Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for *Arabidopsis* flower development

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Transcription repression plays important roles in preventing crucial regulatory proteins from being expressed in inappropriate temporal or spatial domains. *LEUNIG* (*LUG*) and *SEUSS* (*SEU*) normally act to prevent ectopic expression of the floral homeotic gene *AGAMOUS* in flowers. *LUG* encodes a protein with sequence similarities to the yeast Tup1 corepressor. *SEU* encodes a plant-specific regulatory protein with sequence similarity in a conserved dimerization domain to the LIM-domain binding 1/Chip proteins in mouse and *Drosophila*. Despite the molecular isolation of *LUG* and *SEU*, the biochemical function of these two proteins remains uncharacterized, and the mechanism of *AGAMOUS* repression remains unknown. Here, we report that LUG and SEU interact directly *in vitro* and *in vivo*. Furthermore, LUG exhibits a strong repressor activity on several heterologous promoters in yeast and plant cells. SEU, in contrast, does not exhibit any direct repressor activity, but can repress reporter gene expression only in the presence of LUG, indicating a possible role of SEU as an adaptor protein for LUG. Our results demonstrate that LUG encodes a functional homologue of Tup1 and that SEU may function similarly to Ssn6, an adaptor protein of Tup1. We have defined the LUG/LUH, Flo8, single-strand DNA-binding protein domain of LUG as both necessary and sufficient for the interaction with SEU and two domains of LUG as important for its repressor function. Our work provides functional insights into plant transcriptional corepressors and reveals both conservation and distinctions between plant corepressors and those of yeast and animals.

Transcription repression is emerging as a key regulatory strategy for both animals and plants to prevent crucial regulatory proteins from being expressed in inappropriate temporal or spatial domains. Repression is a dynamic process that regulates gene expression at two points. First, a gene can be repressed but primed for transcription, derepression resulting in rapid up-regulation of expression. Second, expression of an actively transcribed gene can be down-regulated rapidly. Repression occurs through two distinct yet overlapping mechanisms: the stabilization of nucleosomes on DNA to form a closed chromatin structure and the inactivation of the transcription machinery. Despite the conservation of repression mechanisms demonstrated in yeast and animals, our understanding of these mechanisms in higher plants remains limited.

In higher plants, normal floral development requires the proper expression and function of the floral homeotic gene *AGAMOUS* (*AG*). *AG* mRNA is normally expressed in the inner two whorls of a flower to specify stamen and carpel identity and to control floral meristem determinancy (1–3). We have previously identified two negative regulators of *AG*, namely *LEUNIG* (*LUG*) and *SEUSS* (*SEU*), which are required for the repression of *AG* transcription in the outer two whorls of a flower (4, 5). In flowers of both *lug* and *seu* mutants, *AG* mRNA is expressed in all four floral whorls, resulting in the ectopic formation of carpels and stamens in the outer two whorls (4, 5). Additionally, *AG* transcription is normally initiated at stage 3 floral meristems in wild type, but is initiated precociously in stage 2 floral meristems in *lug* and *seu* mutants. Furthermore, synergistic genetic interactions have been observed between *lug* and *seu*, resulting in a more severe degree of *AG* misexpression in *lug seu* double-mutant flowers (5). Genetic epistasis indicated that precocious and ectopic *AG* expression is responsible for the floral organ identity transformation and organ loss observed in *lug* and *seu* single and double mutants.

LUG encodes a nuclear localized protein with an N-terminal LUG/LUH, Flo8, single-strand DNA-binding protein (SSDP) (LUFS) domain, two central glutamine (Q)-rich domains, and a C terminus 7-WD repeat domain (6). The LUFS domain is a protein motif present in LUG, LUH, Flo8, and Ssdp. However, the similarity between LUG/LUH and Flo8 or Ssdp is limited only to the LUFS domain. The Q-rich and WD-repeat domains of LUG are conserved in a class of transcriptional corepressors including Tup1 in yeast (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Candida albicans*), Groucho (Gro) in *Drosophila*, and transducin-like enhancer of split (TLE) in mammals (7–9). These corepressor proteins, collectively called the Gro-TLE family proteins (10), do not possess a DNA-binding motif but repress a diverse number of target genes through targeted recruitment by site-specific DNA-binding transcription factors. Whereas the *Drosophila* Gro directly binds to a VWPRY pentapeptide present in the C terminus of some transcription factors (11), yeast Tup1 interacts with DNA-binding factors through an adaptor protein, Ssn6 (12). The N-terminal domain of Tup1 interacts directly with Ssn6, which binds to specific transcription factors. Once recruited to a promoter, GroTLE proteins interact with chromatin modifying factors or components of the RNA polymerase II holoenzyme, leading to the silencing of target gene expression. The sequence and motif similarity between LUG and GroTLE family proteins as well as defects in *AG* repression in *lug* mutants suggest that *LUG* may encode a transcription corepressor.

