Arabidopsis LEAFY COTYLEDON2 induces maturation traits and auxin activity: Implications for somatic embryogenesis

Sandra L. Stone*, Siobhan A. Braybrook*†, Stephanie L. Paula*‡, Linda W. Kwong*, Jonathan Meuser*§, Julie Pelletier*, Tzung-Fu Hsieh¶, Robert L. Fischer¶, Robert B. Goldberg‖**, and John J. Harada*†**

*Section of Plant Biology, College of Biological Sciences, and †Graduate Program in Plant Biology, University of California, Davis, CA 95616; ¶Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720; and ‖Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA 90024

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LEAFY COTYLEDON2 (LEC2) is a central regulator of embryogenesis sufficient to induce somatic cells to form embryos when expressed ectopically. Here, we analyze the cellular processes induced by LEC2, a B3 domain transcription factor, that may underlie its ability to promote somatic embryogenesis. We show auxin-responsive genes are induced after LEC2 activation in seedlings. Genes encoding enzymes involved in auxin biosynthesis, *YUC2* and *YUC4*, are activated within 1 h after induction of LEC2 activity, and *YUC4* appears to be a direct transcriptional target of LEC2. We also show ectopic *LEC2* expression induces accumulation of seed storage protein and oil bodies in vegetative and reproductive organs, events that normally occur during the maturation phase of embryogenesis. Furthermore, LEC2 activates seed protein genes before an increase in RNAs encoding LEC1 or FUS3 is observed. Thus, LEC2 causes rapid changes in auxin responses and induces cellular differentiation characteristic of the maturation phase. The relevance of these changes to the ability of LEC2 to promote somatic embryogenesis is discussed.

seed development | totipotency

An outstanding characteristic of plants is their totipotency. That is, a variety of cells can be induced to regenerate the adult organism, and several cell types do so by undergoing embryogenesis. For example, the fertilized egg cell undergoes zygotic embryogenesis, a number of differentiated cells of the sporophyte can be induced to undergo somatic embryogenesis, microspores can be diverted from their development into pollen grains to enter microspore embryogenesis, and a number of ovule cell types undergo asexual embryo development in a suite of processes known collectively as apomixis (1–4). Evidence suggests the morphological pathways of development used by these different types of embryos are similar to those of zygotic embryos (5, 6). However, the processes that induce a cell to change its fate and enter an embryonic program of development are not known, nor is it known whether a common pathway is used to initiate embryonic development in all of these diverse cell types.

To gain insight into these questions, we focused on *Arabidopsis* LEAFY COTYLEDON2 (LEC2). LEC2 regulates many distinct aspects of embryogenesis (7, 8). For example, during the early morphogenesis phase of embryogenesis in which the basic body plan of the embryo is established, loss-of-function mutations in *LEC2* affect the maintenance of embryonic cell fate and specification of cotyledon identity. Later in embryogenesis, *lec2* mutants have cotyledon tips that do not accumulate storage reserves nor acquire desiccation tolerance, indicating defects in the initiation and/or maintenance of the maturation phase. Consistent with the pleiotropic effects of the *lec2* mutation, *LEC2* encodes a transcription factor with a B3 domain, a DNA binding region found thus far only in plant proteins (8–10). Two transcription factors most closely related to LEC2, ABA INSENSITIVE3 (ABI3) and another LEC protein, FUSCA3 (FUS3), also play critical roles in embryogenesis (11, 12).

To gain insight into the mechanisms by which cells change their fate and become embryogenic, we analyzed postembryonic *35S:LEC2* plants. We showed previously *LEC2* is expressed primarily during seed development and ectopic postembryonic expression of *LEC2* induces vegetative cells to undergo somatic embryogenesis (8). We hypothesized LEC2 establishes a cellular environment that promotes embryo development. Thus, cellular processes induced postembryonically by ectopic LEC2 activity may provide insight into the mechanisms by which LEC2 induces somatic embryogenesis. We show LEC2 is sufficient to confer maturation traits to postembryonic cells, consistent with its role as a direct regulator of maturation-specific genes (9). Furthermore, we show LEC2 activity causes alterations in auxin responses. Implications of these results for the mechanisms by which LEC2 induces somatic embryogenesis are discussed.

