In plants, the developmental mechanisms that regulate the positioning of lateral organs along the primary root are currently unknown. We present evidence on how lateral root initiation is controlled in a spatiotemporal manner in the model plant Arabidopsis thaliana. First, lateral roots are spaced along the main axis in a regular left-right alternating pattern that correlates with gravity-induced waving and depends on AUX1, an auxin influx carrier essential for gravitropic response. Second, we found evidence that the priming of pericycle cells for lateral root initiation might take place in the basal meristem, correlating with elevated auxin sensitivity in this part of the root. This local auxin responsiveness oscillates with peaks of expression at regular intervals of 15 hours. Each peak in the auxin-reporter maximum correlates with the formation of a consecutive lateral root. Third, auxin signaling in the basal meristem triggers pericycle cells for lateral root initiation prior to the action of INDOLE-3-ACETIC ACID14 (SOLITARY ROOT).

KEY WORDS: Arabidopsis, Auxin, Basal meristem, Lateral root, Root branching

INTRODUCTION

Lateral roots maximize the ability of a root system to acquire nutrients and water. In several plant species, lateral roots along the main root axis seem to be formed according to a regular pattern (Mallory et al., 1970; Charlton, 1983; Barlow and Adam, 1988). New lateral roots are continuously initiated at a predictable distance above the growing root tip (reviewed by Charlton, 1996). Lateral root primordia and the youngest lateral roots can be found nearest to the root tip, whereas more mature lateral roots are encountered higher in the root (Fahn, 1974).

Prior to emergence in the mature zone, lateral root primordia go through an extensive series of cell divisions (Malamy and Benfey, 1997). Due to the acropetal development of lateral roots, early stages can be traced back at more distal positions. Furthermore, a G2-to-M-specific promoter-reporter construct, CYCB1;1::GUS, marks the very first divisions in the pericycle during lateral root initiation. In Arabidopsis thaliana, the first lateral root that is initiated after embryogenesis is observed in the differentiation zone, at a fixed distance above the root tip (Casimiro et al., 2001). This position corresponds with the region where pericycle cells progress via S phase to G2 (Beeckman et al., 2001). Initiation of Arabidopsis lateral roots occurs in a strict acropetal pattern and only in a relatively short zone distal to the youngest lateral root primordium (Dubrovsky et al., 2006).

Here, we provide evidence that the events which determine lateral root positioning take place in a region at the transition between the meristem and the elongation zone, referred to as the basal meristem (Beemster et al., 2003), where several other physiological and growth responses occur, including responses to gravity, touch and moisture (Ishikawa and Evans, 1995). The data presented suggest that auxin signaling in the central cylinder of the basal meristem correlates with regular lateral root spacing. Furthermore, we show that lateral root initiation is regulated by periodic fluctuations in DR5 activity. We hypothesise such fluctuations might be representative for fluctuations in auxin distribution mediating regular longitudinal spacing of lateral roots. Our data provide a new model for root branching that highlights the involvement of the root apex.

MATERIALS AND METHODS

Used materials

We analyzed Arabidopsis thaliana (L.) Heynh. ecotype Columbia (Col-0); mutants aux1-7 (Pickett et al., 1990) and aux1-22 (both null alleles), aux1-22; promoter fusions CYCB1;1::GUS (Colón-Carmona et al., 1999); DR5::GUS (Ulmasov et al., 1997), IAA2::GUS (Swarup et al., 2001), IAA14::GUS (Fukaki et al., 2002); GAL4-GFP enhancer trap lines J0121, J0951, J1701, M0013 and Q1220 (http://www.plantsci.cam.ac.uk/Haseloff/geneControl/catalogues/lines); the promoter trap QC184 (Sabatini et al., 2003); UAS:AUX1::aux1-22, J0951>>AUX1::aux1-22, UAS:axr3-1, J0121>>UAS:axr3-1 (Swarup et al., 2005), and pIAA14::mlA14::GR lines (Fukaki et al., 2005).

Growth conditions and drug treatments

Seeds were germinated on standard Murashige and Skoog (MS)-derived medium on vertically or at 45° oriented square plates (Greiner Labortechnik, Kremmünster, Austria) under growth conditions described by Vanneste et al. (Vanneste et al., 2005). Supplements were 10 μM N1-naphthylphthalamic acid (NPA; Duchefa, Haarlem, The Netherlands), 10 μM α-naphthaleneacetic acid (NAA; Sigma-Aldrich, St Louis, MO), or 1 μM dexamethasone (Dex; Sigma-Aldrich).

