipulation of the CSN3 abundance should directly and specifically affect the COP9 signalosome level in vivo.

A role of the COP9 signalosome in multifaceted development processes

It is striking that reduction of the cellular COP9 signalosome abundance resulted in such a variety of developmental defects in *Arabidopsis*. The most common phenotypes include alterations in pattern formation, phyllotaxy, homeotic transformation and organ size or number modifications from embryogenesis to flower development (see Table 2). Although the phenotype among individual progenies of the transgenic lines varies greatly, several constant features are evident throughout our studies. First, the spectra of the phenotypes for most of the reduction-of-function lines are quite similar when the population is large enough. Second, for a given transgenic line and with the exception of embryogenesis defects, the spectrum of phenotypes is constant from generation to generation and independent of the phenotype of the parent

plant. Third, the phenotypic defects apparent at late developmental stages were in general milder, while the early phenotypes are more severe and often detrimental to plant development.

The phenotypes observed in the transgenic lines provided us with the opportunity to systematically document the role of COP9 signalosome in plant development. It is likely that specific regulatory proteins are targets of the COP9 signalosome during plant development and the reduction of the COP9 signalosome resulted in altered regulation of the protein abundance and defects in their associated developmental processes. The diverse phenotypes we observed would be consistent with the notion that the COP9 signalosome interacts and regulates multiple E3 ligases and thus their substrate protein degradation. Besides the well-documented role of the COP9 signalosome in SCF^{TIR1}-mediated protein degradation and auxin response, some of our co-suppression line phenotypes also indicate that other SCF-type E3 ligases also require the COP9 signalosome for their proper function. For example, some aspects of the observed flower defects resemble those of the *ufo* mutants (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995; Ingram et al., 1995). UFO is a F-box protein and has been suggested to be part of a SCF-type E3 ligase complex involved in flower development (Samach et al., 1999). Most likely, the *ufo* mutations cause a defect in the degradation of proteins critical for flower development, thus leading to an abnormal flower phenotype. It is thus plausible that some of the flower defects we observed in our *CSN3* co-suppression lines are caused by an improper function of the SCF^{UFO} as a result of reduced COP9 signalosome levels. Beside TIR1 and UFO, several other F-box proteins have been reported to regulate plant defense (Xie et al., 1998), flower timing (Somers et al., 2000; Nelson et al., 2000) and light regulation (Dieterle et al., 2001). The different F-box proteins seem to be involved in distinct developmental processes but all share an ability to form a specific SCF E3 ligase complex with the same core subunits. Since *Arabidopsis* has more than 300 F-box proteins, it is quite possible that the COP9 signalosome could accomplish most, if not all, its function in regulating many developmental processes by modulating the activities of these distinct F-box-containing SCF E3 ligases.

How can individual progenies of the same transgenic line have different phenotypes and the different parts of the same transgenic plant have different phenotypes? It is well established that proteasome-mediated proteolysis plays a crucial rule in plant development, by ensuring that the activity level of its targets is controlled in a precise temporal and spatial manner according to developmental and environmental cues. Some of these protein degradation events likely require the function of the COP9 signalosome. The reduction of the COP9 signalosome would result in inappropriate activities of key developmental regulators above or below their critical thresholds, thus causing defects in developmental processes controlled by them. The stochastic nature of the phenotypes of CSN3 transgenic lines could be caused by variation of the levels of the COP9 signalosome among individual plants at different time points of the plant development, or in different cells and tissues within a plant, due to the temporal and spatial pattern of gene silencing. It is worth noting that the phenotype of the *ufo* mutant also appeared to be stochastic (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). UFO is a F-box protein involved in protein degradation as a component of the SCF E3 ligase complex (Samach et al., 1999). Thus it is not entirely impossible that stochastic phenotype defects might be a characteristic feature of protein degradation malfunction.

