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Endosperm rupture as a model for lateral root emergence in Arabidopsis?

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Endosperm rupture and lateral root emergence are biological processes involving organ emergence out of cellular layers. We review here the main similarities and differences between these two developmental processes and discuss the possibility that both could be associated with auxin-dependent regulation of cell wall remodelling gene expression. We speculate that endosperm rupture may serve as a model system for the study of certain aspects of lateral root emergence.

Higher plants have developed unique structures within the seed to efficiently protect and disseminate their fragile progeny. Indeed, for most angiosperm species, the seed consists of an osmotolerant and metabolically inert embryo surrounded by a layer of living endosperm cells surrounded by an outer layer of maternal dead tissue (testa). Hence, upon imbibition, the embryo must emerge from this protective structure in order to develop into a seedling. This is achieved by rupturing the testa and the micropylar endosperm (i.e., germination per se), thus allowing embryonic axis protrusion and growth. For most plant species, including *Arabidopsis thaliana*, testa and endosperm rupture occur successively, and endosperm rupture is the final barrier for radicle protrusion and germination.[1]

In many plant species, endosperm rupture does not only result from the mechanical pressure exerted by the elongating embryonic axis, but is also accompanied by endosperm weakening prior to radicle protrusion. Indeed, tensile measurements have revealed a biphasic (tomato, coffee, pepper) or monophasic (lepidium) decrease in mechanical strength of the micropylar endosperm.[1-4] Studies on lettuce also revealed structural changes in the micropylar endosperm prior to radicle protrusion.[5] The mechanisms underlying the decrease in mechanical resistance of the endosperm layer are largely unknown. However, several studies in tomato, tobacco or Arabidopsis reported the specific localization of cell wall remodelling enzymes in the micropylar area of the endosperm.[6-8] It therefore suggested that during endosperm rupture neighbouring endosperm cells detach from each other by loosening the molecular attachment linking juxtaposed cell walls.

This situation is remarkably similar to that occurring during lateral root emergence. Indeed, the lateral root primordium initiates its growth within the inner tissues of the primary root (e.g., pericycle in many species including Arabidopsis) and forces its way through several cell layers prior to its protrusion out of the root, as reviewed recently.[9,10] In Arabidopsis, it has been proposed that lateral root emergence involves the specific expression of cell wall remodelling genes in the cells of the different layers of the root faced by the lateral root primordium.[8,11-13] This would trigger the weakening of the cell wall attachments linking two adjacent cells to allow their separation as the lateral root primordium forces its way through.

It was recently shown that shoot-derived auxin in the lateral root primordium is required for lateral root emergence.[13] The Aux/IAA negative regulator of auxin signalling *SHY2/IAA3* is specifically expressed in the endodermal cells facing the lateral root primordium. Genetic

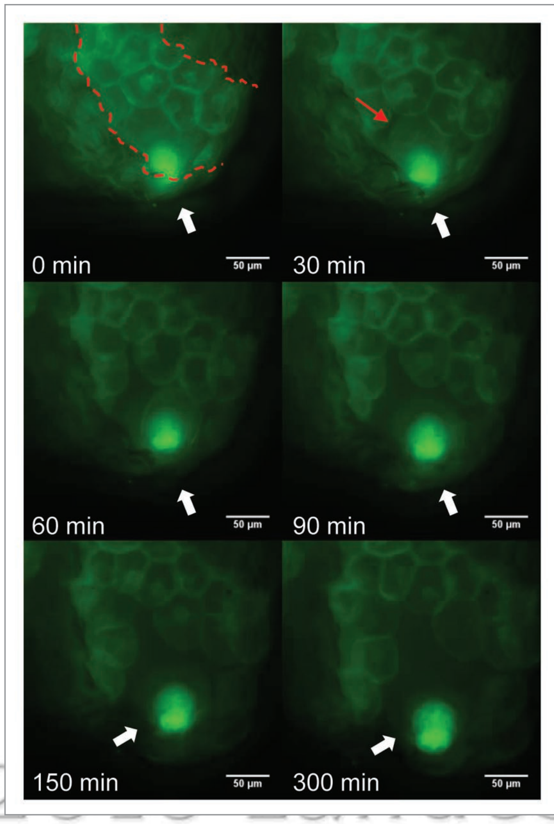

Figure 1. Endosperm cell detachment allows embryo radicle tip to protrude. Snapshot pictures of a representative movie showing the radicle protruding out of the endosperm layer (see Supplemental movies online). For this purpose, we used an Arabidopsis line expressing the Green Fluorescent Protein (GFP) under the control of the *DR5* auxin-responsive synthetic promoter in a wild-type background. White arrows indicate strong fluorescence present in the embryonic radicle tip. Fluorescence of GFP is also visible in all visible endosperm cells. Testa covers part of the endosperm (testa borders are indicated by red dashed lines in the first picture). Note how the endosperm cells close to the radicle tip start to detach from each other (endosperm rupture is indicated by a red arrow in the second picture).

analyses using both loss-of-function and gain-of-function *shy2* mutants confirmed that this auxin signalling component is regulating lateral root emergence, and that *SHY2* is regulating auxin-dependent cell wall remodelling gene expression.