In contrast, *SEU* encodes a plant protein with two Q-rich domains and a conserved central domain. This conserved central domain shows sequence similarity to the dimerization domain of LIM domain-binding (Ldb) family of transcriptional coregulators such as the Ldb1 in mouse and Chip in *Drosophila* (5, 13). Ldb proteins regulate transcription by means of direct physical interactions with LIM-homeodomain proteins (14–16). It was thought that the Ldb1/Chip cofactors homodimerize and thereby bridge two LIM-homeodomain proteins to form a tetrameric complex (17). Recently a third protein, Ssdp, was discovered to be a functional component of the complex (13, 18).

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Abbreviations: LUG, LEUNIG; SEU, SEUSS; AG, AGAMOUS; SSDP, single-strand DNA-binding protein; LUFS, LUG/LUH, Flo8, SSDP; Ldb1, LIM domain-binding 1; Q, glutamine; Gro, Groucho; TLE, transducin-like enhancer of split; TSA, trichostatin-A; HDAC, histone deacetylase; MBP, maltose-binding protein; GAL4BD, DNA-binding domain of GAL4; GAL4AD, activation domain of GAL4; LUC, luciferase; β-gal, β-galactosidase.

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Interestingly, Ssdp contains a N-terminal LUFS domain shown to interact with Ldb1/Chip. Hence, the Ldb1/Chip acts to bridge the interaction between Ssdp and LIM-homeodomain.

Despite the sequence similarity between LUG and the GroTLE corepressors and between SEU and Ldb1/Chip, it is not known whether *LUG* can function as a transcription repressor, nor do we have any understanding of the molecular function of *SEU*. Furthermore, the molecular basis underlying the synergistic genetic interactions between *seu* and *lug* remains to be characterized. The similar single mutant phenotype, the strong synergistic genetic interaction between *lug* and *seu* suggest that *SEU* could function together with *LUG* as components of the same corepressor complex. *SEU* may function to bridge the interaction between LUG, a LUFS domain protein, and other as-yet-unidentified DNA-binding factors. Alternatively, *LUG* and *SEU* could act in parallel and partially overlapping pathways to regulate *AG* transcription.

Here, we demonstrate that *LUG* functions as a transcriptional repressor by means of an apparently conserved eukaryotic transcription repression mechanism, demonstrating that LUG is a bona fide plant homologue of the GroTLE transcription corepressors. We also define a functional role of SEU, which does not exhibit any repressor activity, but rather, acts as an adaptor protein for LUG. In addition, we demonstrate a physical interaction between LUG and SEU, which parallels those between Ssdp and Ldb1/Chip and suggests that the LUFS domain is an evolutionary conserved protein–protein interaction domain. Together, these data provide insights into how plant corepressors interact to regulate target gene expression and help define the biochemical functions of SEU, the founding member of a plant-specific regulatory protein family. By understanding how *LUG* and *SEU* represses *AG* transcription we hope to shed light on general transcriptional repression mechanisms in higher plant development.

Methods

Plasmid Construction. The procedures of plasmid construction for the yeast two-hybrid assays, yeast repression assays, plant repression assays, and maltose-binding protein (MBP)- or GST-tagged proteins are described in *Supporting Methods*, which is published as supporting information on the PNAS web site. Primer sequences and primer pair combinations are listed in Tables 1 and 2, which are published as supporting information on the PNAS web site.

Yeast Two-Hybrid Assays. Yeast strain PJ69-4A (*MAT**a** trp1-901 leu2-3,12 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2*::*GAL1-HIS3 met2*::*GAL7-lacZ*) harbors three reporters *HIS3*, *ADE2*, and *lacZ*, each under the control of a different GAL4-responsive promoter (19). BD-bait and AD-prey plasmids were cotransformed into PJ69-4A according to the protocol described in www.umanitoba.ca/faculties/medicine/biochem/gietz/2HS.html, and were plated on selection medium before being incubated at 30°C for 3 days. Primary transformants were subsequently streaked onto plates selective for the reporter gene(s): −histidine (+3 mM 3-amino-1,2,4-triazole) and −adenine plates. White colonies grown on the selection plates were assayed for the β-galactosidase (β-gal) activities. For β-gal liquid assays, transformants were inoculated into selective liquid media and grown at 30°C until the OD_{600} reached ≈1. Samples were analyzed in triplicate by using the Galacto Light Plus kit (Applied Biosystems) and the TD-20/20 luminometer (Turner Designs, Sunnyvale, CA) according to the manufacturer's instructions.

Yeast Repression Assays. FT5::LG312Δ S and FT5::JK1621 yeast strains contain integrated *lacZ* reporter genes with or without four LexA operator sites upstream of the *CYC1* promoter, respectively (20). Yeast cells were transformed with either *LexA-LUG (*or *LUG* derivatives), *LexA-SEU*, or *LexA-SEU+LUG*. After overnight growth in liquid medium to an early log phase, cells were harvested and assayed for β-gal activity. Values were normalized to OD_{600}.