Possible mechanism of the COP9 signalosome in modulating the ubiquitin-proteasome pathway

A direct role of the COP9 signalosome in the ubiquitin/proteasome pathway is supported by several observations. In addition to its essential role in HY5 degradation in darkness (Osterlund et al., 2000; Fig. 4B), the COP9 signalosome was reported to physically interact with a SCF-type ubiquitin E3 ligase and is essential for the E3 ligase mediated process (Schwechheimer et al., 2001). In this study, we provide evidence that there is an overall increase in cellular multi-ubiquitin-conjugated proteins in the COP9 signalosome-deficient mutants and in *CSN3* transgenic plants with reduced levels of the COP9 signalosome (Figs 4A, 5B). As the multi-ubiquitin-conjugated proteins are the substrates for the proteasome, their over accumulation in the COP9 signalosome-defective plants indicates a defect in the presentation of those substrates to the proteasome. Since the COP9 signalosome and SCF-type E3 ligases associate with each other physically in vivo (Schwechheimer et al., 2001), it is possible that the COP9 signalosome could function in releasing the multi-ubiquitin conjugated proteins from the E3 ligases and presenting them in a proper form to the 26S proteasome. This specific role of the COP9 signalosome could be related to its ability to promote RUB1 de-conjugation from the AtCUL1 subunit of the SCF ligase complex (Lyapina et al., 2001; Schwechheimer et al., 2001). The best case to be considered is the SCF^{TIR1}-mediated protein degradation and auxin response, where both AXR1-mediated RUB1 conjugation to AtCUL1 and the COP9 signalosome-promoted RUB1 de-conjugation are essential. It is feasible that this cycling of RUB1 conjugation and de-conjugation of the SCF ligase provide the dynamic conformation changes necessary for releasing the multi-ubiquitin conjugated substrate and presenting to the 26S proteasome. Clearly, this area should be a fertile ground for future study.

It is not clear at this point at which level the specificity of the COP9 signalosome to the substrate is conferred. We have shown that while the COP9 signalosome is essential for proteasome-mediated HY5 degradation, it does not play an observable role in proteasome-mediated phytochrome A degradation (Fig. 4; Jabben et al., 1989; Osterlund et al., 2000). There are two possible ways by which the COP9 signalosome could act specificity toward selected substrates. First, the COP9 signalosome may only interact and regulate a selected group of E3 ligases in vivo. This is possible but not supported by the fact that the core subunits of the SCF complexes are responsible for contacting the COP9 signalosome (Lyapina et al., 2001; Schwechheimer et al., 2001). Alternatively, the COP9 signalosome could directly confer its specificity toward substrates. This could be achieved by directly influencing the E3 ligase conformation through its association, and/or enhance the interaction of the E3 ligase with selective substrates. Further studies will be required to sort out these alternative models.

We are grateful to Dr Magnus Holm for commenting this manuscript. This research is supported by a NSF grant (MCD-

0077217) and a BARD grant (IS-3123-99) to X.-W. D. X.-W. D. is a NSF Presidential Faculty Fellow. ZP is a recipient of a National Institute of Health postdoctoral fellowship.

REFERENCES

Bech-Otschir, D., Kraft, R., Huang, X., Henklein, P., Kapelari, B., Pollmann, C. and Dubiel, W. (2001). COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. *EMBO J.* **20**, 1630-1639.

Bennett, S. R. M., Alvarez, J., Bossinger, G. and Smyth, D. R. (1995). Morphogenesis in pinoid mutants of *Arabidopsis thaliana*. *Plant J.* **8**, 505-520.

Dieterle, M., Zhou, Y. C., Schäfer, E., Funk, M. and Kretsch, T. (2001). EID1, an F-box protein involved in phytochrome A-specific light signaling. *Genes Dev.* **15**, 939-944.

Castle, L. and Meinke, D. (1994). A *FUSCA* gene of *Arabidopsis* encodes a novel protein essential for plant development. *Plant Cell* **6**, 25-41.

Chamovitz, D. A., Wei, N., Osterlund, M. T., von Arnim, A. G., Staub, J. M., Matsui, M. and Deng, X.-W. (1996). The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* **86**, 115-121.

Chory, J., Peto, C. A., Feinbaum, R., Pratt, L. and Ausubel, F. (1989). An Arabidopsis thaliana mutant that develops as a light-grown plant in the absence of light. *Cell* **58**, 991-999.

Deng, X.-W., Caspar, T. and Quail, P. H. (1991). cop1: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. *Genes Dev.* **5**, 1172-1182.

Deng, X.-W., Dubiel, W., Wei, N., Hofmann, K., Mundt, K., Colicelli, J., Kato, J. Y., Naumann, M., Segal, D., Seeger, M. et al. (2000). Unified nomenclature for the COP9 signalosome: an essential regulator of development. *Trends Genet.* **16**, 289.

del Pozo, J. C. and Estelle, M. (1999). The *Arabidopsis* cullin AtCUL1 is modified by the ubiquitin-related protein RUB1. *Proc. Natl. Acad. Sci. USA* **96**, 15342-15347.