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For all time-course experiments, the highest synchronization level was obtained by incubating the agar plates, after sowing, for 2 days at 4°C in the dark and then under continuous light at 22°C. Under these conditions, germination started at the earliest 20 hours after transfer to the growth chamber and was nearly 100% at 48 hours. After transfer to the growth chamber, the plates were screened for germinated seeds with a dissecting microscope, to indicate the early (at 24 hours) and late (at 34 hours) germinating population. The positions of germinating seeds (i.e. seeds with a radicle protruding the seed coat) were marked on the plate using a felt-tip pen. Only the marked seedlings were used for further analyses. In each time course, samples were taken at intervals of 7.5 hours (see Fig. S1 in the supplementary material for corresponding seedling stages). By considering the appearance of the radicle as time 0 hours, we obtained highly uniform seedling stages as supported by the homogenous seedling size at each time point determined by time-lapse recordings (see Fig. S1 in the supplementary material).

### Histochemical and histological analysis

The β-glucuronidase (GUS) assays were performed as described by Beeckman and Engler (Beeckman and Engler, 1994) or according to the protocol of Malamy and Benfey (Malamy and Benfey, 1997). For anatomic sections, GUS-stained samples were treated as described previously (Beeckman and Viane, 2000; De Smet et al., 2004).

### Microscopic analyses

For whole-mount microscopic analysis, samples were cleared by mounting in lactic acid (Acros Organics, Geel, Belgium) or according to Malamy and Benfey (Malamy and Benfey, 1997). All samples were analyzed by differential interference contrast microscopy (DMLB; Leica Microsystems). For fluorescence microscopy, whole seedlings were stained with 10 µg/mL propidium iodide (Sigma-Aldrich) and mounted in water under glass coverslips for green fluorescent protein (GFP) signal analysis with a confocal microscope 100M with software package LSM 510 version 3.2 (Zeiss, Jena, Germany). Images were collected with a 488-nm emission filter.

### Imaging and root length measurements

Photographs were taken with a CAMEDIA C-3040 zoom digital camera (Olympus, Tokyo, Japan) and processed with Photoshop 7.0 (Adobe Systems, San José, CA). Whole plates were scanned on a color copier CLC-iR C3200 (Canon, Tokyo, Japan). For measuring the interlateral root distances, the positions of lateral roots and emerged primordia were indicated under a dissecting microscope (Stemi SV11 Apo, Zeiss) on the back of the plates using a felt-tip pen prior to scanning. Plate scans were measured with ImageJ (http://rsb.info.nih.gov/ij/).

#### Toner labeling

Distances from root tips (including root cap) to the start of DR5::GUS expression at 10, 25, 40 and 55 hours after germination (HAG) were first determined using a stereomicroscope (Stemi SV11 Apo, Zeiss) with an eyepiece and measurement unit. To label that part of the root tip where DR5::GUS expression is anticipated, black toner particles from a copier eyepiece were placed on the root cap. The toner was rinsed off and the plates were placed in the growth chamber and was nearly 100% at 48 hours. After transfer to the growth chamber, the plates were screened for germinated seeds with a dissecting microscope, to indicate the early (at 24 hours) and late (at 34 hours) germinating population. The positions of germinating seeds (i.e. seeds with a radicle protruding the seed coat) were marked on the plate using a felt-tip pen. Only the marked seedlings were used for further analyses. In each time course, samples were taken at intervals of 7.5 hours (see Fig. S1 in the supplementary material for corresponding seedling stages). By considering the appearance of the radicle as time 0 hours, we obtained highly uniform seedling stages as supported by the homogenous seedling size at each time point determined by time-lapse recordings (see Fig. S1 in the supplementary material).

