Lateral root emergence: a difficult birth

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Abstract

Lateral root initiation takes place deep within the parental root, requiring new primordia to break through the overlying tissues before they emerge into the soil. Lateral root emergence has been well described at the cellular level but, until recently, the molecular mechanisms involved were unclear. Scientists in the 19th and 20th centuries hypothesized that the cell wall of the overlying tissues was modified by enzymes released by cells within the primordium. Recent studies in the model plant Arabidopsis thaliana revealed the existence of a complex transcellular signalling network regulated by auxin that controls cell wall remodelling in cells overlying lateral root primordia. In the first part of this review, early observations on the cell biology of lateral root formation and emergence are summarized, and in the following two sections recent observations in Arabidopsis that led to the identification of the molecular mechanism regulating lateral root emergence are described.

Key words: Arabidopsis, auxin, cell separation, cell wall, lateral root emergence, rice.

Introduction

Roots provide plants with water, minerals, and anchorage (Lloret, 2002). In order to adapt to a very heterogeneous environment, root architecture is extremely plastic, responding to nutrient concentrations in the soil (López-Bucio et al., 2003), soil matrix heterogeneity (Hinsinger et al., 2005; Hodge, 2006) and biotic interactions (Osmont et al., 2007).

The major part of the root system originates from the primary root through production of lateral roots (LR). LR formation can be divided into three major steps defined as pre-initiation, initiation, and post-initiation (Péret et al., 2009). LR pre-initiation takes place very close to the root tip, in the basal meristem (De Smet et al., 2007). The first divisions leading to the formation of the lateral root primordium (LRP) occur higher up in the mature part of the root (Dubrovsky et al., 2000, 2001). Subsequent controlled cell divisions give rise to a typical dome-shaped primordium. As these processes happen deep within the parental root, it is critical that overlying tissues undergo cell separation to allow primordium emergence. In Arabidopsis thaliana, three different tissues have to be crossed: the endodermis, the cortex, and the epidermis, each of them composed of one cell layer, resulting in a dramatic impact on primary root structure (Fig. 1).

The reasons for such a peculiar developmental pattern remain unclear: protection of the meristematic tissues from external damage, adaptation to a very heterogeneous environment to integrate numerous external and internal signals or simply connection to the primary root vasculature? The fact is that the mechanisms by which LR emergence is controlled has puzzled scientists for centuries.

Several recent reviews have described LR formation as a whole in Arabidopsis (Nibau et al., 2008; Péret et al., 2009) and other species (Hochholdinger and Zimmermann, 2008), have focused on the effect of nutrients on root architecture (López-Bucio et al., 2003; Osmont et al., 2007; Zhang et al., 2007) or on the role of the hormone auxin (Fukaki et al., 2007). This review focuses on the LR emergence process. The first section will summarize the information gathered by cell biologists over the past decades on lateral root emergence in different plant species. The second section will focus on more recent work demonstrating the importance of shoot-derived auxin during emergence in Arabidopsis. Finally, the last section will summarize recent findings.
is defined as lateral root emergence (Peşet et al., 1993). Since the pericycle is located deep within the root, new primordia have to break through the two outermost tissues, its progression towards the rhizosphere is blocked (Armstrong and Armstrong, 2005). Amazingly, the lateral root starts growing upward within the primary root mesodermis (Armstrong and Armstrong, 2005). This example clearly illustrates the importance of cell wall weakening during emergence. 

In summary, diverse strategies have evolved to promote LR emergence in higher plants. The very existence of these regulatory mechanisms shows that an important selective pressure must exist on the ability for a LR to emerge.

Hormonal regulation of LR emergence

Considerable progress has recently been made describing the role of auxin during lateral root formation in Arabidopsis (Fukaki et al., 2007; Peşet et al., 2009). More precisely, the importance of auxin accumulation for LR initiation has been described by the molecular mechanism of auxin action during LR emergence.
studied extensively since it is the first visible event leading to the production of a new root (Benkova et al., 2003). However, auxin is also required for both pre- and post-initiation events including emergence (Péret et al., 2009).

In the young seedling up to 4 d after germination, auxin is mainly synthesized in the shoot and transported to the root via two pathways of equal importance: phloem-mediated transport and polar transport. By 8 d, phloem transport becomes the dominant pathway (Ljung et al., 2005). This aerial source of auxin is required to promote LR emergence (Bhalerao et al., 2002). Indeed, removal of the leaves and cotyledons blocks LR emergence (Swarup et al., 2008). Roots are also a source of auxin, however LR acquire the ability to synthesize their own auxin only after emergence (Ljung et al., 2005). It is therefore unlikely that this source of auxin plays a role in emergence.