Repression Assays in Plant Cells. Isolation and transfection of *Arabidopsis* mesophyll protoplasts was as described, and can be accessed at http://genetics.mgh.harvard.edu/sheenweb/protocols_reg.html. In cotransfection assays, 10 μg of reporter, 10 or 20 μg of effector constructs, and 0.1 μg of control plasmid *35S*::*LUC* or *35S*::*RenillaLUC* were used for each transfection. The total amount of DNA for each transfection remained constant (40 μg) by adding the appropriate amount of vector pART7 DNA that contains the 35S promoter and 3′ Ocs site (21). In cases where trichostatin-A (TSA) was used, the protoplasts were first transfected with DNA for 12 h, and then 20 μM TSA was added to the transfection reaction for an additional 12 h before the reporter assay. Luciferase assays were performed with the Promega dual-luciferase reporter assay system and the TD-20/20 luminometer. The fluorometric β-glucuronidase (*GUS*) assay was performed with the substrate MUG as described (22), and fluorescence was measured by using a Picofluor fluorometer (Turner Designs).

***In Vitro* Pulldown.** The procedures for the purification of MBP- and GST-fusion proteins are described in *Supporting Methods*. Five micrograms of GST-SEU immobilized on the glutathione resin was mixed with 5 μg of MBP-fusion proteins and was incubated overnight at 4°C in 100 μl of binding buffer (50 mM KH_2PO_4, pH 7.5/50 mM NaCl/50 mM KCl/5 mM $MgCl_2$/0.2% Triton X-100/1% BSA). Samples were washed five times in PBS buffer before being resuspended in 1× NuPage LDS sample buffer (Invitrogen), boiled, and then separated by 4–12% NuPage gel (Invitrogen). The retention of MBP-fusion proteins by GST-SEU was detected by Western blots with anti-MBP antibody (NEB, Beverly, MA).

Results

The LUFS Domain of LUG Is Sufficient for Interaction with SEU. To illuminate the molecular basis underlying the synergistic genetic interactions between *seu* and *lug*, a yeast two-hybrid assay was used to test whether *SEU* could physically interact with *LUG*. The LUG protein can be divided into three domains: the N-terminal LUFS domain, the central Q-rich domain, and the C-terminal 7-WD repeat domain. Full-length LUG, LUFS, LUFS+Q, and Q+WD were each fused to the DNA-binding domain of GAL4 (GAL4BD) and were tested for interaction with full-length *SEU* which was fused to the activation domain of GAL4 (GAL4AD). Whereas full-length LUG, LUFS, and LUFS+Q interacted with full-length SEU, Q+WD failed to interact with SEU (Fig. 1*A*). This finding suggested that the LUFS domain is both necessary and sufficient for interacting with SEU. Interestingly, the strongest interaction was observed between the LUFS and SEU (Fig. 1*A*, lane 4). Two alternative explanations could account for the difference in interaction strength between LUFS alone and other interacting LUG truncations. First, any repressor activity conferred by the full-length LUG-BD or LUFS+Q-BD may antagonize the activation capability of SEU-AD. Alternatively, the LUG-BD or LUFS+Q-BD may interfere with or partially block the accessibility of LUFS by SEU.

To establish whether the two-hybrid interactions between LUG and SEU represented a direct physical contact *in vitro*, we carried out GST affinity chromatography. Immobilized GST-SEU was incubated with MBP, MBP-LUFS, and MBP-LUFS+Q. In agreement with the two-hybrid data, GST-SEU (but not GST alone) interacted specifically with MBP-LUFS and