Auxin promotes the cellular expression of LAX3, an auxin influx transporter, in the cortical and epidermal adjacent cells juxtaposing the root primordium and the endodermal cells expressing *SHY2*.[13] Therefore, it was proposed that auxin originating from the lateral root primordium propagates to overlaying cells, in part through LAX3, where it triggers cell wall remodelling gene expression to allow cell separation as the lateral root elongates.

Although auxin is known to regulate almost all developmental processes during plant life cycle, its role during seed germination has remained elusive. We recently showed that auxin is essential to promote the abscisic acid (ABA)-dependent repression of embryonic axis growth upon endosperm rupture.[14] This supports an important role for auxin to control plant development in response to environmental cues shortly upon seed germination.

To illustrate this notion further, we used a line expressing the *DR5:GFP* auxin response reporter to follow the dynamics of endosperm rupture by fluorescence microscopy (**Fig. 1 and Suppl. movies online**). The *DR5* synthetic reporter was active in the endosperm prior to and after endosperm rupture, indicating that active auxin signalling pathways are present in the endosperm during germination. Endosperm cells facing the radicle tip progressively detached from each other, thus allowing the endosperm layer to open as the embryonic axis elongated (**Fig. 1 and Suppl. movies online**). Those cells, which were still alive, became round-shaped and loosely connected to the rest of the endosperm, consistent with a previous description of spherical endosperm cells close to the root tip.[15]

It is therefore tempting to speculate that concomitant endosperm rupture and radicle protrusion may involve processes similar to those taking place during lateral root emergence, notably those involving auxin-dependent regulation of cell wall remodelling gene expression in juxtaposed cells. This hypothesis is mainly based on three observations: (1) the structural similarity between the two developmental processes, i.e., the occurrence of an elongating axis forcing its passage through covering tissues whose living cells detach from each other; (2) the occurrence of auxin responses observed in the tip of the emerging organ and in the covering cells; (3) the local specific expression of cell wall remodelling genes in cells overlaying the protruding organ.

Interestingly, osmotic stress represses lateral root emergence through an ABA-dependent mechanism.[16] It is also well documented that ABA plays a key role to repress endosperm rupture.[1,17] Major ABA signalling components, such as *ABI3*, were reported to be expressed in both the embryo and the lateral root primordium.[18,19] Thus, it is plausible that endosperm rupture and lateral root emergence not only share common mechanisms but are also similarly controlled by the environment.

Here we wished to bring the attention to some of the striking similarities encountered in the processes of endosperm rupture and lateral root emergence. We speculate that the study of endosperm rupture may serve as a convenient model for the study of lateral root emergence. Indeed, in Arabidopsis the radicle must protrude out of a single cell layer, the endosperm, while the lateral root primordium faces three root layers. Moreover, under conditions of seed stratification, endosperm rupture occurs in a nearly synchronized manner in seed populations within time frames that are significantly shorter compared to those encountered during lateral root emergence. Finally, some genetic screens may be more convenient to perform in germinating seeds rather than in growing roots.

Clearly, endosperm rupture and lateral root emergence are processes with specific features. The main difference resides in the need to maintain the cellular integrity of the main root upon emergence of the lateral root. In contrast, the endosperm tissue is a priori not essential for plant survival as soon as the young seedling emerges from the seed coat. Therefore we could expect that cell detachment during lateral root emergence is tightly regulated and may involve specific developmental checkpoints. This could involve specific cell wall components and signalling pathways. Indeed, cell wall remodelling genes and auxin signalling components are part of multigene families and different members of these families might act during germination and lateral root emergence. However, this does not preclude that common signalling and mechanistic themes may emerge by studying the apparently simpler process of endosperm rupture.

Methods

Histological GUS staining and image capture were performed as previously described.[14] Fluorescence microscopy was performed using an AF6000 LX widefield fluorescence microscope (Leica), in the NCCR Frontiers in Genetics bioimaging service (http://www.frontiers-in-genetics.org/). Pictures for each time-point were obtained by combination of 8 Z-stacks images using the public domain image analysis program ImageJ version 1.43l (http://rsb.info.nih.gov/ij/) and its plugin Extended Depth-of-Field—Model based Method,[20] developed by the Biomedical Imaging Group (EPFL Lausanne—http://bigwww.epfl.ch/).

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Note

Supplementary materials can be found at: www.landesbioscience.com/supplement/BelinPSB5-5-Sup.pdf

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