### RESULTS

**Arabidopsis** roots exhibit alternating left-right lateral root positioning correlated with **AUX1-dependent root waving**

In response to gravity, roots display positive gravitropic growth (reviewed by Morita and Tasaka, 2004), resulting in an enhanced waving of the root when seedlings are grown at a 45° angle (Okada and Shimura, 1990). On agar plates, vertically grown *Arabidopsis* roots also display a wavy pattern (Fig. 1A), which is accompanied by lateral root development at outer sides of bends (Fig. 1B). To investigate the correlation between these two processes, *Arabidopsis* seedlings were grown on 1.5% hard agar at an inclination of 45° (Fig. 1C). At 12 days after germination (DAG), seedlings showed on average 20 measurable curves per root representing 16% of the total root length (Fig. 1D; Table 1). Of the total number of lateral roots, approximately 51% were positioned precisely in this 16% region of the root (Fig. 1D, red mark). A χ2 test (with one degree of freedom) revealed that this peculiar lateral root distribution does not occur by chance in wild type (*P*<0.001) and suggested a correlation between lateral root formation and root waving.

In vertically grown Col-0 seedlings, the root length between two consecutive curve tops (Fig. 1D) was 2740±146 µm (*n*=35). Because under our growth conditions the growth rate of Col-0 is fairly constant and represents 182±11 µm h⁻¹ (Beemster et al., 2002), the time to bridge this distance could be calculated to approximately 15 hours.

The wavy growth pattern is the consequence of an alternation between right-turn and left-turn root bending (Rutherford and Masson, 1996). As lateral roots are formed on top of the bends, the wavy growth will result in a left-right alternation of lateral roots and in an equal distribution of laterals over both sides of the root. In vertically grown 10-DAG-old *Arabidopsis* seedlings (*n*=11), lateral roots (including primordia) were indeed distributed equally at both sides (49.6% left and 50.4% right) (Fig. 1B,E), with 66% of the roots in a strict left-right alternating sequence. This result is in agreement with previous analyses in tomato, another species with lateral roots positioned on two longitudinal rows (Newson et al., 1993).

The gravitropic aux1 mutant (Bennett et al., 1996) lacks the normal wavy growth pattern. Instead of the left-right bending found in wild-type roots, aux1 roots mainly bent constitutively to the right, with a right-handed root coiling as a consequence (Fig. 1F). In 10-DAG-old aux1 roots (*n*=18), lateral roots predominantly appeared on the outer (left) side of the coiling root (69.7% left and 30.3% right; Fig. 1E). This uneven positioning of lateral roots resulted in a clear deviation from left-right alternation in 66% of the successive lateral roots investigated, representing a significantly higher percentage than was found in wild-type roots as determined by a Student’s *t*-test (*P*<0.001).

Both lateral root initiation and gravitropic response depend on AUX1-facilitated auxin transport (Casimiro et al., 2001; Swarup et al., 2005), so we asked whether lateral root initiation might be controlled by local activity of AUX1. Targeted expression of *AUX1* to the lateral root cap and epidermal tissues of *aux1* roots fully restores the *aux1* gravitropic defect (Swarup et al., 2005). Hence, we analyzed whether the same targeted expression of *AUX1*, using a GAL4 driver line (J0951; Fig. 2A) could also restore the lateral root initiation defect of *aux1* (Marchant et al., 2002). Seedlings expressing *UAS:AUX1* under the control of the GAL4 driver line J0951 in an *aux1-22* mutant background (Swarup et al., 2005) were grown for 10 DAG on 1.5% agar at 45° inclination. The number of lateral roots per cm in the *aux1-22* mutant was significantly reduced compared with that of the Col-0 control (Fig. 2B; Table 2). In contrast, targeted expression of *AUX1* to the lateral

| Table 1. Correlation between curve tops and lateral root positioning |
|----------------------|-----------------|
| **n** | **54**|
| **Main-root length (mm)** | **50.6±2.2**|
| **Total number of emerged primordia and lateral roots** | **10.8±1.0**|
| **Total number of curves (amplitude of at least 0.4 mm)** | **20±1**|
| **Number of lateral roots positioned precisely on top of curve** | **5.5±0.4**|
root cap and epidermis of aux1 restored the lateral root number to that of the wild type (Fig. 2B; Table 2). Furthermore, the left-right alternation in lateral root formation could be rescued in vertically grown J0951>>AUX1, aux1-22 plants to levels similar to those of the Col-0 control (Fig. 2C; Table 2). Other GFP driver lines restoring AUX1 functioning only in the lateral root cap (M0013) or in stele and columella tissues (J1701) (Swarup et al., 2005) did not complement the lateral root initiation defect in the mutant background, but complementation could be obtained with another lateral root cap and epidermis-specific driver line (Q1220) (see Fig. S2 in the supplementary material). In the absence of an epidermis-specific driver line, we conclude that AUX1 action in lateral root cap and/or epidermal cells influences lateral root initiation and positioning.