LR initiation is regulated by auxin originating from the root tip (Casimiro et al., 2001; De Smet et al., 2007) whereas emergence depends exclusively on auxin derived from the shoot (Bhalerao et al., 2002). Despite these distinct sources, a competition appears to exist between the two processes as shown by statistical analysis and in silico modelling backed up by experimental evidences (Lucas et al., 2008). This suggests that LR initiation and emergence are distinct but yet interconnected developmental processes competing for the same source of auxin. Auxin modifies cell fate and activates cell division during LR initiation whereas during emergence, auxin is linked with cell separation (Boerjan et al., 1995; Laskowski et al., 2006).

Expression profiling of auxin-treated roots revealed increased expression of pectate lyase, pectin methyl esterase, expansin, and β-xylanosidase genes (Laskowski et al., 2006). However, the induction by auxin of a set of genes involved in cell separation brings the question whether the primordium is producing its own enzymes or if they are produced by the outer tissues. Recently, several genes potentially

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**Fig. 2.** Root sections of emerging lateral root primodia in the two model species *Arabidopsis thaliana* and *Oryza sativa* (rice). The *Arabidopsis* root has a simple structure composed of the stele surrounded by three one-cell layers (A). The emerging LR primordium reprogrammes the outer cells to promote cell separation (B). Rice has a complex root system made of different root types (radicle, embryonic crown root, crown root, large lateral root, and small lateral root). The rice crown root is composed of the stele that is surrounded by five layers accounting for 5–15 cells in total (C). Emergence of a rice LR primordium involves more cell layers and is probably highly regulated (D). *Arabidopsis* root diameter is 100 μm (A, B) and rice root diameter is 300 μm (C, D).
involved in cell wall remodelling were reported to be expressed in front of the emerging LRP including polygalacturonase (González-Carranza et al., 2007; Ogawa et al., 2009), expansin, subtilisin-like protease (Neuteboom et al., 1999), xyloglucan endotransglucosylase/hydrolase (Swarup et al., 2008), and pectate lyase (Swarup et al., 2008). Moreover, the activity of xyloglucan endotransglucosylase/hydrolase has been demonstrated in the epidermal cells at sites of lateral root emergence using fluorescent assays (Vissenberg et al., 2000). Polygalacturonases and pectate lyases can cleave pectin polymers within the cell wall (Marín-Rodríguez et al., 2002; Kim et al., 2006) and xyloglucan endotransglycosylase/hydrolase can trigger cell wall loosening (Van Sandt et al., 2007). Associated with LR primordium growth, these enzymes can facilitate cell separation during emergence.

However, the massive production of cell wall remodelling enzymes at sites of LR emergence does not affect LR primordium integrity. This might be explained by a difference in cell wall composition between the LR primordium and the parental root. The pectin in the developing LR is largely methylated while that in the overlying cells of the parent root has become demethylated under the action of pectin methyl esterases (Laskowski et al., 2006). Together with targeted gene expression, this would restrict cell wall remodelling activity to cells in the outer tissues.

The mechanism responsible for the localized pattern of expression of cell wall remodelling genes was recently deciphered in Arabidopsis.

**Genetic dissection of LR emergence**

Polar transport of auxin is achieved by the co-ordinated action of influx transporters encoded by aux1-LAX genes and efflux transporters encoded by PIN and PGP genes (Kramer and Bennett, 2006; Vanneste and Friml, 2009). AUX1 promotes LR initiation as shown by the reduced number of primordia in the aux1 mutant (Marchant et al., 2002; De Smet et al., 2007; Laskowski et al., 2008). Recently, the molecular characterization of the Aux1 homologue LAX3 also revealed a role for auxin influx during LR emergence (Swarup et al., 2008). The lax3 mutant shows a reduction in LR number, however LR initiation is not reduced. Instead LR primordia are blocked during their development resulting in an increased number of stage I primordia and a decrease in emerged laterals (Swarup et al., 2008). LAX3 has a striking expression pattern in the cortical and epidermal cells overlying the LR but not in the primordium itself. The fact that the cortical cells facing LR can induce genes that are normally not expressed in this tissue suggests that the primordium is reprogramming these cells. Interestingly, auxin originating from the primordium is proposed to act as a local inductive signal that reprograms these overlying cells (Swarup et al., 2008). Consistent with this model, the pin2 mutant (which exhibits a higher auxin maximum at the tip of the LR primordium) is associated with faster emergence and increased auxin response (as shown by the DR5 reporter) in the outer tissues (Swarup et al., 2008) (Fig. 3A). In agreement with this hypothesis, LAX3 expression is induced by auxin in the cortex and epidermis. The auxin induction of LAX3 triggers a positive feedback loop (Fig. 3B) that is likely to increase auxin accumulation in the overlying cells whilst reducing diffusion to adjacent outer tissues.