Deshaies, R. J. (1999). SCF and Cullin/Ring H2-based ubiquitin ligases. *Annu. Rev. Cell Dev. Biol.* **15**, 435-467.

Estelle, M. A. and Somerville, C. R. (1987). Auxin resistant mutants in Arabidopsis with an altered morphology. *Mol. Gen. Genet.* **206**, 200-206.

Freilich, S., Oron, E., Kapp, Y., Nevo-Caspi, Y., Orgad, S., Segal, D. and Chamovitz, D. A. (1999). The COP9 signalosome is essential for development of *Drosophila melanogaster*. *Curr. Biol.* **9**, 1187-1190.

Glickman, M. H., Rubin, D. M., Coux, O., Wefes, I., Pfeifer, G., Cjeka, Z., Baumeister, W., Fried, V. A. and Finley, D. (1998). A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to COP9 signalosome and eIF3. *Cell* **94**, 615-623.

Goto, N., Starke, M. and Kranz, A. R. (1987). Effect of gibberellins on flower development of the pin-formed mutant of *Arabidopsis thaliana*. *Arab. Inf. Serv.* **23**, 66-71.

Ingram, G. C., Goodrich, J., Wilkinson, M. D., Simon, R., Haughn, G. W. and Coen, E. S. (1995). Parallels between *UNUSUAL FLORAL ORGANS* and *FIMBRIATA*, genes controlling flower development in *Arabidopsis* and *Antirrhinum*. *Plant Cell* **7**, 1501-1510.

Jabben, M., Shanklin, J. and Vierstra, R. D. (1989). Ubiquitin-phytochrome conjugates. Pool dynamics during *in vivo* phytochrome degradation. *J. Biol. Chem.* **264**, 4998-5005.

Karniol, B., Malec, P. and Chamovitz, D. A. (1999). *Arabidopsis FUSCA5* encodes a novel phosphoprotein that is a component of COP9 complex. *Plant Cell* **11**, 839-848.

Kendrick, R. E. and Kronenberg, G. H. M. (eds) (1994). *Photomorphogenesis in Plants*. 2nd edn. Dordrecht, The Netherlands: Kluwer.

Kwok, S. F., Piekos, B., Misera, S. and Deng, X.-W. (1996). A complement of ten essential and pleiotropic *Arabidopsis COP/DET/FUS* genes is necessary for repression of photomorphogenesis in darkness. *Plant Physiol.* **110**, 732-742.

Kwok, S. F., Solano, R., Tsuge, T., Chamovitz, D. A., Matsui, M., Ecker, J. R. and Deng, X.-W. (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.

Levin, J. Z. and Meyerowitz, E. M. (1995). UFO: An Arabidopsis gene involved in both floral meristem and floral organ development. *Plant Cell* **7**, 529-548.

Li, S., Liu, X. and Ascoli, M. (2000). $P38^{JAB1}$ binds to the intracellular precursor of the lutropin/Chroriogonadotropin receptor and promotes its degradation. *J. Biol. Chem.* **275**, 13386-13393.

Lyapina, S., Cope, G., Shevchenko, A., Serino, G., Tsuge, T., Zhou, C., Wolf, D. A., Wei, N., Shevchenko, A. and Deshiaes, R. J. (2001). Promotion of NEDD8-CUL1 Conjugate Cleavage by COP9 Signalosome. *Science* (in press).

McNellis, T. W., von Arnim, A. G., Araki, T., Komeda, Y., Misera, S. and Deng, X.-W. (1994). Genetic and molecular analysis of an allelic series of cop1 mutants suggests functional roles for the multiple protein domains. *Plant Cell* **6**, 487-500.

Medford, J. I., Behringer, F. J., Callos, J. D. and Feldmann, K. A. (1992). Normal and abnormal development in the Arabidopsis vegetative shoot apex. *Plant Cell* **4**, 631-643.

Miséra, S., Müller, A. J., Weiland-Heidecker, U. and Jürgens, G. (1994). The *FUSCA* genes of *Arabidopsis*: negative regulators of light responses. *Mol. Gen. Genet.* **244**, 242-252.

Nelson, D. C., Lasswell, J., Rogg, L. E., Cohen, M. A. and Bartel, B. (2000). FKF1, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* **101**, 331-340.

