Multifunctionality of the LEC1 transcription factor during plant development

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LEC1 acts as a key regulator of embryogenesis in *Arabidopsis thaliana*, but is involved in a wide range of functions, all the way from embryo morphogenesis to seed maturation. New data show that LEC1, partially in conjunction with abscisic acid, affects auxin synthesis, and both brassinosteroid and light signaling. The phenotype of *LEC1* overexpressors confirms LEC1's known participation in the regulation of somatic embryogenesis, but also indicates additional roles in embryonic and extra-embryonic cell elongation. Here we present an integrated model of LEC1 function and suggest potential directions to be taken in future research in this important area of plant science.

The regulation of transcription by transcription factors (TFs) relies on their specific interaction with sequence motifs in their target gene promoter, a process which triggers the downstream transcriptional machinery. Many TFs have been shown to be involved in multiple developmental events and/or responses to environmental cues, through the variability of their spatio-temporal expression and their interaction with other TFs. The LEAFY COTYLEDON1 (LEC1) TF is a CCAAT-box-binding factor, whose expression in the *Arabidopsis thaliana* embryo and endosperm peaks at an early stage of seed development and then declines up to the green pre-mature seed stage.[1,2] The multifaceted phenotype of LEC1 loss-of-function mutants demonstrates this TF's involvement in the morphogenesis of the embryo, the specification of the suspensor and cotyledons,

the synthesis and accumulation of seed storage compounds, and in various desiccation- and dormancy-related processes. Both in vitro and in vivo experiments have established that LEC1 binds with NF-Y subunits,[3,4] with TFs of other classes[4,5] and with a non-TF protein.[6] An additional level of understanding of LEC1 function has been provided by the demonstration that LEC1 has functional relations outside the known seed maturation-related LEC1-AFL/B3 network[7,8] and even outside the process of seed development itself.[9] Here, we have attempted to integrate its known with its more novel proposed functions to assemble a model for LEC1 function. The picture is one where a crosstalk network involves both transcriptional and hormonal control (**Fig. 1**).

LEC1 and Embryogenesis

LEC1 transcript abundance peaks at the globular-to-heart stage transition and then declines steadily toward early seed maturation[10] (**Fig. 1A, B**). During embryo morphogenesis, the most prominent function of LEC1 is the specification of cotyledon identity. The *lec1* mutant produces leafy cotyledons,[11] whereas the ectopic expression of *LEC1* results in the formation of cotyledonary leaves[9] (**Fig. 1C**). During seed maturation, *LEC1* expression is required for the synthesis of storage compounds, including components of lipid synthesis and storage (such as oleosins).[9,12] As part of the NF-YC2 complex, the *LEC1* product activates the promoter of certain storage protein encoding genes.[4,13] These seed
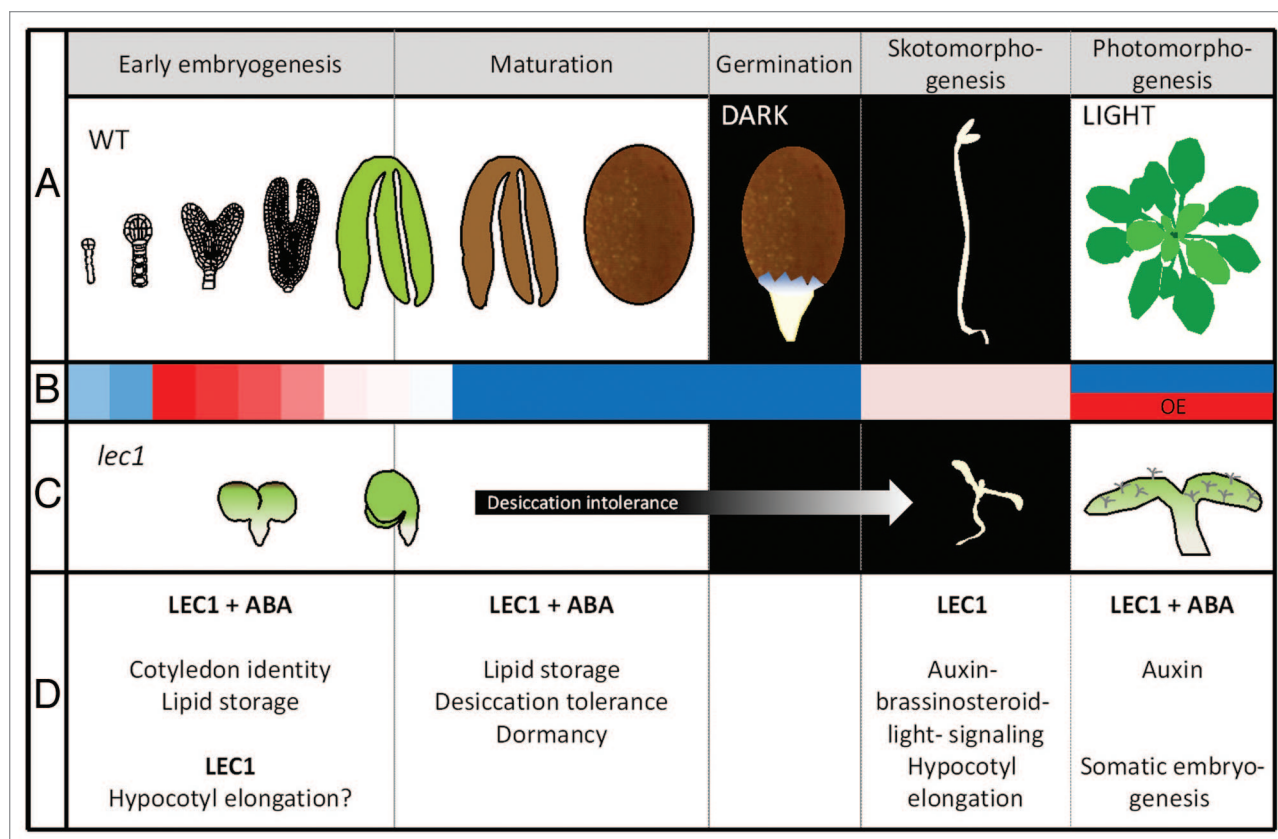
Figure 1. Model of LEC1 functions during the development of *Arabidopsis thaliana*. (A) Schematic representations of Arabidopsis seed developmental stages starting from the globular stage (early embryogenesis) until seed maturation resulting in a fully desiccated seed. Upon germination in the dark the elongating radicle protrudes the seed coat. In order to reach the light, during etiolation hypocotyl elongation and apical hook formation drive the seedling through the soil and protect the apical meristem from mechanical damage, respectively. (B) *LEC1* expression in the respective developmental stages. During embryogenesis *LEC1* mRNA can be detected during early stages declining until maturation. Further on weak expression can be found in the etiolated seedling. *LEC1* expression is incompatible with vegetative growth and the artificial system of ectopic expression of *LEC1* outside of its natural expression domains is indicated by OE (overexpression). (C) Schematic representations of *lec1* mutant phenotypes. *Lec1* embryos are characterized by round cotyledons that do not curl. The embryonic axis is shorter due to missing hypocotyl elongation. The *lec1* embryo is desiccation-intolerant and has to be rescued before maturation in order to continue growth. In the dark *lec1* seedlings fail to elongate and light-grown *lec1* seedlings develop trichomes on their cotyledons. (D) Functional relations of LEC1.