Results

We showed previously ectopic *LEC2* expression induces somatic embryo formation in seedlings, generates masses of shoots and roots, and causes reduced apical dominance (8). More detailed analyses revealed even greater pleiotropy of LEC2 action. As shown in supporting information (SI) Fig. 5, *35S:LEC2* plants displayed reduced organ elongation, direct organogenesis of shoots and roots, and expansion of plant organs. Although aspects of the morphological changes caused by ectopic *LEC2* expression resembled those resulting from changes in hormone signaling, we could not collectively attribute the Lec2$^+$ overexpression phenotype to enhancement or repression of responses to a single hormone. Because LEC2 induces somatic embryogenesis and somatic embryogenesis is induced with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) (13–15), we asked whether LEC2 affects auxin responses.

Ectopic LEC2 Activity Alters Auxin-Responsive Gene Expression. To monitor changes in auxin activity, we used the auxin-responsive

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‡Present address: School of Pharmacy, University of California, San Francisco, CA 94143.

§Present address: Environmental Science and Engineering Division, Colorado School of Mines, Golden, CO 80401.

**To whom correspondence may be addressed. E-mail: jjharada@ucdavis.edu or bobg@ucla.edu.

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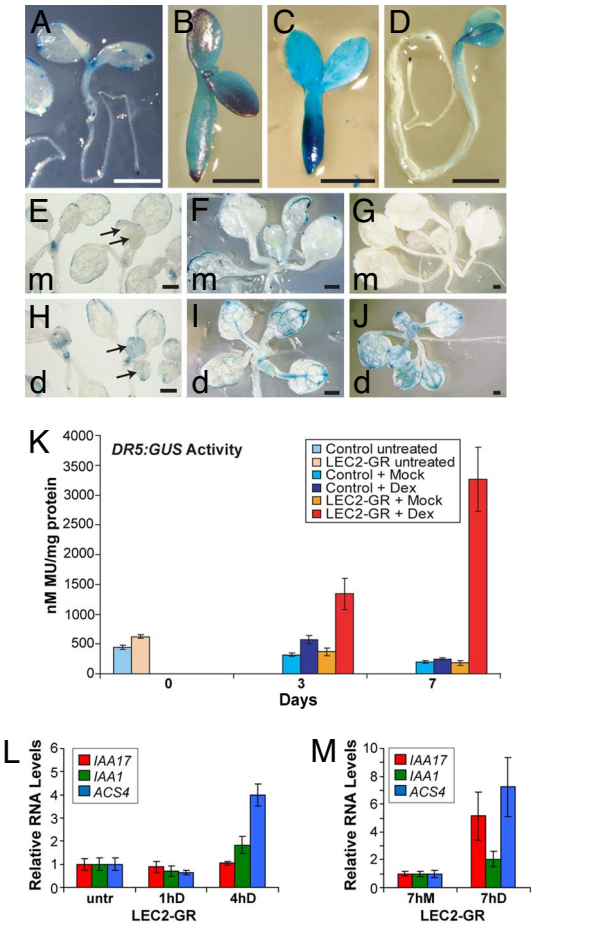

Fig. 1. Auxin responses are enhanced by ectopic LEC2 activity. (*A–J*) *DR5*:*GUS* plants were stained for GUS activity. Seedling without (*A*) and with (*B*) *35S*:*LEC2* grown in the light on hormone-free media. Seedlings lacking *35S*:*LEC2* were grown in the dark to reduce endogenous auxin levels in the presence (*C*) or absence (*D*) of 5 μM 2,4-D. Temporal changes in LEC2-induced auxin responses are shown. (*E–J*) Eight-day *35S*:*LEC2-GR* seedlings were grown in the presence (*H–J*) or absence (*E–G*) of Dex for 7 h (*E* and *H*), 3 days (*F* and *I*), and 7 days (*G* and *J*). Arrows indicate young vegetative leaves stained differently for GUS activity. (*K*) *DR5*:*GUS* activities in 8-day *35S*:*LEC2-GR* seedlings treated with Dex for 3 or 7 days. Control seedlings contained the *DR5*:*GUS* but not *35S*:*LEC2-GR*. (*L*) Relative levels of three auxin-responsive RNAs in 8-day *35S*:*LEC2-GR* seedlings untreated or treated with Dex for 1 and 4 h as determined by qRT-PCR. *IAA1* and *ACS4* RNA levels differed in Dex-treated and untreated samples at the 0.05 significance level. (*M*) Relative RNA levels in shoots of nontransgenic and *35S*:*LEC2-GR* seedlings either mock or Dex-treated for 7 h. RNAs from Dex-treated and mock-treated samples differed at the 0.05 significance level. (Scale bars: *B* and *C*, 0.5 mm; *A* and *D–J*, 1 mm.)