The basal meristem exhibits an auxin reporter maximum

The basal meristem has been recently proposed to recycle auxin coming from the root tip via the root cap (Blilou et al., 2005), and the basipetal transport towards this region also involves AUX1 (Swarup et al., 2001; Swarup et al., 2005). Therefore we investigated whether the basal meristem displayed an increased susceptibility for auxin-induced lateral root initiation. Wild-type seedlings (5 DAG) that had already formed a few lateral roots in a left-right alternating fashion were transferred to a high concentration of NAA (10 \( \mu \)M) and incubated for 1 week. Although lateral roots were induced along the entire length of the root, proliferation was especially excessive in the basal meristem (Fig. 3A). The same experiment was repeated with a quiescent center-expressed promoter trap (QC184) (Sabatini et al., 2003). Even though no clear discrete quiescent centers could be visualized in the proliferating cell population in the basal meristem (Fig. 3B), reflecting some level of apical differentiation and suggesting intensive primordia formation in this part of the root with fused structures as a consequence. We analyzed whether this increased sensitivity was also reflected in the expression of the synthetic auxin-responsive marker DR5::GUS (Ulmasov et al., 1997). At 40 HAG, transgenic seedlings were transferred from MS medium to medium containing 10 \( \mu \)M NAA. On MS medium, after 20 minutes of GUS staining, the auxin reporter DR5::GUS was detected in two short strands, just above the
meristem (Fig. 3C). After 6 hours of NAA treatment, DR5::GUS expression had increased all over the root of the seedlings, but more intensely in the basal meristem (Fig. 3D).

An important regulatory mechanism to obtain the recycling of auxin in the basal meristem is auxin transport (Blilou et al., 2005). Therefore, the DR5::GUS reporter line was grown under conditions where auxin transport was blocked with 10 μM NPA. After 72 hours on NPA and subsequent GUS staining, no expression was detected in the basal meristem (Fig. 3E), arguing for auxin transport-dependent expression of DR5::GUS in the basal meristem.

A detailed anatomical analysis of DR5::GUS stelle expression on MS medium revealed that the GUS reporter was restricted to the two protoxylem strands and was absent from the adjacent pericycle cells (Fig. 3F). To confirm this pattern, the expression of a more sensitive auxin-responsive marker, IAA2::GUS (Swarup et al., 2001), was analyzed in detail in the basal meristem (Fig. 3E), arguing for auxin transport-dependent expression of DR5::GUS in the basal meristem.

A recurrent auxin signal in the basal meristem controls regular longitudinal lateral root initiation

The above lines of evidence suggested that the basal meristem might represent a site of auxin accumulation distinct from the distal auxin maximum in the quiescent center and surrounding cells (Sabatini et al., 1999). To elaborate on its potential significance for lateral root initiation, we monitored spatial and temporal expression patterns of the DR5::GUS reporter line in the basal meristem. Starting from 10 HAG, seedlings were harvested every 7.5 hours and subsequently stained for GUS activity. Temporal changes were observed in the staining pattern of the GUS-positive strands in the stelle of the basal meristem. The recorded temporal variations revealed an oscillating DR5::GUS expression pattern in the basal meristem with an interval of approximately 15 hours (Fig. 4A; Table 3). Over the entire time course we obtained two populations of seedlings, one with a high and one with a low percentage of strong DR5::GUS staining.