The expression of cell wall remodelling genes such as a polygalacturonase and a xyloglucan endotransglycosylase/hydrolase at sites of LR emergence was shown to be LAX3 dependent (Swarup et al., 2008). Therefore, the auxin influx transporter LAX3 promotes LR emergence by increasing the auxin content of cortical and epidermal cells directly facing the primordium. The high auxin concentration in these cells induces a set of cell wall remodelling enzymes and facilitates local remodelling of the cell wall. The LAX3 positive feedback loop ensures that only cells in contact with the primordium undergo cell separation.

LAX3 expression is dependent on the auxin response factors ARF7 and ARF19 and the Aux/IAA repressor SOLITARY ROOT SLR/IAA14 (Swarup et al., 2008). These transcription factors positively and negatively regulate LAX3, respectively (Fig. 3B). Interestingly, all these genes have previously been demonstrated to play a role in
LR initiation. However, because of the strong mutant phenotype associated with these genes (i.e. no LR), no effect on LR emergence had been previously described (Fukaki et al., 2002, 2005; Okushima et al., 2005). This shows the limit of conventional genetic approaches and stresses the need for new conditional approaches to overcome this issue, such as chemical biology (Stockwell, 2000; Spring, 2005; Lehar et al., 2008).

**LAX3** expression is strictly limited to the cortex and epidermis but LR primordia must first pass through the endodermal layer. In this layer, no auxin influx transporter seems to be involved, probably due to its close proximity to the primordium allowing efficient diffusion. Instead, auxin directly induces a distinct set of cell wall remodelling genes under the control of the Aux/IAA repressor SHY2/IAA3. Consistent with this model, IAA3 is expressed in the endodermis but not in the cortex and epidermis (Swarup et al., 2008). The reason for a different regulatory network in this layer is currently unclear. One reason could be the presence of the Casparian strip that necessitates different remodelling enzymes due to the presence of suberin and lignin.

**Perspectives**

Major advances have been made in the description of LR emergence in the model plant *Arabidopsis*. Systems biology approaches should further improve our understanding of this process. For instance, modelling cereals multilayered roots would allow for the prediction of the existence of similar mechanisms and/or the need for new components. It therefore appears important to build multiscale models to apprehend the behaviour of such complex systems.

Such models should include mechanical data in order to take into account the pressure at the interface between the LR primordium and the overlying tissues. It is not clear whether the outer tissues actually bear a high level of resistance or if the cell wall remodelling is efficient enough to allow a smooth emergence of the LRP. It is also possible that the mechanical pressure produced by the primordium growth acts as a signal to reinforce the hormonal signal.

The effect of nutrient availability on root architecture is dramatic (López-Bucio et al., 2003). However, the regulatory mechanisms involved have not been studied in great detail. Recently, it was shown that phosphate deprivation increases LR initiation and emergence by modulating auxin sensitivity. In low phosphate conditions, the expression of TIR1 is increased which increases auxin sensitivity of the pericycle cells without the need for more auxin transport/production (Pérez-Torres et al., 2008). Such studies will help in building molecular frameworks to explain how environmental signals can impact LR formation. It is probable that other checkpoints are involved in altering LR initiation and/or emergence in response to different nutrients.

The regulation of LR emergence in plants is of major importance for the integrity of the entire root system. LR emergence sites are often used by soil root pathogens as entry points for infection (Sprague et al., 2007). This evolutionary pressure may have accelerated the appearance of the regulatory network controlling emergence. The production of defence molecules at LR emergence sites in order to reduce pathogenic infections (Park et al., 2004) may also be linked with this regulatory network.

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**References**