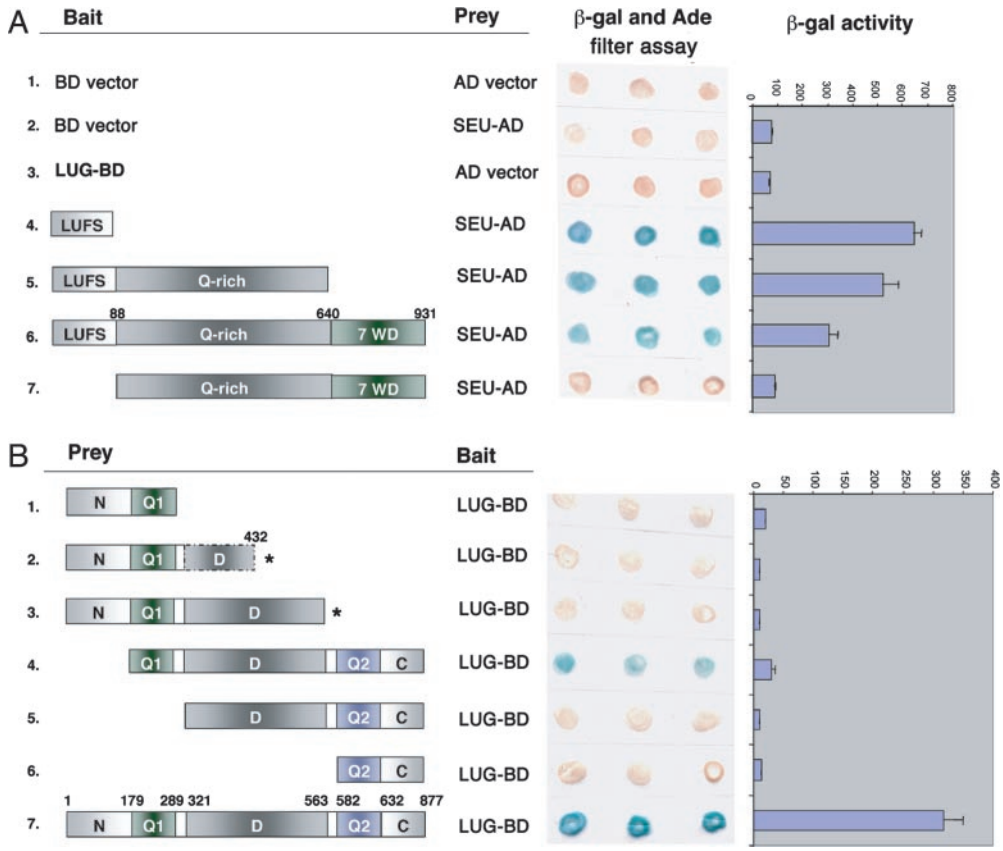

Fig. 1. The LUFS domain of LUG is both necessary and sufficient for interacting with full-length SEU. (*A*) Yeast two-hybrid assays testing the interaction between various LUG truncations and full-length SEU. (*Center* and *Right*) Yeast two-hybrid assay results of reporter gene (*lacZ* and *ADE2*) expression. Blue colonies indicate a positive β-gal activity and pink colonies indicate a negative *ADE2* activity. Quantitative measurements of β-gal activities (average of three independent transformants) are indicated on the right. (*B*) Yeast two-hybrid assays testing the interaction between various SEU truncations and full-length LUG. With the exception of two SEU derivatives shown in lanes 2 and 3 (marked by an asterisk), all other SEU derivatives and full-length SEU can self-activate reporter gene expression when fused to the GAL4BD domain. SEU protein is divided into the following five regions: N and C, N- and C-terminal domains; Q1 and Q2, the two Q-rich domains; D domain, the highly conserved dimerization domain.

MBP-LUFS+Q (Fig. 2), demonstrating a direct protein–protein interaction between LUG and SEU.

To define the domain(s) within SEU responsible for the interaction with LUG, similar two-hybrid analyses were performed with various SEU truncations. Full-length SEU as well as several SEU truncations were able to self-activate reporter *lacZ* expression when they were fused to the GAL4BD (Fig. 1*B*). Hence, SEU truncations were all cloned as AD fusions. Only full-length SEU was found to interact efficiently with LUG-BD (Fig. 1*B*, lane 7), although a very weak interaction was detected with a truncated SEU lacking the N-terminal region (Fig. 1*B*, lane 4). These results suggest that the entire SEU protein is needed for the interaction with LUG. It is possible that multiple regions of SEU are required to maintain proper protein conformation needed for the interaction with LUG.

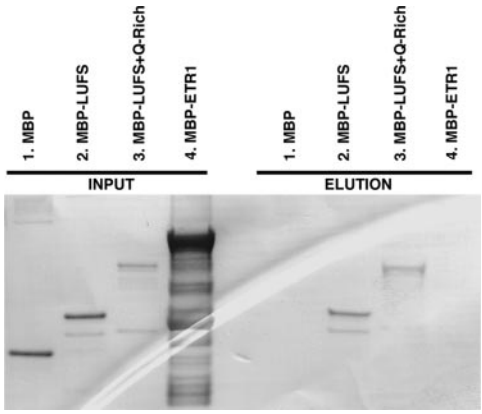

Fig. 2. SEU and LUG interact directly *in vitro*. Shown is a Western blot with anti-MBP antibody. Input lanes: ≈5 μg of MBP-tagged proteins (except MBP-ETR1) were used in the pull-down assay. Elution lanes: MBP-tagged proteins were first added to immobilized GST-SEU and were then eluted and analyzed by SDS/PAGE. Only MBP-LUFS or MBP-LUFS+Q-rich are retained by the GST-SEU. MBP and MBP-ETR1 are negative controls. Similar experiments using immobilized GST failed to retain any MBP-tagged proteins (data not shown).