reporter gene, *DR5*:*GUS*. Activity of the *DR5* promoter parallels changes in auxin levels (16). We analyzed light-grown, *DR5*:*GUS* seedlings with and without the *35S*:*LEC2* transgene. As shown in Fig. 1*A*, seedlings without the *35S*:*LEC2* gene had expanded cotyledons and extended hypocotyls and roots, and β-glucuronidase (GUS) activity was detected in hydathodes, shoot apices, and root tips. By contrast, *35S*:*LEC2 DR5*:*GUS* seedlings were fleshy and had unexpanded cotyledons and unextended hypocotyls and roots, and the entire embryo stained intensely (Fig. 1*B*). Although there were differences in GUS staining intensities, the morphological phenotype of *35S*:*LEC2* seedlings was mimicked by dark-grown seedlings grown in the presence (Fig. 1*C*) but not the absence (Fig. 1*D*) of 2,4-D. Thus, LEC2 induces the activity of an auxin-responsive promoter, and 2,4-D phenocopies morphological effects of ectopic *LEC2* expression. We conclude LEC2 causes changes in auxin activity in seedlings.

We measured the timing of LEC2-induced changes in *DR5* promoter activity to determine whether LEC2 affects auxin responses directly. An inducible *LEC2* gene encoding LEC2 fused with the steroid-binding domain of glucocorticoid receptor (*35S*:*LEC2-GR*) was constructed and transferred into WT and *lec2-1* mutant plants. Treatment of *35S*:*LEC2-GR* plants with the mammalian steroid hormone analog dexamethasone (Dex) induced characteristics of *35S*:*LEC2* plants (SI Fig. 6) and suppressed the *lec2* mutation. Thus, Dex treatment induced high levels of LEC2 activity. We monitored the time course of *DR5*:*GUS* expression after Dex treatment of *35S*:*LEC2-GR* plants. As shown in Fig. 1*K*, induction of LEC2 activity for 3 days resulted in a 4- to 5-fold increase in GUS activity over mock treatment. By 7 days of Dex induction, GUS activity had increased 12- to 18-fold over mock treatment. GUS staining was more apparent in cotyledons and leaves, primarily in the vascular tissue and hydathodes (Fig. 1 *I* and *J*) than in mock-treated seedlings (Fig. 1 *F* and *G*). We visually observed an increase in GUS staining after 7 h of Dex treatment in the young primary leaves and the margins and vasculature of the cotyledons (Fig. 1*H*) relative to mock-treated plants (Fig. 1*E*), although no significant increase in GUS activity was detected fluorometrically before 3 days.

To validate results with the *DR5* promoter and determine whether auxin responses occurred even earlier than 7 h, we measured RNAs for three auxin-responsive genes in 8-day *35S*:*LEC2-GR* seedlings treated with Dex for 1, 4, and 7 h. Fig. 1*M* shows *ACS4*, *IAA17*, and *IAA1* RNAs were induced 7-, 5-, and 2-fold, respectively, by 7 h of Dex induction in *35S*:*LEC2-GR* shoots. These results confirm LEC2 induction of auxin activity at 7 h. Moreover, *ACS4* and *IAA1* but not *IAA17* RNAs were at significantly higher levels ($P < 0.05$) in seedlings treated for 4 h with Dex than in uninduced seedlings (Fig. 1*L*), suggesting that LEC2 induction of some auxin-responsive genes is a rapid response.

LEC2 Activates Genes Involved in Auxin Biosynthesis. We searched our previously published DNA microarray results that identified RNAs up-regulated in seedings 1 and 4 h after induction of LEC2 activity to determine whether genes involved in auxin biosynthesis are activated by LEC2 (9). RNAs encoding YUCCA2 (YUC2) and YUC4, two flavin monooxygenase enzymes involved in auxin biosynthesis (17, 18), were identified. As shown in Fig. 2*A*, *YUC2* and *YUC4* RNA levels were induced ~8- and 30-fold, respectively, within 1 h of Dex treatment. Thus, LEC2 rapidly activates genes encoding auxin biosynthetic enzymes.

