

Joseph G. Dubrovsky · Thomas L. Rost
Adán Colón-Carmona · Peter Doerner

Early primordium morphogenesis during lateral root initiation in *Arabidopsis thaliana*

Received: 21 February 2001 / Accepted: 21 March 2001 / Published online: 30 June 2001
© Springer-Verlag 2001

Abstract The first morphogenetic events of lateral root primordium (LRP) formation in the *Arabidopsis thaliana* (L.) Heynh. pericycle occur soon after cells of the primary root complete elongation. Pericycle cells in direct contact with underlying protoxylem cells participate in LRP formation. Two types of LRP initiation were found, longitudinal uni- and bi-cellular. These occur when a single or two pericycle cells within a file, respectively, become founder cells for the entire longitudinal extent of the LRP. Histochemical and cytological analysis suggests that three is the minimum number of cells required to initiate an LRP. In young primordia comprising less than 32 cells, the average cell-doubling time was 3.7 h, indicating a drastic acceleration of cell cycle progression after lateral root initiation. Early in LRP development, cell growth is limited and therefore cytokinesis leads to a reduction of cell volume, similar to cleavage division cycles during animal and plant embryogenesis. The striking coordination of proliferation

between pericycle cells in adjacent files in direct contact with the underlying protoxylem implies that intercellular signaling mechanisms act in the root apical meristem or later in development.

Keywords *Arabidopsis* (root initiation) · Development · Cell cycle · Lateral root initiation · Meristem · Pericycle

Abbreviations LRP: lateral root primordium · GUS: β -glucuronidase

Introduction

The control of root branch initiation and development remains an essential problem in our understanding of plant development (Sussex et al. 1995; Malamy and Benfey 1997b). The first growth control point in lateral root formation is defined by the specification of those pericycle cells (founder cells) that subsequently give rise to a lateral root primordium (LRP; Dubrovsky et al. 2000). After cell flux through the elongation zone, pericycle cells adjacent to the protoxylem in the *Arabidopsis* root continue to divide (Dubrovsky et al. 2000). Cyclin genes *BI;1* (expressed during G2 and mitosis) and *D4;1* (expressed during G1-S) were found to be expressed in single pericycle cells in the protoxylem radius (Colón-Carmona et al. 1999; De Veylder et al. 1999; Dubrovsky et al. 2000). The majority of these divisions lead to a reduction in cell length and are therefore proliferative. However, around 11.3% of the divisions are developmentally asymmetric divisions, which result in founder cell formation and hence establish a lineage leading to lateral root formation (Dubrovsky et al. 2000).

In some species, including those in the *Brassicaceae*, two longitudinally adjacent pericycle cells become LRP founder cells. They participate in LRP formation by asymmetric divisions in such a way that two shorter cells are produced adjacently, which then form the center of a

J.G. Dubrovsky (✉)
Centro de Investigaciones Biológicas del Noroeste (CIBNOR),
La Paz B.C.S., A. P. 128, México 23000
E-mail: jdubrov@cibnor.mx
Fax: +52-73-172388

J.G. Dubrovsky² · T.L. Rost
Section of Plant Biology, Division of Biological Sciences,
University of California, Davis, CA 95616, USA

A. Colón-Carmona¹ · P. Doerner
Plant Biology Laboratory, Salk Institute for Biological Studies,
P.O. Box 85800, San Diego, CA 92186-5800, USA

P. Doerner
Institute for Cell and Molecular Biology,
University of Edinburgh, Edinburgh EH9 3JR, Scotland, UK

Present addresses:

¹Department of Biology, University of Massachusetts,
Boston, MA 02125-3393, USA

²Departamento de Biología Molecular de Plantas,
Instituto de Biotecnología, UNAM, A. P. 510-3,
Cuernavaca Morelos 62271, México

future primordium (Casero et al. 1993, 1995; Laskowski et al. 1995; Demchenko 1999). Single β -glucuronidase (GUS)-positive cells in the pericycle of *CycB1;1::uidA* transgenic plants, observed in the young differentiation zone (Dubrovsky et al. 2000), suggested that a longitudinally uni-cellular morphogenetic type of LRP initiation might also occur in the *Arabidopsis* root.