LUG Possesses Repressor Activities. Based on genetic and structural data, we predicted that *LUG* would function as a transcription repressor. By using an *in vivo* yeast transcription repression assay, we tested the ability of *LUG* or *SEU* to repress transcription. Full-length *LUG* cDNA and four truncated *LUG* derivatives were fused in-frame and downstream of the bacterial LexA DNA-binding domain and tested for their ability to repress *lacZ* expression (Fig. 3*A*). When directly recruited to the test promoter, *LexA-LUG* reduced reporter gene expression by 45% compared with LexA alone, demonstrating that *LUG* has a repressor activity, and suggesting that *LUG* can repress transcription through a conserved eukaryotic repression mechanism.

To determine whether the observed repression activity could be attributed to specific domains within the LUG protein,

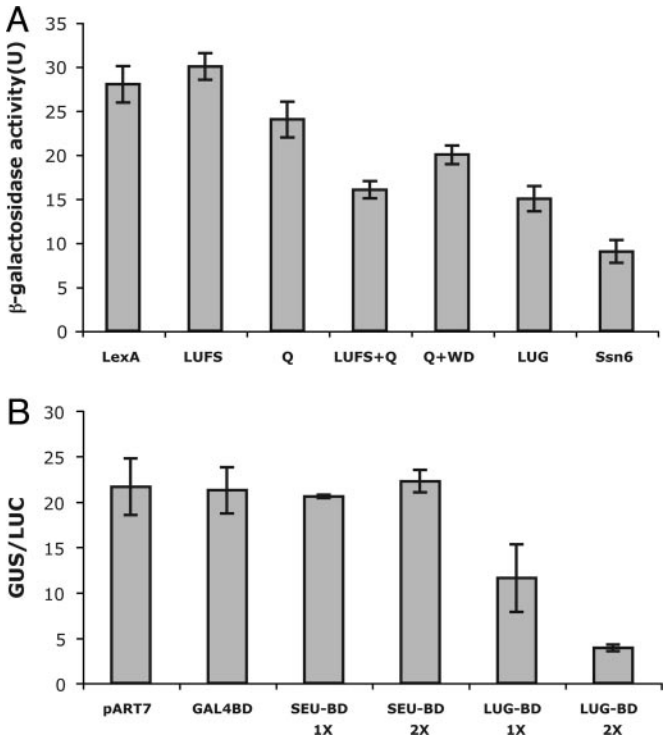

Fig. 3. *LUG* represses reporter gene expression in yeast and plant cells when tethered to test promoters. (*A*) Yeast repression assays with a *lacZ* reporter containing LexA operator sites (lop) upstream of the $CYC1_{UAS}$-$CYC1_{TATA}$ promoter integrated into the yeast genome. Yeast cells were independently transformed with the indicated LexA-fusion proteins, and β-gal activities (average of five independent transformants) are shown. No effect was seen with reporters lacking lop. LexA alone is a negative control. LexA-Ssn6 serves as a positive control. *LUFS*, *Q*, *LUFS+Q*, and *Q+WD* are four different *LUG* truncations each fused to the LexA DNA-binding domain; *LUG* indicates the full-length *LUG* fused to LexA. (*B*) Transient plant repression assays. *2XUAS-tCUP*::*GUS* reporter plasmid was mixed with *35S*::*LUC* plasmid and was transfected into *Arabidopsis* protoplasts. *GUS*/*LUC* ratio equalizes differences in transfection efficiency. Different effector DNAs were introduced simultaneously with the reporter DNA. *pART7*: vector alone; *SEU-BD*, *GAL4BD* fused to full-length *SEU*; *LUG-BD*, *GAL4BD* fused to full-length *LUG*. All effector proteins were expressed from the CaMV 35S promoter. 1× and 2× indicate equal (10 μg) and twice (20 μg) the amount of effector DNA compared with the *2XUAS-tCUP*::*GUS* reporter DNA. (Bar, SD.)