We used ChIP experiments to determine whether *YUC2* and *YUC4* are direct transcriptional targets of LEC2. LEC2 activity was induced for 8.5 h in seedlings with a *35S*:*FLAG-LEC2-GR* chimeric gene encoding LEC2-GR fused with a FLAG peptide. LEC2-bound chromatin was measured by comparing the ratio of DNA fragments immunoprecipitated with FLAG antibody versus a nonspecific control GST antibody. To validate the method, we showed DNA fragments for oleosin and *2S3* storage protein genes were enriched in the ChIP experiments, whereas fragments for two control DNAs, actin and *Ta3*, were not (Fig. 2*B*). Given that oleosin and *2S3* genes are activated rapidly by LEC2 and are bound by LEC2 both *in vitro* (9) and *in planta*, these results suggest strongly that they are LEC2 target genes. *YUC4* DNA fragments were bound by LEC2 (Fig. 2*B*), although *YUC2* DNA was not enriched significantly (data not shown). Thus, LEC2 appears to directly activate a gene involved in auxin biosynthesis, *YUC4*, although it is not clear whether *YUC2* is a direct target or activated indirectly by LEC2.

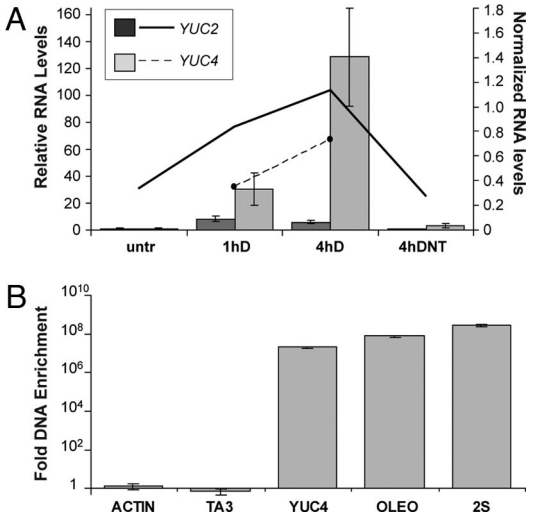

Fig. 2. LEC2 activates *YUC2* and *YUC4* genes. (*A*) Relative levels of *YUC2* and *YUC4* RNA in *35S:LEC2-GR* seedlings either not treated with Dex or Dex-treated for 1 or 4 h and in nontransgenic seedlings Dex-treated for 4 h. Bars show relative RNA levels determined by qRT-PCR, and lines show normalized RNA levels determined previously (9). (*B*) ChIP of *35S:FLAG-LEC2-GR* seedlings treated with Dex for 8.5 h. Fold DNA enrichment represents the ratios of DNA amplicons for the indicated genes in samples immunoprecipitated with anti-FLAG and anti-GST (control) antibodies. Oleosin and *2S3* storage protein genes are known LEC2 targets, and actin and *Ta3* are negative controls not induced by LEC2.

LEC2 Induces Maturation Processes in Vegetative and Reproductive Organs. We investigated the basis for organ enlargement in *35S:LEC2* plants. *35S:LEC2* pistils enlarged to a greater extent than WT because unfertilized ovules developed into fleshy seed-like structures similar in size to WT seeds (Fig. 3*A*). As shown in Fig. 3 *B–D* and SI Fig. 7, enlargement of *35S:LEC2* ovules relative to WT resulted primarily from cell expansion although an increase in cell number was also observed. Cell expansion was associated with the accumulation of storage macromolecules normally present in embryos. For example, oil bodies were prevalent in the integuments of *35S:LEC2* ovules (Fig. 3*C*), but they were not detected in nontransgenic ovules and seed coats (Fig. 3 *D* and *E*). The oil content of *35S:LEC2* ovules, measured by fatty acid methyl ester analysis, was 23.6% on a dry weight basis, a value more similar to the 33.5% oil content of mature, nontransgenic seeds in which embryos and endosperms are packed with oil bodies (Fig. 3*E*) than that of rosette leaves, a nonstorage organ (2.3%). Moreover, the fatty acid composition of *35S:LEC2* ovules, summarized in Fig. 3*I*, was very similar to that of mature seeds and differed significantly from that of rosette leaves (19). Consistent with these observations, RNA encoding the seed oil body protein oleosin was detected in *35S:LEC2* pistils that contained enlarged ovules but not in nontransgenic pistils (Fig. 4). Similarly, SI Fig. 8 shows expanded root cells in enlarged *35S:LEC2* roots contained oil bodies not present in WT roots.