The analysis of the earliest stages of LRP initiation is difficult because primordia are initiated only infrequently along the parent root (Laskowski et al. 1995). Auxin treatments are often used to increase the frequency of LRP initiation. However, they confound the analysis of LRP founder cells in initiation because practically all pericycle cells in protoxylem radii can become mitotically active (Laskowski et al. 1995). Laskowski et al. (1995) determined the number of LRP founder cells in auxin-treated roots to be 11, but the minimum number of founder cells in *Arabidopsis* plants not treated with auxin is still not known.

Prior to founder cell formation, the average length of pericycle cells opposite the protoxylem is about 200 μm (Dubrovsky et al. 2000). The first cytokinesis of founder cells and the following few initial anticlinal cell divisions during LRP formation lead to a progressive reduction in cell length (Malamy and Benfy 1997a). Thus, cytokinesis does not alternate with cell growth and therefore these divisions are similar to the very rapid cleavage divisions observed during early plant and animal embryogenesis. In animals, early embryonic division cycles often dispense with the gap phases of the cell cycle (Edgar et al. 1994) to allow for the dramatic shortening of the time between nuclear divisions.

To better understand the early steps of LRP development in *Arabidopsis*, we undertook a detailed cytological and histochemical analysis of the first few cell divisions leading to LRP formation. We analyzed these first morphogenetic events in *Arabidopsis* roots not treated with auxin. Transgenic plants carrying the *CycB1;1::uidA* reporter construct (Colón-Carmona et al. 1999) were used to examine cell division patterns. We determined the minimum number of founder cells in the pericycle required for LRP formation, the patterns of division, and we analyzed the duration of cell division cycle in nascent LRPs. Three-dimensional analysis by optical sectioning of a young LRP observed under Nomarski optics and analysis of serial histological sections were used to determine the minimum number of founder cells in the pericycle.

Materials and methods

Plant growth conditions, sectioning, and whole mounts

Seeds of *Arabidopsis thaliana* (L.) Heynh., Col-2, were sterilized and germinated under the same conditions as previously reported (Dubrovsky et al. 2000). Unless otherwise indicated, all observations and measurements were done on roots 8 days after the start of germination. Histological preparations and whole mounts of the roots were done as described earlier (Dubrovsky et al. 2000).

Arabidopsis transgenic lines carrying the mitotic cyclin *CycB1;1::uidA* reporter construct (Colón-Carmona et al. 1999) were used to identify cells undergoing cell division. To detect GUS activity the material was stained as described by Hemery et al. (1993), and cleared by using the method of Malamy and Benfy (1997a).

The material was analyzed and photographed with an Olympus Vanox AHB photomicroscope using Kodak 160T Ektachrome film or images were captured with a Sony XC-75 CCD camera. Whole mounts of roots stained by Feulgen and embedded on a slide (Dubrovsky et al. 2000) in Histo-resin (Leica Instruments, Heidelberg, Germany) were also viewed under a Leica TCS confocal microscope using a Krypton laser at 568 nm. The number of replicates of each experiment is indicated in the text.

Determination of the cell doubling time in young LRPs

Cell-doubling time in young LRPs initiated by the uni-cellular longitudinal type (see *Results*) was determined by relating the number of progeny of a single founder cell to the duration of LRP formation. For example, it was assumed that in LRPs composed of 3–4, 5–8, 9–16, and 16–32 cells, a founder cell passed 2, 3, 4, and 5 cell cycles, respectively. The location of the most apical mitoses was estimated on Feulgen-stained preparations. The duration of LRP formation (T_{lrp}) was determined by the equation

$$T_{lrp} = (L_p - L_{am})/V$$

where L_p is the distance from the root body-root cap junction to the primordium in a root, L_{am} is the distance from the root body-root cap junction to the first detected mitosis in the pericycle in the differentiation zone, and V is the rate of root growth. For the determination of V , the position of the root tip was marked on the surface of the Petri dish and the growth increments in 24 h were measured to the nearest 0.5 mm under a binocular microscope. The L_p was determined on the cleared roots mounted in 50% glycerol (Malamy and Benfy 1997a) using Nomarski optics. All data (L_p , L_{am} , V) were collected on individual roots. Cell-doubling time was determined by dividing T_{lrp} by the estimated number of cell cycles.