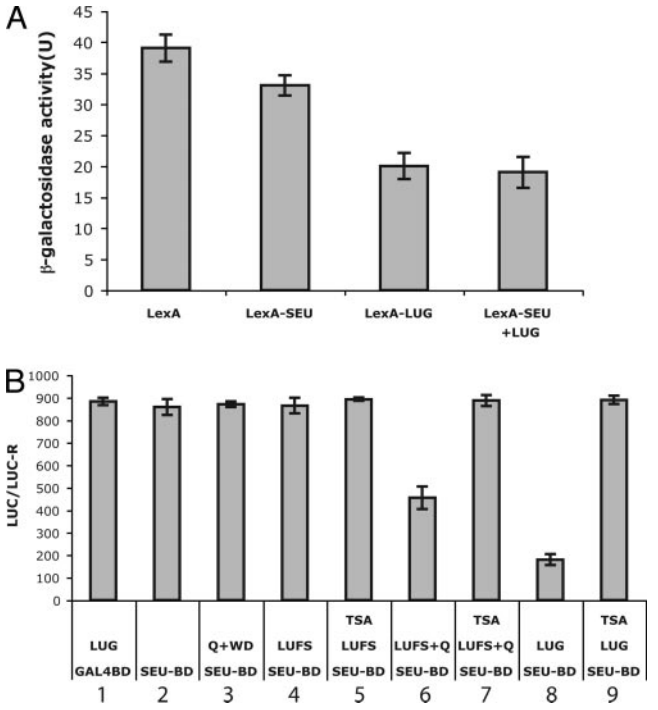

Fig. 4. SEU acts as an adaptor protein for LUG. (*A*) Yeast repression assays using reporters described in Fig. 3*A*. *LexA-SEU* (full-length), *LexA-LUG* (full-length), as well as *LexA-SEU+LUG* are tested for their repressor activities. Whereas *LexA-SEU* alone showed no repressor activity, cotransfection of *LexA-SEU* and *LUG* together showed repressor activity. (*B*) Transient *Arabidopsis* protoplast repression assays using reporter *2XUAS-35S*::*LUC* mixed with *35S*::*RenillaLUC* (*35S*::*LUC-R*). *LUC*/*LUC-R* ratio was used to indicate reporter gene expression and to control for transfection efficiency. Ten micrograms of *GAL4BD* DNA or *35S*::*SEU-BD* DNA was mixed with (or without) 20 μg of DNA of various *35S*::*LUG* derivatives. In lanes 5, 7, and 9, 20 μM TSA was used. (Bar, SD.)

truncated LUG derivatives were assayed. Two distinct domains were found to have repression function. The LUFS+Q repressed transcription to the same extent as full-length LUG (Fig. 3*A*), whereas the Q+WD repressed transcription to a lesser but significant extent. This repression function cannot be attributed solely to the Q-rich domain because the Q-rich domain alone cannot repress transcription.

To test whether LUG could function as a transcription repressor *in planta*, a transient *Arabidopsis* protoplast repression assay using the $2XUAS_{GAL4}$-*tCUP*::*GUS* reporter (23) was adopted. A $2XUAS_{GAL4}$ element is located immediately upstream of the *tCUP*, a constitutive tobacco promoter, driving the *GUS* reporter gene. *Arabidopsis* leaf protoplasts were transfected with 35S::*LUG-BD* or *35S*::*SEU-BD* together with the reporter, and the effects on *GUS* expression were quantified (Fig. 3*B*). Whereas *SEU-BD* did not show any effect on the level of *GUS* expression, *LUG-BD* significantly reduced the *GUS* expression level in a concentration-dependent manner. Specifically, doubling the amount of *LUG-BD* plasmid DNA used in the transfection resulted in an additional 3-fold reduction of *GUS* reporter activity (Fig. 3*B*). The reduced *GUS* activity is unlikely due to simple steric interference of the transcription machinery by increased *LUG-BD* at the promoter region because a similar increase in *SEU-BD* DNA did not result in any decrease in *GUS* expression (Fig. 3*B*). Clearly, *LUG* functions as a strong repressor of transcription *in planta* and can repress transcription more efficiently in a homologous system than the heterologous yeast system.

LUG Is Required by SEU to Repress Transcription in Yeast and *in Planta*. In contrast to *LUG*, the protein sequence of *SEU* provided scant clues as to the biochemical function of *SEU*. *SEU* was unable to repress transcription when fused to the LexA in yeast repression assays (Fig. 4*A*). However, when *LUG* was overexpressed together with *LexA-SEU*, a similar level of repression to *LexA-LUG* was observed (Fig. 4*A*), indicating a functional molecular interaction between these two proteins.

What then is the biological significance of the SEU–LUG interaction? One possibility as suggested by the above yeast repression assay is that SEU may serve as an adaptor protein facilitating the interaction between LUG and DNA-binding transcription factors just as the yeast corepressor Ssn6 and the mouse and *Drosophila* Ldb1/Chip proteins. To assess this possibility *in planta*, *Arabidopsis* protoplasts were cotransfected with a reporter and *35S*::*SEU-BD* in the absence or presence of *35S*::*LUG* (or various *35S*::*LUG* truncations; Fig. 4*B*). The reporter, $2XUAS_{GAL4}$-*35S*::*LUC*, contains the luciferase (LUC) gene under the control of a constitutive 35S promoter with $2XUAS_{GAL4}$ located immediately upstream of the 35S promoter. The use of *35S*::*SEU-BD* alone had no effect on the reporter

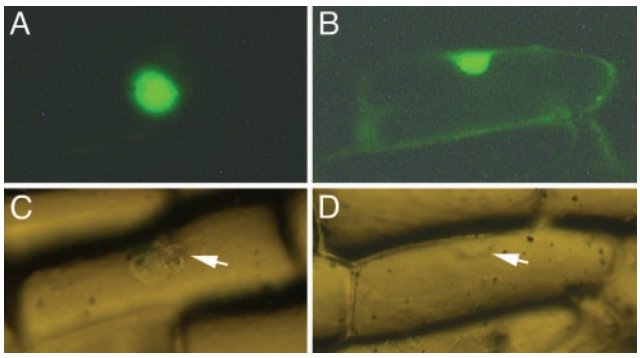

Fig. 5. Nuclear localization of $_{\text{deltaLUFS}}$LUG-GFP in plant cells. (*A*) A LUG with the LUFS domain removed was fused to GFP to yield $_{\text{deltaLUFS}}$LUG-GFP. This chimeric protein is localized to the nucleus of onion epidermal cells. (*B*) GFP alone is localized to both the nucleus and the cytoplasm. (*C*) Bright-field image of the onion epidermal cell shown in *A*. (*D*) Bright-field image of the onion epidermal cell shown in *B*. Arrow indicates the nucleus.