Table 2. Involvement of AUX1 in lateral root initiation and positioning

<table>
<thead>
<tr>
<th>Lateral root density</th>
<th>Lateral root positioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Col-0</td>
<td>32</td>
</tr>
<tr>
<td>aux1-22</td>
<td>32</td>
</tr>
<tr>
<td>J0951,aux1-22</td>
<td>32</td>
</tr>
<tr>
<td>UAS:AUX1,aux1-22</td>
<td>31</td>
</tr>
<tr>
<td>J0951&gt;&gt;AUX1,aux1-22</td>
<td>32</td>
</tr>
</tbody>
</table>

n.d., not determined.
Having determined the timing of the \textit{DR5} activity in the basal meristem, we designed a toner ink labeling experiment to assess the possible correlation between lateral root initiation and the observed \textit{DR5} activity. As the size of the root apical meristem is increasing during early seedling growth (Beemster and Baskin, 1998) we observed a basipetal shift of the \textit{DR5::GUS} expression in the later time points compared with the early ones. To be able to label the correct zone of the root tips where \textit{DR5 activity} is expected to occur, the distances between the root tip, including the root cap, to the start of the \textit{DR5::GUS} staining were measured at the time points with high level of expression (Table 3). Taking these distances into account, toner ink particles were positioned on the roots of seedlings of the \textit{CYCB1;1::GUS} reporter line, as described in Materials and methods. After the seedlings had been labeled, they were allowed to grow for another 30 hours, sufficient for development of at least one lateral root initiation site (Dhooge et al., 1999), and subsequently stained histochemically for GUS (Fig. 4B). By assuming that the auxin signal in this part of the root that corresponds to the basal meristem triggers lateral root initiation, the label that remained attached to the epidermis after 30 hours of growth would be expected to colocalize with an early lateral root initiation site (Fig. 4B,C). Indeed, toner particles were detected at the position of a lateral root.
Table 3. Oscillating DR5 activity in the basal meristem correlates with lateral root initiation

<table>
<thead>
<tr>
<th>HAG</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>%</th>
<th>n</th>
<th>Distance (mm)</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>56</td>
<td>10</td>
<td>80±12</td>
<td>52</td>
<td>0.121±0.050</td>
<td>12</td>
<td>15</td>
<td>89±13</td>
</tr>
<tr>
<td>17.5</td>
<td>51</td>
<td>24</td>
<td>34±20</td>
<td>n.d.</td>
<td>n.d.</td>
<td>21</td>
<td>15</td>
<td>33±6</td>
</tr>
<tr>
<td>25.0</td>
<td>54</td>
<td>76</td>
<td>76±3</td>
<td>44</td>
<td>0.169±0.053</td>
<td>28</td>
<td>13</td>
<td>59±3</td>
</tr>
<tr>
<td>32.5</td>
<td>38</td>
<td>31</td>
<td>38±1</td>
<td>31</td>
<td>n.d.</td>
<td>31</td>
<td>12</td>
<td>26±6</td>
</tr>
<tr>
<td>40.0</td>
<td>34</td>
<td>50</td>
<td>74±13</td>
<td>19</td>
<td>0.198±0.063</td>
<td>18</td>
<td>21</td>
<td>74±8</td>
</tr>
<tr>
<td>47.5</td>
<td>91</td>
<td>30</td>
<td>21±12</td>
<td>n.d.</td>
<td>n.d.</td>
<td>34</td>
<td>24</td>
<td>33±15</td>
</tr>
<tr>
<td>55.0</td>
<td>85</td>
<td>22</td>
<td>82±8</td>
<td>21</td>
<td>0.221±0.046</td>
<td>44</td>
<td>36</td>
<td>73±10</td>
</tr>
</tbody>
</table>

HAG, hours after germination; n.d., not determined; Exp. 1/Exp. 2, number of individuals for two independent experiments; LRI, lateral root initiation.

Table 4. Effects of abolished auxin response in xylem pole pericycle cells during lateral root initiation

<table>
<thead>
<tr>
<th>Lateral root density</th>
<th>Lateral root stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Lateral roots (cm)</td>
</tr>
<tr>
<td>Col-0</td>
<td>33</td>
</tr>
<tr>
<td>J0121</td>
<td>32</td>
</tr>
<tr>
<td>UAS:axr3-1</td>
<td>33</td>
</tr>
<tr>
<td>J0121&gt;&gt;UAS:axr3-1</td>
<td>33</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

n.d., not determined; LRI, lateral root initiation.
region just above the root meristem demonstrated that both J0121 and J0121>>UAS:axr3-1 revealed the same frequency of lateral root initiation events per seedling, but the portion of displaced nuclei per seedling in J0121 was significantly lower than that in J0121>>UAS:axr3-1 (Fig. 5E; Table 4).