Osterlund, M. T., Hardtke, C. H. and Deng, X.-W. (2000). Targeted destabilization of HY5 during light-regulated *Arabidopsis* development. *Nature* **405**, 462-466.

Peng, Z., Lu, Q. and Verma, D. P. S. (1996). Reciprocal regulation of Δ^1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. *Mol. Gen. Genet.* **253**, 334-341.

Samach, A., Klenz, J. E., Kohalmi, S. E., Risseeuw, G. W. H. and Crosby, W. L. (1999). The UNUSUAL FLORAL ORGANS gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. *Plant J.* **20**, 433-445.

Schwechheimer, C., Serino, G., Callis, J., Crosby, W., Lyapina, S., Deshaies, R. J., Gray, W. M., Estelle, M. and Deng, X.-W. (2001). Interaction of the COP9 signalosome with the E3 ubiquitin ligase SCF^{Tir1} in mediating auxin-response. *Science* (in press)

Seeger, M., Kraft, R., Ferrell, K., Bech-Otschir, D., Dumdey, R. and Schade, R. (1998). A novel protein complex involved in signal transduction possessing similarities to 26S proteasome subunits. *FASEB J.* **12**, 469-478.

Serino, G., Tsuge, T., Kwok, S. F., Matsui, M., Wei, W. and Deng, X.-W. (1999). *Arabidopsis cop8* and *fus4* mutations define the same locus that encodes subunit 4 of the COP9 signalosome. *Plant Cell* **11**, 1967-1980.

Sieburth, L. E., Running, M. P. and Meyerowitz, E. M. (1995). Genetic separation of third and fourth whorl functions of *Agamous*. *Plant Cell* **7**, 1249-1258.

Somers, D. E., Schultz, T. F., Milnamow, M. and Kay, S. (2000). ZEITLUPE encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* **101**, 319-329.

Staub, J. M., Wei, N. and Deng, X.-W. (1996). Evidence for FUS6 as a component of the nuclear-localized COP9 complex in *Arabidopsis*. *Plant Cell* **8**, 2047-2056.

Tomoda, K., Kuboto, Y. and Kato, J.-Y. (1999). Degradation of the cyclin-dependent-kinase inhibitor $p27^{Kip1}$ is instigated by Jab1. *Nature* **398**, 160-165.

Tsuge, T., Matsui, M. and Wei, N. (2001). The subunit 1 of the COP9 signalosome suppresses gene expression through its N-terminal domain and incorporates into the complex through the PCI domain. *J. Mol. Biol.* **305**, 1-9.

Vierstra, R. D. and Callis, J. (1999). Polypeptide tags, ubiquitous modifiers for plant protein regulation. *Plant Mol. Biol.* **41**, 435-442.

Wei, N. and Deng, X.-W. (1992). COP9: a new genetic locus involved in light-regulated development and gene expression in *Arabidopsis*. *Plant Cell* **4**, 1507-1518.

Wei, N. and Deng, X.-W. (1996). The role of pleiotropic COP/DET/FUS genes in light control of *Arabidopsis* seedling development. *Plant Physiol.* **112**, 871-878.

Wei, N. and Deng, X.-W. (1999). Making sense of the COP9 signalosome, a conserved regulatory protein complex from *Arabidopsis* to human. *Trends Genet.* **15**, 98-103.

Wei, N., Chamovitz, D. A. and Deng, X.-W. (1994a). *Arabidopsis* COP9 is

a component of a novel signaling complex mediating light control of development. *Cell* **78**, 117-124.

Wei, N., Kwok, S. F., von Arnim, A. G. and Deng, X.-W. (1994b). *Arabidopsis* COP8, COP10, and COP11 genes are involved in repression of photomorphogenic development in darkness. *Plant Cell* **6**, 629-643.

Wei, N., Tomohiko, T., Serino, G., Dohmae, N., Takio, K., Matsui, M. and Deng, X.-W. (1998). The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. *Curr. Biol.* **8**, 919-922.

Wilkinson, M. D. and Haughn, G. W. (1995). UNUSUAL FLORAL ORGANS controls meristem identity and organ primordia fate in *Arabidopsis*. *Plant Cell* **7**, 1485-1499.

Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M. and Turner, J. G. (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science*, **280**, 1091-1094.

Yamamoto, Y. Y., Matsui, M., Ang, L. H. and Deng, X.-W. (1998). Role of COP1 interactive protein in mediating light-regulated gene expression in *Arabidopsis*. *Plant Cell* **10**, 1083-1094.