gene expression (Fig. 4*B*, lane 2). However, cotransfecting *35S*::*LUG* and *35S*::*SEU-BD* together significantly reduced reporter gene expression (Fig. 4*B*, lane 8). This repression appears dependent on direct physical interactions between SEU-BD and LUG as cotransfection with *35S*::*SEU-BD* and *35S*::*Q+WD* failed to reduce reporter gene expression (Fig. 4*B*, lane 3) due to the inability of Q+WD to interact with SEU-BD. In addition, whereas *35S*::*SEU-BD* combined with *35S*::*LUFS* failed to repress reporter expression (Fig. 4*B*, lane 4), cotransfection of *35S*::*SEU-BD* and *35S*::*LUFS+Q* provided a significant repressor activity (Fig. 4*B*, lane 6), supporting the earlier observations made in yeast (Fig. 3*A*) that the LUFS+Q derivative exhibited a strong repressor activity but LUFS or Q-rich domain alone did not. These results not only indicated a role of SEU as an adaptor protein for LUG but also supported a direct physical interaction between LUG and SEU occurring in plant cells.

Toward understanding the mechanism underlying the repressor activity of *LUG*, we tested whether TSA, an inhibitor of histone deacetylases (HDACs), could reduce or abolish the repressor activity of *LUG*. The addition of 20 μM TSA completely eliminated the repressor activity of *LUG* or *LUFS+Q* (Fig. 4*B*, lanes 7 and 9). However, 20 μM TSA did not affect reporter gene expression with *35S*::*SEU-BD* plus *35S*::*LUFS* (Fig. 4*B*, lane 5). Together, these results suggest that HDACs are required for the repressor activity of *LUG*.

LUG Localizes to the Nucleus Independent of SEU. In addition to being an adaptor protein, another possible role of SEU could be that SEU is required for the cytoplasm to nucleus translocation of LUG, as Ldb1/Chip has been shown to be required for the cytoplasm to nucleus translocation of Ssdp (13). SEU protein has a typical bipartite nuclear localization signal (NLS) within the conserved dimerization domain (5), whereas LUG has an atypical NLS located in the Q-rich domain (6). The *35S*::*LUG-GFP* was previously shown to localize in the nucleus of onion epidermal cells (6), but this result could depend on the activity of SEU-like proteins present in the onion cells. To investigate whether the SEU–LUG interaction is necessary for the nuclear localization of LUG, a truncated LUG (LUFS domain deleted) was fused to GFP. This $_{\text{deltaLUFS}}$LUG-GFP protein was able to localize to the nucleus of onion epidermal cells (Fig. 5), suggesting that the LUG–SEU interaction was not required for the nuclear localization of LUG. Taken together, our results indicate that SEU functions primarily as an adaptor protein for LUG in this plant corepressor complex.

Discussion

LUG Acts as a Transcriptional Repressor. Transcription corepressor proteins play important roles in the correct expression of many genes. Aberrant corepressor function has been shown to cause severe developmental and physiological defects in many eukaryotic systems. In this investigation, we demonstrated that *LUG* can directly repress transcription *in vivo*, demonstrating its structural and functional similarities to the GroTLE transcriptional corepressor proteins. The identification of two functional repression domains within LUG further extends the specific structural and functional conservation with the yeast corepressor Tup1; two nonoverlapping repression domains of Tup1 were mapped to the N- and C-terminal regions of Tup1 (20).

Because *LUG* is capable of repressing transcription in both homologous and heterologous systems, *LUG* may regulate transcription through a conserved eukaryotic repression mechanism such as stabilizing chromatin and/or negatively regulating RNA polymerase II function. Tup1, the yeast homologue of *LUG*, represses transcription through specific recruitment of Rpd3, a member of the class I HDACs (24). Our observation that the repressor activity of *LUG* in *Arabidopsis* protoplasts was abolished by the HDAC inhibitor TSA (Fig. 4*B*) suggests that *LUG*, like Tup1, may repress transcription by recruiting HDACs to alter the accessibility of target promoters by means of nucleosome stabilization. The *Arabidopsis* genome contains 16 putative HDACs, of which 10 belong to the RPD3/HDA1 superfamily (25). Antisense or T-DNA knockout of *HD1* in the RPD3/HAD1 superfamily caused a variety of developmental abnormalities, including a loss of sepals and petals in flowers and the development of serrated leaves (26). These phenotypes are similar to those of *lug* mutants. Future experiments will aim at identifying the specific HDACs whose activities are required for the LUG/SEU corepressor function.