The strong reduction in the number of lateral roots in J0121>>UAS:axr3-1 is in agreement with recent data obtained with xylem pole pericycle-specific expression of a stabilized form of IAA14 also resulting in the absence of lateral root initiation (Fukaki et al., 2005). IAA14 (SOLITARY ROOT, SLR) has been shown to play a prominent role in lateral root initiation (Fukaki et al., 2002). Although IAA14 is expressed in the pericycle and during lateral root initiation (Fukaki et al., 2002) expression analysis of IAA14::GUS revealed that IAA14 is not expressed in the basal meristem (Fig. 6A). Therefore, auxin response in the xylem pole pericycle cells in the basal meristem is most likely still intact in the slr-1 mutant and initial priming of pericycle cells might still occur. To demonstrate this possibility we made use of plants expressing the stabilized mutant mIAA14 under the native IAA14 promoter controlled by an inducible system with the glucocorticoid receptor (pIAA14::mIAA14-GR) (Fukaki et al., 2005). This allows the evaluation of a temporal effect of the mutated IAA14 protein on the initiation of lateral roots, as release from medium containing Dex to medium without Dex restores the wild-type IAA14 functioning. We asked more specifically whether pericycle cells that passed through the basal meristem in plants having a stabilized mutant mIAA14, were still competent to initiate lateral roots when the wild-type IAA14 was restored. As reported previously, 10-DAG-old seedlings expressing pIAA14::mIAA14-GR in the presence of Dex did not form lateral roots (Fukaki et al., 2005). Subsequently, seedlings (10 DAG) grown in the presence of Dex were transferred to media with and without Dex. Seedlings that continued to grow on Dex had no lateral roots (Fig. 6B), but pIAA14::mIAA14-GR seedlings that had been transferred onto Dex-free media formed lateral roots (Fig. 6C). Interestingly, also in the distal part of the region previously subjected to Dex (8.3±0.7 mm, n=10), roots could be formed whereas this was not the case in the more proximal regions of the roots where the inhibition appeared to be permanent (Fig. 6C).

**DISCUSSION**

**Auxin accumulation in the basal meristem is essential for lateral root initiation**

Both lateral root initiation and gravitropic response rely on unimpaired auxin transport and redistribution (Casimiro et al., 2003; Swarup et al., 2005). Here, we demonstrate that the processes for lateral root initiation and gravitropic response are intertwined and...
operate in the same zone of the root tip: gravitropic response-mediated waving of the primary root is correlated with the formation of lateral root primordia. As a consequence, lateral root development displays a left-right alternating pattern, which is disturbed in auxl mutants. Several mutations that simultaneously affect lateral root initiation and gravitropic response corroborate this connection (Hobbie and Estelle, 1995; Muday et al., 1995; Simmons et al., 1995; Marchant et al., 2002; Benková et al., 2003; Lin and Wang, 2005).

A temporal delay exists between the fastest auxin responsive gene expression and the initial divisions (Himanen et al., 2002; Vanneste et al., 2005) that could hitherto not be explained. Our new data support the physiological relevance of this time lag. The initial auxin signal transduction takes place in the basal meristem already at 10 HAG, subsequently priming divisions of founder cells will occur at late time stages higher up in the root. Recently, the basal meristem has been shown to cycle auxin channeling back from the root tip through the root cap (Blilou et al., 2005; Leyser, 2005). Redistribution of this recycled pool might generate an auxin response in the basal meristem as visualized by the DR5::GUS reporter, which also revealed the existence of an auxin maximum in columella initials (Sabatini et al., 1999). Fundamental changes in cell fate, cell division plane, and cell polarity have been observed when this root tip auxin maximum is disturbed. Although we could not demonstrate directly the existence of a second concentration maximum in the basal meristem, it is tempting to speculate that auxin accumulation itself primes the initiation of a new lateral organ. The occurrence of an auxin maximum in the basal meristem fits with the auxin signaling center defined in the basal part of the elongation zone based on genome-wide expression profiles in the root (Birnbaum et al., 2003; Beeckman, 2004) and is in agreement with the previously proposed inductive signal (hormonal or environmental) that affects founder cell formation and/or division of pericycle cells close to the root tip (Barlow and Adam, 1988; Dubrovsky et al., 2001; Dubrovsky et al., 2006).