35S:LEC2 enlarged ovules and roots also accumulated protein bodies (Fig. 3 *G* and *H* and SI Fig. 8) that were not observed in the seed coat (Fig. 3*F*) or roots of nontransgenic plants (SI Fig. 8). SDS/PAGE analysis indicated seed-specific 2S and 12S storage proteins accumulated in the protein bodies of *35S:LEC2* ovules (data not shown). In support of this observation, we detected *CRA1* RNA encoding 12S storage protein in unpollinated *35S:LEC2* pistils (Fig. 4). Taken together, we conclude LEC2 is sufficient to induce processes that normally occur during the maturation phase of embryogenesis.

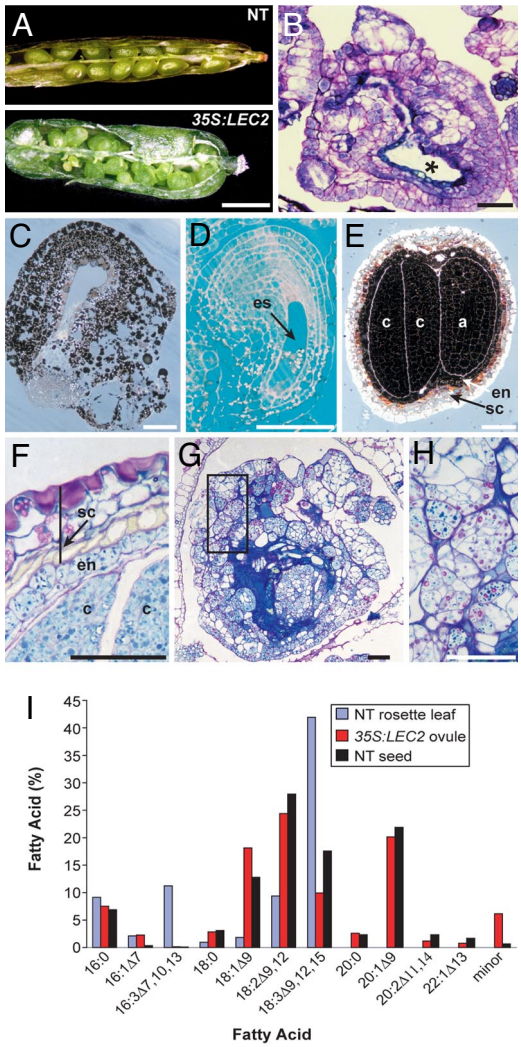

Fig. 3. Macromolecular reserves characteristic of seeds accumulate in unpollinated, enlarged *35S:LEC2* ovules. (*A*) Maturing seeds in the upper third of a nontransgenic (NT) silique and enlarged *35S:LEC2* ovules from an unpollinated pistil. (*B*) *35S:LEC2* ovules 20 days after flower emasculation (DAE). Embryo sac contents (*) have degenerated and integument cells have divided and enlarged. (*C–E*) Lipid staining (black) of an enlarged, unfertilized *35S:LEC2* ovule (*C*) with oil reserves, a nontransgenic ovule at 1 DAE (*D*) lacking oil reserves, and a nontransgenic mature green-stage seed (*E*) with oil reserves in embryonic cotyledons (c), axis (a) and endosperm (en) but not seed coat (sc). (*F–H*) Protein bodies (blue) and insoluble carbohydrate-stained (pink) tissues. (*F*) Protein bodies in nontransgenic seeds are detected only in the endosperm (en) and embryo (c), whereas starch grains (pink) are visible in the seed coat (sc). (*G*) Enlarged *35S:LEC2* ovule. (*H*) Higher-magnification view of region from *G* showing the presence of protein bodies and starch grains in ovule integument cells. (*I*) Fatty acid methyl ester analysis showing profiles from enlarged *35S:LEC2* ovules, nontransgenic rosette leaves, and seeds. For clarity, only fatty acids that constitute >0.1% of the total are shown. Minor components are either not fatty acids or fatty acids of unknown identity. (Scale bars: *A*, 1 mm; *B–H*, 50 μm.)