LUG Physically Interacts with SEU. Our *in vivo* and *in vitro* data showing that *SEU* interacts directly with the LUFS domain of *LUG* suggest that the genetic synergy observed between *lug* and *seu* is due to the disruption of the functional components of this plant corepressor complex. Specifically, the LUFS domain of LUG is both necessary and sufficient for interaction with SEU. The LUFS domain was originally identified and named by us, based on the high degree of conservation of this domain between LUG and LUH in *Arabidopsis*, Flo8 in yeast, and Ssdp in humans (6). The first half of the LUFS domain exhibits significant sequence similarity to the lissencephaly type-1-like homology (LisH) domain found in numerous animal, fungi, and plant proteins. It has been suggested that the LisH domain may be involved in mediating protein dimerization (27, 28). The second half of the LUFS domain comprises of a core amino acid sequence motif P-X-GFX-XX-WW-X-VFWD (13). Hence, the LUFS domain represents an evolutionarily conserved protein interface for transcriptional regulation in plants as well as in animals.

Whereas the LUFS domain of LUG interacts specifically with SEU, our *in vivo* and *in vitro* data demonstrate that the entire SEU protein is required for LUG–SEU interaction. SEU contains a Ldb1/Chip conserved domain (LCCD), a stretch of 49 highly conserved residues located C-terminal to the dimerization domain, which is essential for the interaction of Chip or Ldb1 with the LUFS domain of Ssdp proteins from *Drosophila* to mice (13). Although this LCCD domain is highly conserved in SEU, our study showed that the interaction with LUFS appears dependent on multiple regions of SEU, which may include, but are not limited to, the LCCD domain.

SEU May Function as an Adaptor Protein for LUG. Despite the sequence similarities between *SEU* and *Ldb1/Chip* in the dimer-

ization and the LCCD domains, both the N- and C-terminal regions flanking these domains are unique to SEU. In addition, SEU does not encode the LIM-interaction-domain that is essential for Ldb1/Chip's interaction with LIM-homeodomain. SEU therefore defines a class of plant-specific transcription factors and is a member of a small gene family in *Arabidopsis* (5), the molecular function of which is largely unknown. The results presented here indicate that SEU does not have any inherent function in repressing transcription and, on the contrary, may have an intrinsic activation potential as revealed in the yeast two-hybrid assays. Additionally, despite the presence of endogenously expressed *LUG*, *35S*::*SEU-BD* alone failed repress reporter gene expression in the *Arabidopsis* protoplasts (likely due to a low level of endogenous *LUG* expression in *Arabidopsis* leaves; ref. 6). Only simultaneous transfection of *SEU-BD* and *LUG* gave measurable repressor activities. This situation is analogous to the Ssn6-Tup1 interaction in yeast, where Ssn6 has an absolute requirement for Tup1 to repress transcription, and Ssn6 was shown to activate transcription in the absence of Tup1 (29). Hence, SEU appears to be a functional homologue of Ssn6, despite the similarity between SEU and Ssn6 proteins being limited to the presence of Q-rich domains.

Whether the apparent functional homology between SEU and Ssn6 extends to SEU interacting with specific DNA-binding transcription factors remains to be determined. Preliminary yeast two-hybrid assays failed to detect an interaction between LUG and any of the tested floral regulatory genes (V.V.S., A.S., and Z.L., unpublished data) including *APETALA2*, *AINTEGUMENTA*, *APETALA1*, *SEPALLATA3*, *BELLRINGER*, and *LEAFY* (30, 31). However, a similar yeast two-hybrid assay used to detect interactions between *SEU*, and the above floral regulatory factors revealed an interaction between *SEU* and *APETALA1*, and between *SEU* and *SEPALLATA3* (V.V.S., A.S., and Z.L., unpublished data). Nevertheless, the biological significance of such interactions remains to be established. Recently, a role of *SEU* in auxin response was revealed (32), and both genetic and direct physical interactions between *SEU* and the *ARF3/ETTIN* were detected, suggesting that *SEU* could interact with the DNA-binding transcription factor *ARF3/ETTIN* to regulation the expression of auxin-responsive genes.

An important function of the Ssdp and Ldb1/Chip interaction is the nuclear import of Ssdp, which lacks a nuclear localization signal. In *Drosophila Chip* mutants, Ssdp remains in the cytoplasm (13). We have eliminated this possible function for the SEU–LUG interaction. Hence, while not having an intrinsic transcription repressor activity, the main function of SEU appears to be an adaptor protein recruiting LUG to target promoters. Our findings demonstrate for the first time, to our knowledge, that plant corepressors function through a mechanism similar to yeast and animal corepressors and underpin future researches exploring the molecular mechanisms used by corepressors in plants.

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