Nomenclature

In this paper, the term ‘founder cell’ is used to describe those cells that initially acquire a developmental fate different from that of their mother and, as a consequence, play a principal role during the first stages of lateral root formation. Thus, the term is used to emphasize how the organ is initiated, in contrast to its use in the context of clonal or lineage analysis and fate-mapping (Poethig 1987; Dawe and Freeling 1991), where the term refers to an estimated number of progenitor cells deduced from the analysis of structures in developed organs. The term ‘asymmetric division’ refers to mitoses that give rise to daughter cells of unequal size. The term ‘developmentally asymmetric divisions’ refers to those mitoses that give rise to daughter cells with different fates (Scheres and Benfy 1999) and is used synonymously with the term ‘formative divisions’.

Results

Two types of LRP initiation in *Arabidopsis*

We found two morphogenetic types of LRP initiation in the first 10-mm section of the primary root, both on histological sections and cleared root specimens. The first type of LRP initiation, similar to that found in other species, was termed “longitudinal bi-cellular”. Here, the first indication of primordium formation is

asymmetrical cell divisions in two adjoining cells of the same file. These divisions are oriented such that the short cells formed from these unequal divisions are juxtaposed and form the center of primordium initiation (Fig. 1A). Subsequent anticlinal divisions occur in the vicinity of the established center of the developing primordium (Fig. 1B).

The second type, termed “longitudinal uni-cellular” initiation, occurs when one pericycle cell becomes a founder cell for the entire longitudinal extent of the LRP (Fig. 2). The founder cell usually is characterized by convex end cell walls (Fig. 2A). Sometimes it can be convex only at one end, indicating that a preceding formative cell division took place in a mother cell giving rise to the founder cell (Fig. 2A). In this case, the transverse cell wall at the other end is perpendicular or oblique to the longitudinal cell wall (Dubrovsky et al. 2000). The cell wall formed as a result of a formative division can be seen at the right-hand end of cells shown in Fig. 2C and D. Founder cell division is anticlinal (Fig. 2A) and their progeny form a few cells of approximately equal lengths that grow radially (Fig. 2B) and then proceed to the first (Fig. 2C) and the second set (Fig. 2D) of periclinal divisions. The longitudinal unicellular type of LRP initiation was found more frequently than the bi-cellular type, but the number of primordia we were able to score unequivocally in whole mounts of Feulgen-stained or cleared roots was too small to make this a general conclusion.

What is the minimum number of pericycle founder cells giving rise to an LRP?

The number of cells that are in direct contact with the protoxylem can be one (not shown), two, or three as seen in transverse sections of the root in Fig. 3C (asterisks). As direct contact with the protoxylem would

facilitate prospective formation of the vascular connection between the parent and lateral roots, hypothetically the pericycle cells in direct contact with the protoxylem would comprise the minimal number of LRP founder cells. This number was estimated from the number of cells participating in the formation of the primordium using three approaches: (i) analyzing the number of cell layers where first mitoses occurred leading to formation of the primordium, (ii) analyzing the number of cell layers in young primordia initiated by the longitudinal unicellular type in the *CycB1;1::uid1A* transgenic line and in wild-type plants, and (iii) spatially analyzing serial sections.

Two (Fig. 3A) or three (Fig. 3B) single mitotic figures were frequently (6 cases in 10 roots, 2- to 6-mm root portions analyzed) found at the same distance from the root tip, but in different cell layers, all associated with a protoxylem strand. These mitotic divisions appeared to be synchronous or almost so, which suggested that they represented the earliest stages of LRP initiation by founder cells. We did not observe a mixed type of initiation (uni- and bi-cellular) within the same LRP. Examination by Nomarski optics of young primordia after completion of the first set of periclinal divisions, in cleared roots lying in the protoxylem plane, showed that the primordia comprise descendants of single parental pericycle cells from three adjacent files (Fig. 3D–F). In roots lying in the protophloem plane, a young primordium was represented by a plate of cells that were descendants of three pericycle cells (Fig. 3G, asterisks). Analysis of serial histological sections of a primordium initiated as a longitudinal unicellular type showed that three pericycle founder cells located near the protoxylem participated in its initiation (Fig. 4B–D). One additional cell with less proliferative activity also participated in the formation of this primordium (Fig. 4A). These data collectively indicate that three pericycle cells are the minimum needed to form a primordium.

What is the average cell-doubling time in young primordia?