LEC2 Activation of Seed Protein Genes Precedes Induction of LEC1 and FUS3. The other LEC transcription factors, LEC1 and FUS3, have been implicated to play roles in controlling seed maturation (reviewed in ref. 20). Because *LEC1* and *FUS3* are expressed predominately during seed development (12, 21), we determined whether LEC2 induced these genes in postembryonic organs. As shown in Fig. 4, *LEC1* and *FUS3* RNAs were detected in organs that displayed maturation traits, such as pistils containing enlarged ovules and roots from *35S:LEC2* plants. These RNAs

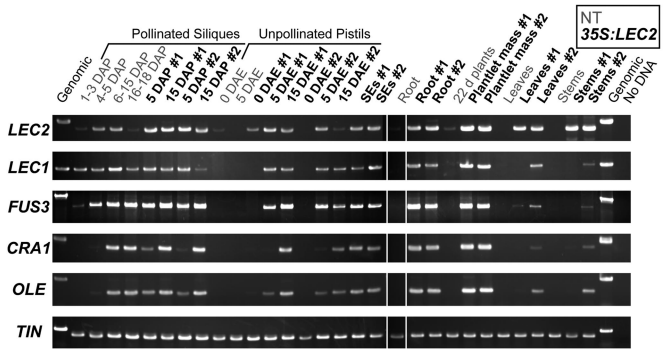

Fig. 4. Seed RNAs accumulate in postembryonic organs of *35S:LEC2* plants. RT-PCR detection of RNAs for genes expressed predominantly during embryogenesis and a control RNA encoding a ribosomal protein (TIN) in organs from two independent *35S:LEC2* transformants and nontransgenic (NT) tissues. Forty PCR cycles were used to detect *LEC2*, *LEC1*, and *FUS3* RNAs, 35 cycles for *CRA1* 12S storage protein and *TIN* RNA, and 30 cycles for oleosin (*OLE*) RNA. DAE, day after emasculation; DAP, day after pollination; SE, somatic embryos.

were detected inconsistently in transgenic leaves and stems that did not display obvious maturation characteristics, suggesting they may be at very low levels.

We showed previously many seed protein genes expressed primarily during the maturation phase are regulated directly by LEC2 (9). Using *35S:LEC2-GR* plants, we asked whether LEC1 and FUS3 are also direct transcriptional targets of LEC2. RNAs from 8-day *LEC2-GR* and nontransgenic seedlings either treated with Dex or mock-treated for 4 h were isolated. We confirmed RNAs encoding the seed proteins oleosin and the 2S and 12S storage proteins accumulated within 4 h of Dex induction (SI Fig. 9 and ref. 9). However, neither *LEC1* nor *FUS3* RNAs were detected. Thus, LEC2 does not appear to require LEC1 or FUS3 to activate seed protein genes. However, because LEC1 and FUS3 are expressed later after Dex induction, they may act in concert with LEC2 to induce other aspects of the maturation phase.

Discussion

LEC2 Affects Auxin Activity. We present compelling evidence that LEC2 activates auxin activity in seedlings. First, auxin-responsive genes became active after the induction of LEC2. Activity of the auxin-responsive promoter, *DR5*, became detectable 7 h after induction of *LEC2* activity (Fig. 1). Because the *DR5* promoter has been reported to be activated by brassinosteroids (22), we also showed auxin-responsive genes, including *IAA1*, *IAA17*, and *ACS4*, were activated within 7 h of LEC2 induction. Although *ACS4* and *IAA1* genes were activated within 4 h of LEC2 induction, neither of those genes validated as direct transcriptional targets of LEC2 based on ChIP assays (data not shown).