At the anatomical level the DR5::GUS staining is restricted in the basal meristem to the two protoxylem cell files neighboring the pericycle cells (Fig. 7A). This peculiar radial staining pattern is easily disturbed in the presence of NPA (Fig. 7B), a treatment known to inhibit lateral root initiation (Casimiro et al., 2001). We hypothesize that a radial gradient with a maximum in the protoxylem cells might be required for lateral root initiation to take place. However, this interpretation is still very speculative and only based on the radial expression pattern of GUS markers. Further studies of the radial auxin distribution patterns and mechanisms in the basal meristem are required to support this hypothesis.

**A recurrent auxin signal in the basal meristem controls longitudinal lateral root distribution**

We demonstrated that an auxin response reporter in the basal meristem shows rhythmic expression with the same periodicity as lateral root initiation. This phasing of approximately 15 hours is in agreement with the temporal window between the initiation of two successive lateral roots as was recently calculated for Arabidopsis by Dubrovsky et al. (Dubrovsky et al., 2006). The recurrence of the auxin signal may be caused (at least in part) from periodic gravitropism-induced fluctuations in auxin redistribution within the root apex. The existence of an auxin signal in the basal meristem stresses the importance of the root tip for the regulation of root branching and supports the idea that the auxin pool in the root tip drives the initial stages of lateral root primordia formation (Bhalerao et al., 2002). As lateral roots are almost never found in opposite positions, the appearance of the auxin signal simultaneously at both protoxylem poles (Fig. 7A) necessitates an attenuation determining the left-right positioning of lateral roots. How this attenuation is brought about is not known.

Auxin-dependent signaling in the basal meristem presumably represents the very first checkpoint toward lateral root initiation (Fig. 7C.D). It cannot be neglected that other auxin sources, such as shoot-derived auxin, play a role in later steps of lateral root formation (Reed et al., 1998; Bhalerao et al., 2002), for instance in triggering the asymmetric division and further primordium development.
Auxin response of xylem pole pericycle cells in the basal meristem required for determination of founder cell identity is independent of IAA14/SLR

Lateral roots were nearly totally absent when auxin response in the xylem pole pericycle cells was abolished by specific expression of a stabilized form of IAA17 (AXR3). Microscopic inspection of such roots revealed a pre-mitotic stage of Arabidopsis lateral root initiation that has, until now, only occasionally been reported (Casero et al., 1993; Barlow et al., 2004). Just prior to the asymmetric cell division, the nuclei of two neighboring pericycle cells migrate to the common anticlinal cell wall (Fig. 7C). In wild-type roots, this process is probably rapidly followed by the division event, explaining the lack of reports on this stage in the literature.

In lateral root initiation, a crucial role has been assigned to IAA14 (Fukaki et al., 2002; Fukaki et al., 2005; Vanneste et al., 2005). Xylem pole pericycle-specific expression of a stabilized form of IAA14, which is closely related to IAA17, can repress lateral root formation (Fukaki et al., 2005). Our data suggest that IAA14 control of lateral root initiation acts downstream of auxin signaling in the basal meristem and is not required for the priming of the founder cells. First, IAA14 is not expressed in the basal meristem. Second, releasing pericycle cells from the repression of a stabilized form of IAA14 (mIAA14) results in the formation of lateral roots even in a region of the root that was earlier subjected to mIAA14. We conclude that expressing the stabilized form of IAA14 from its native promoter does not interfere with the very early phases of lateral root initiation and that priming of the pericycle cells could take place in the absence of normal IAA14 functioning.

In Fig. 7D a model is proposed that illustrates the possible spatiotemporal events that occur in the root tip prior to lateral root initiation. Our results suggest that via AUX1 action in lateral root cap and/or epidermal cells, the pericycle cells in the basal meristem might be primed through an IAA14-independent pathway (Fig. 7D). Next, in more proximal regions of the root, an IAA14-dependent auxin response is required for initiation of cell division of pericycle cells as can be visualized by the expression of CYCB1;1. It is likely that IAA14 is not the only Aux/IAA protein involved in this process but further functional characterization of other Aux/IAA proteins such as IAA17 during lateral root initiation is required.

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Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/4/681/DC1

References

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