Insights into the role of LEC1 in Arabidopsis embryogenesis

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The LEAFY COTYLEDON gene LEC1 is a central regulator of early and late embryogenesis [1]. Using a glucocorticoid-inducible system (GR) we were able to show that permanent induction of LEC1:GR under the control of the 35S Cauliflower Mosaic Virus promoter leads to morphological alterations in all parts of the seedling. Over time these become reminiscent of tissues of embryonic origin (cotyledons) rather than vegetative tissues.

Leaves and hypocotyls of 14 days old, permanently induced LEC1::GR seedlings reveal features of storage organs. Their cells are largely filled with storage components and also in ultrastructure they are similar to the pre-germination condition. At the same time the primary root tip transformed into a swollen and greenish structure, identical to what has been described for the pkl mutant [2] and is therefore referred to as pickle root (Fig 1). Eventually the induction of LEC1 triggers callus formation and subsequent somatic embryogenesis (SE) at the root-hypocotyl junction indicating a local activation of auxin.

LEC1 induced embryonic structures only when present in the first 48 h after seed imbibition. In the presence of ABA, however, these LEC1 effects could also be observed during vegetative development. In this case LEC1 induced embryonic structures never emerged from already differentiated tissues or organs but always developed de novo, although meristems themselves seemed unaffected (Fig. 2).

To better understand these LEC1 effects, homozygous LEC1::GR was crossed with a line expressing the auxin-induceble artificial promoter DR5 linked to GFP as a reporter gene (DR5::GFP). DR5::GFP x LEC1::GR seeds were germinated on induction medium and analyzed by confocal laser scanning microscopy at different time intervals.

In non-induced seedlings, GFP fluorescence showed maxima in the shoot apical meristem (SAM) and in the root apical meristem (RAM). LEC1-induced seedlings showed an additional GFP maximum at the junction between hypocotyl and root, the sites of future SE (Fig. 3). Interestingly, the GFP maximum in the root tip remained also after the onset of pickle root formation (Fig. 4). These observations indicate that LEC1 induction does not affect meristem identity (quiescence center, organizing center). We therefore hypothesize that LEC1 induces embryonic differentiation in dividing stem cells (RAM/SAM). LEC1 may exert this effect by changes in the auxin sensitivity or auxin balance in these cells. Support for this theory comes from the observation that the LEC1 induced callus formation and SE at the root-hypocotyl junction was preceded by local auxin accumulations.

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2. J. Ogas et al., Science **277** (1997) p91.

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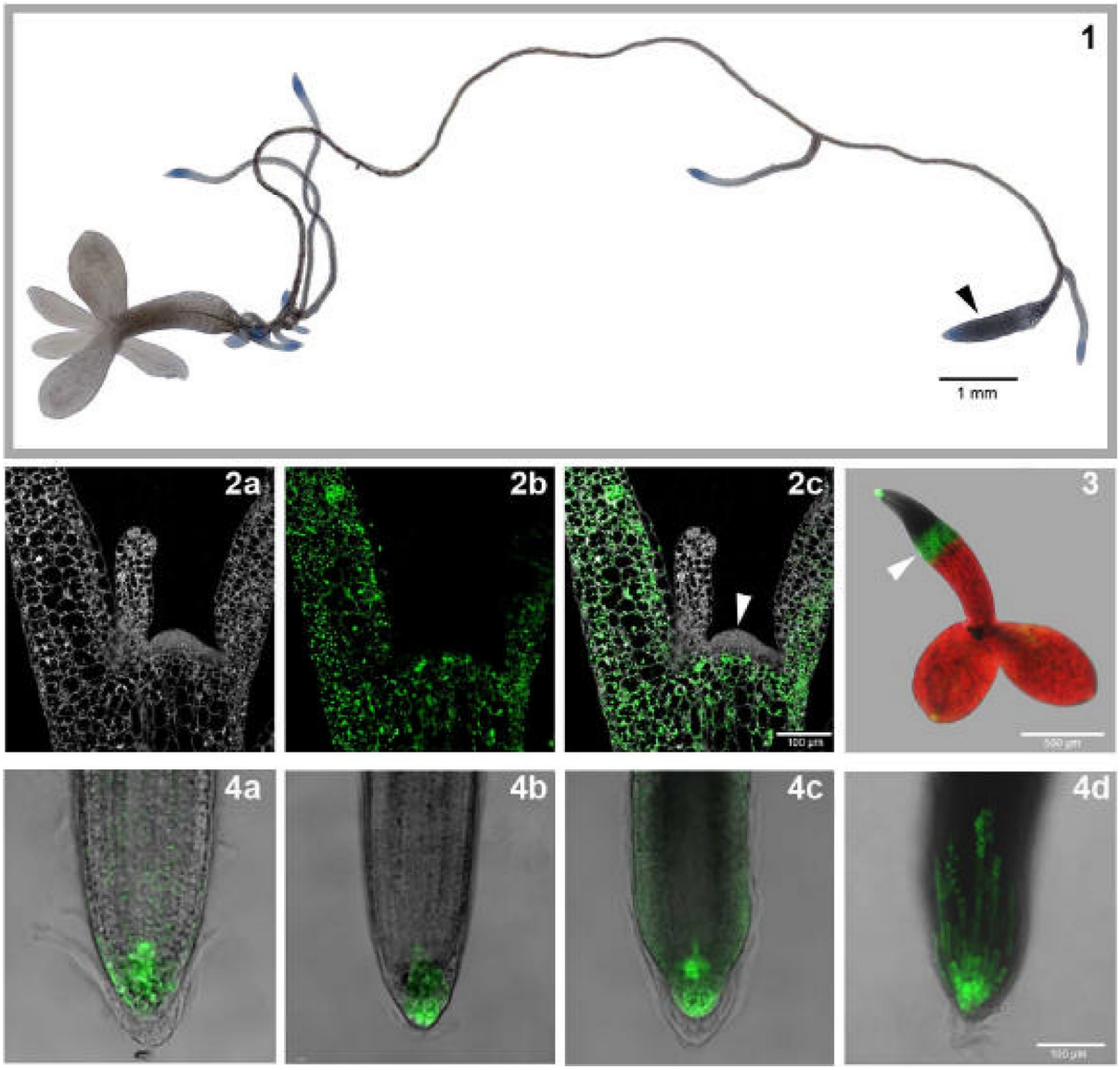

Figure 1. Arabidopsis thaliana seedling after 14 days of permanent LEC1 induction showing pickle root (arrowhead).

Figure 2a-c. Fluorescence microscopy images of semi thin sections through the SAM of LEC1::GR plants. Seedlings were grown 14 days without induction followed by an additional 10 days on induction medium with ABA. a) = UV autofluorescence showing all tissues; b) = labelling for the storage protein cruciferin; c) = a) + b) combined show the absence of cruciferin from the meristem and youngest leave (Arrowhead)

Figure 3. GFP expression in LEC1::GR x DR5::GFP seedling after 10 days of permanent LEC1 induction showing accumulations in the RAM and at the root-hypocotyl junction (arrowhead).

Figure 4a-d. GFP expression in the root tip of LEC1::GR x DR5::GFP seedlings. a) = uninduced; b) = 4 days of induction; c) = 7 days of induction; d) = 17 days of induction. Note that in bright field the root becomes progressively darker due to the transformation into a pickle root.

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