Second, induction of *LEC2* expression caused rapid increases in *YUC2* and *YUC4* gene expression (Fig. 2). YUC flavin monooxygenases are key enzymes in Trp-dependent auxin biosynthesis (17, 18). Overexpression of *YUC* genes causes overproduction of auxin, and loss-of-function multigenic mutations in *YUC* genes cause defects that could be rescued with a bacterial auxin biosynthetic gene. A simple interpretation of this result is that LEC2 induces auxin biosynthesis. In preliminary experiments, we detected a small, but statistically insignificant, increase in indoleacetic acid (IAA) levels in seedlings 7 h after induction of LEC2 activity and a significant increase in IAA-Asp conjugates (S.L.S., A. R. Kermode, S. R. Abrams, and J.J.H., unpublished results). Thus, we cannot exclude the possibility that LEC2 may also affect sensitivity to auxin. We previously showed LEC2 is a DNA-binding transcription factor (9). The rapid inducibility of *YUC4* and its binding with LEC2 in planta provides strong evidence that *YUC4* is a LEC2 transcriptional target (Fig. 2). Thus, a simple model is that LEC2 activates genes including *YUC4* encoding auxin biosynthesis enzymes and that the increase in auxin levels up-regulates auxin-responsive genes. However, it remains possible that LEC2 directly activates at least some auxin-responsive genes.

Although LEC2 or FUS3, another B3 domain transcription factor, have not been previously reported to influence auxin activity, they affect gibberellic acid (GA) and abscisic acid (ABA) responses. Relative to WT, *lec2* and *fus3* mutant embryos possess elevated levels of GA and RNA encoding a GA biosynthetic enzyme (23), and *FUS3* induction in vegetative plants causes a decrease in RNA encoding a GA biosynthetic enzyme (24). Consistent with the diametrical relationship between GA and ABA, experiments with loss-of-function and gain-of-function alleles of *FUS3* indicate that ABA levels correlate positively with FUS3 activity (24). Pleiotropic defects caused by ectopic *LEC2* expression are also likely to result from changes in other hormone signaling pathways. For example, we found induction of LEC2 activity causes down-regulation of the cytokinin-responsive promoter, *ARR5* (S.L.S. and J.J.H., unpublished results).

LEC2 Induces Cellular Maturation Processes. LEC2 confers maturation characteristics to vegetative and reproductive organs. We previously showed ectopic *LEC2* expression activates genes encoding seed proteins in seedlings (8). Here, we show LEC2 is sufficient to cause the transformation of unfertilized ovule integuments and roots from *35S:LEC2* plants into storage tissues that accumulate lipid and protein reserves characteristic of developing seeds (Fig. 3 and SI Fig. 8). This result is consistent with a report indicating that ectopic *LEC2* expression induces storage lipid accumulation (10). Genes encoding seed proteins normally expressed primarily during seed development were active in vegetative tissues of *35S:LEC2* plants (Fig. 4). We cannot exclude the possibility that LEC2 may induce cells to undergo somatic embryogenesis and induction of maturation processes results from progression of somatic embryos into the maturation phase. However, a more likely explanation is that LEC2 is sufficient to induce maturation directly, because maturation traits are induced in plant organs without visible formation of somatic embryos (Fig. 3). Furthermore, we and others reported LEC2 directly activates genes involved in maturation processes (9, 10). These results provide strong support for the conclusion LEC2 is a direct regulator of the maturation phase.

In addition to *LEC2*, ectopic expression of the other *LEC* genes, *LEC1* and *FUS3*, results in the accumulation of seed proteins in seedlings and cotyledon-like rosette leaves (21, 24). We showed ectopic *LEC2* expression caused accumulation of *LEC1* and *FUS3* RNAs (Fig. 4). Similarly, ectopic expression of *LEC1* causes activation of *LEC2* and *FUS3* genes (25). Thus, the *LEC* genes may act in concert to regulate maturation programs. Although *LEC1* and *FUS3* RNAs are detected in organs from *35S:LEC2* plants displaying maturation characteristics, their accumulation occurred after seed protein RNAs accumulated (Fig. 4 and SI Fig. 9). Similarly, RNA encoding ABI3, another regulator of the maturation phase, is detected in vegetative organs of *35S:LEC2* plants but does not accumulate within 4 h of LEC2 induction (S.L.S. and J.J.H., unpublished results). The *FUS3* promoter is active in immature seeds and seedling roots within 24 h of exogenous auxin application (24), opening the possibility that LEC2 induction of *FUS3* RNA levels is mediated through auxin activity. We conclude LEC2 is sufficient to initiate seed protein gene expression and likely acts in concert with ABI3, FUS3, and LEC1 to control the initiation and maintenance of the maturation phase.