maturation-related functions are dependent on the presence of abscisic acid (ABA) (**Fig. 1D**). Since the relevant ABA responsive elements (ABREs) are over-represented in LEC1-regulated target promoters,[9] it is conceivable that LEC1 probably confers ABA inducibility to its target genes by the recruitment of additional ABRE binding transcription factors such as bZIP. An example of this type of interaction is represented by the substitutability of bZIP67 for ABA, as a cofactor of LEC1 in the regulation of a specific storage protein encoding gene.[4] A comprehensive genome-wide analysis of the LEC1 interactome would therefore be useful to allow the identification of further co-operating factors.

LEC1 and Etiolation

The expression of *LEC1* has also been detected in etiolated seedlings,[6,14] an observation fully consistent with the de-etiolated, short hypocotyl phenotype of dark-grown *lec1* seedlings[15] (**Fig. 1B, C**) and the mimicking of etiolation (including hypocotyl elongation and hook formation) shown by *LEC1* overexpressing seedlings.[9] Given that LEC1 activity regulates genes implicated with auxin, brassinosteroid (BR) and light, the hypothesis has been mooted that both hypocotyl elongation and hook formation may be integrated by LEC1.[9] Hypocotyl elongation as well as certain other processes appear to be enhanced via a positive synergy between

BR and auxin.[16-18] Light signaling factors which form part of the photomorphogenic response have also been implicated with hypocotyl elongation in dark-grown plants, a process which is additionally influenced by the fatty acid reserve of the endosperm.[19] These observations are suggestive of a connection between the seed maturation- and seedling elongation-related functions of LEC1. While the global role of ABA in the elongation process is only poorly understood, it has been established that ABA inhibits both BR signaling[20] and embryonic stem elongation.[21] This elongation-inhibiting role of ABA led us to assume that LEC1 during etiolation may act independent of ABA (**Fig. 1D**). Discriminating between the

ABA-dependent and ABA-independent functions of LEC1 is a priority topic for further experimentation.

LEC1 and Vegetative Plant Development

The formation by the vegetative seedling of cotyledonary leaves and *pickle*-like root tips able to accumulate storage compounds can be induced by the ectopic expression of *LEC1* and the provision of exogenous ABA.[9] The supply of auxin induces the initiation of somatic embryogenesis from competent tissues, a phenomenon consistent with the activation of transcription of the auxin synthesis gene *YUCCA10*[22] when *LEC1* is expressed in the presence of ABA.[9] The seed-specific expression of *YUCCA10* may affect the elongation of the embryo downstream of the activity of LEC1, and opposing phenotypes due to varying auxin concentrations have been described. Enhanced auxin signaling is known to inhibit the elongation of the embryonic axis during germination[21] and auxin synthesis suppresses hypocotyl growth post germination.[23] However, some elongation-promoting effects of auxin have also been described.[24]

The LEC1 induced and auxin-mediated embryonic differentiation of structures associated with the shoot (cotyledonary leaves) and root (*pickle* roots) raises the question of their cellular origin. Currently it is unclear whether these structures are either derived from relict undifferentiated stem cells or whether a process of trans-differentiation (possibly via a de-differentiated state) is required. The analysis of stem cell marker expression in a system of controlled activation of LEC1 expression[9] will help to answer this question.

Conclusions

Recent insights into the function of LEC1 have revealed the complexity of downstream regulatory interactions involving this TF, acting at various stages of plant development. LEC1 appears to be an integrator of a number of regulatory events, including the action of heterologous TFs as well as both light and hormone signaling. At present it is only possible to investigate a minor part of the full combinatorial

potential associated with this TF, which only emphasizes the need for developing more holistic analytical approaches to better understand plant differentiation and development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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