Roles of LEC2 in Somatic Embryogenesis. Somatic embryogenesis is typically induced by treatment of tissues with auxin. The synthetic auxin 2,4-D is required to induce somatic embryogenesis in many plants, including *Arabidopsis* zygotic embryos, although different explants exhibit differential competence to respond to 2,4-D (4, 15). Given that LEC2 induces the expression of genes involved in auxin biosynthesis and auxin-responsive genes, we propose LEC2 induces somatic embryogenesis in vegetative tissues, in part, through its enhancement of auxin activity. The ability of LEC2 to induce *YUC4* is significant because this gene is expressed in specific groups of cells in developing embryos, and multigenic mutations in *YUC4* and three other *YUC* genes causes defects in embryo development (26). Consistent with a role for LEC2 in the induction of auxin-related processes, *LEC2* promoter activity colocalizes with auxin maxima in the cells displaying embryogenic patterns of cell division in tissues undergoing somatic embryogenesis (27).

Given the pleiotropic effects of ectopic LEC2 expression, we considered whether the induction of maturation processes by LEC2 is relevant to the initiation of somatic embryogenesis. Because the maturation phase occurs after fertilization and the morphogenesis phase, an interaction between maturation processes and the initiation of embryogenesis is not expected. However, several lines of evidence suggest a relationship between establishment of a maturation environment and the competence of tissues to undergo somatic embryogenesis. First, *Arabidopsis* zygotic embryos at the maturation phase are the most efficient explants for auxin-induced somatic embryogenesis (13, 15), suggesting maturation processes enhance embryogenic competence. Second, dehydration and osmotic stresses increase the competence of *Arabidopsis* seedlings to undergo embryogenesis in response to 2,4-D (28). Because tissues accumulating storage reserves also experience a decrease in water content (29, 30), a dehydration stress induced by maturation processes could account for the increase in embryogenic competence. Third, we recently showed that some RNAs induced by LEC2 and normally present at high levels during the maturation phase are also detected in seeds containing zygotes (9). In particular, one of these RNAs, *AGL15*, is known to confer embryogenic competence to cells. Ectopic expression of *AGL15* induces secondary embryogenic calli that express maturation genes and enhances competence of shoot apical meristems to undergo somatic embryogenesis in response to auxin treatment (31). Thus, LEC2 may work through AGL15 to enhance competence to undergo somatic embryogenesis. Wang *et al.* (32) showed AGL15 induces an enzyme that inactivates GA and reduction in GA levels increases the frequency of somatic embryo formation. Thus, reduction of GA levels during the maturation phase (33) may relate to the competence of tissues to undergo somatic embryogenesis.

Taken together, we hypothesize LEC2 induces somatic embryogenesis through two mechanisms. First, LEC2 appears to increase the competence of cells to become embryogenic by activating genes that are also expressed during the maturation phase. Second, LEC2 activity promotes auxin activity in competent cells to induce somatic embryogenesis, although it is not clear whether this increase in activity reflects auxin levels or sensitivity. Because LEC2 is expressed at the earliest stage of embryogenesis tested (8), it remains to be determined whether LEC2 fulfills a similar function during zygotic embryogenesis.

Materials and Methods

Plant Material. WT, *lec2–1*, and *35S:LEC2* (8) *Arabidopsis thaliana* (L.) Heynh plants were Ws-0 ecotype, and *DR5:GUS* (16) plants were Col-0 ecotype. *35S:LEC2-GR* and *35S:FLAG-LEC2-GR* plants were constructed as described in *SI Text*. Plants were grown as described (34). Details of methods used to induce LEC2-GR activity are given in *SI Text*.

Analyses of Storage Product Accumulation. Staining of histological sections for lipids, proteins, and starch was done as described (35, 36). Fatty acid composition and quantification were done by the Lipid Analysis Unit of the Scottish Crop Research Institute (Invergowrie, Dundee, Scotland). Additional details are provided in *SI Text*.

Gene Expression Analyses. Total RNA isolation and RT-PCR were done as described (8) except that Concert Plant RNA Reagent (Invitrogen) was used for some RNA isolations and reverse transcription products were labeled with radioactivity and PCRs were performed with equivalent amounts of cDNA. Quantitative RT-PCR (qRT-PCR) was done essentially as described (37). Primers used are in SI Table 1. At least two biological replicates were used for all experiments. GUS activity was assayed as described (38) as detailed in *SI Text*.

ChIP. ChIP experiments were done as described (39, 40) with the modifications given in *SI Text*.

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