Longitudinal unicellular LRP initiation allows us to estimate the average cell-doubling time in a cell packet formed by proliferation of one founder cell. A cell packet is the progeny of one cell enclosed in its cell wall

Fig. 1A, B Longitudinal bi-cellular type of LRP initiation in *Arabidopsis thaliana*. **A** Earliest stage of LRP initiation on a histological section of the root portion 2–4 mm from the root tip. Note the first asymmetrical division in two pericycle cells leading to LRP formation. **B** Young LRP in the unstained, cleared root portion 2–4 mm from the tip where the second set of anticlinal divisions took place (Nomarski optics). *Arrowheads* indicate end cell walls of pericycle founder cells; *arrows* indicate position of cell walls resulting from the division of LRP founder cells. Bars = 20 μm (A), 10 μm (B)



Fig. 2A–D Longitudinal uni-cellular type of LRP initiation and early stages of LRP development in *A. thaliana*. **A** A founder cell that passed one cell cycle. Note convex end walls of the founder cell. **B** Young stage of LRP development when a founder cell had passed a few cell cycles; the cells produced are relatively similar in size. Note some cells grew radially. **C** The first set of periclinal divisions in the LRP. LRP cells are enclosed in a cell wall of the founder cell. Note, the right end wall is not convex and can be a result of division of a larger parent cell. **D** The second set of periclinal divisions in the LRP. Primordia are in the root portion 6–8 mm from the root tip (**A–C**) and at 9 mm from the root tip (**D**). **A, C** Histological sections; **B, D** cleared roots, Nomarski optics. *Arrowheads* indicate end cell walls of pericycle founder cells. Bars = 20 μm (**A, C, D**), 10 μm (**B**)



(Barlow 1983). A packet shown on Fig. 2C consists of 16 cells, so the parent cell (founder cell in this case) passed through 4 cell division cycles. This particular primordium is located 7,100 μm from the root tip. If we assume that founder cell division in this root started at a similar distance from the root tip ($\approx 2,205 \mu\text{m}$), where the earliest mitoses in this set of root samples were observed ($n=12$), then we can estimate the cell-doubling time during LRP formation, if we know the rate of root growth. In this example, the cell-doubling time (T_d) was 3.4 h. The average distance to the earliest mitoses in the pericycle was, however, equal to $3,194 \pm 224 \mu\text{m}$ ($n=10$, mean \pm SE), indicating the T_d can be even shorter (2.7 h for the mentioned primordium).

In a sample of individually fixed and cleared roots with a known rate of root growth where the primordia of the longitudinal uni-cellular type of initiation were found and had passed two to five cell division cycles, cell-doubling time was estimated. T_d ranged from 2.7 h to 4.9 h (Fig. 5). The average cell-doubling time in such primordia was 3.7 ± 0.2 h ($n=13$, mean \pm SE).

Discussion

Morphogenetic types of LRP initiation and inter-cellular interactions

We have described and defined two types of LRP initiation in *Arabidopsis*, longitudinal uni-cellular and longitudinal bi-cellular. The latter type represents

asymmetrical anticlinal divisions of two abutting pericycle cells within a file and has been previously reported for other species (see *Introduction*). The longitudinal uni-cellular type of LRP initiation is rarely found because of general difficulties of documenting spontaneously initiated primordia in intact plants (Blakely et al. 1982). Demchenko (1999) observed the longitudinal uni-cellular type of initiation in wheat roots and proposed that this type of initiation depended on the relative position of prospective founder cells in different cell files. Generally, cells that leave the meristem are in different phases of the cell cycle (Ivanov 1981; Demchenko 1987). However, because founder cells are formed soon after pericycle cells leave the meristem (Dubrovsky et al. 2000), and founder cell division in up to three adjacent cell files can be activated apparently simultaneously during longitudinal uni-cellular LRP initiation, we propose the existence of inter-cellular signaling mechanisms to coordinate cell cycle activity in three adjacent cell files.

Pericycle cells adjacent to the protoxylem poles maintain their competence for cell division without interruption following their exit from the meristem (Dubrovsky et al. 2000). Founder cell fate affects only a minority of pericycle cells in stochastic patterns in a discrete zone of the root (Dubrovsky et al. 2000), but always appears to proceed in a highly coordinated manner in cells from neighboring pericycle cell files. We suggest that founder cells that are committed to their first division must determine their position longitudinally and circumferentially relative to the neighboring

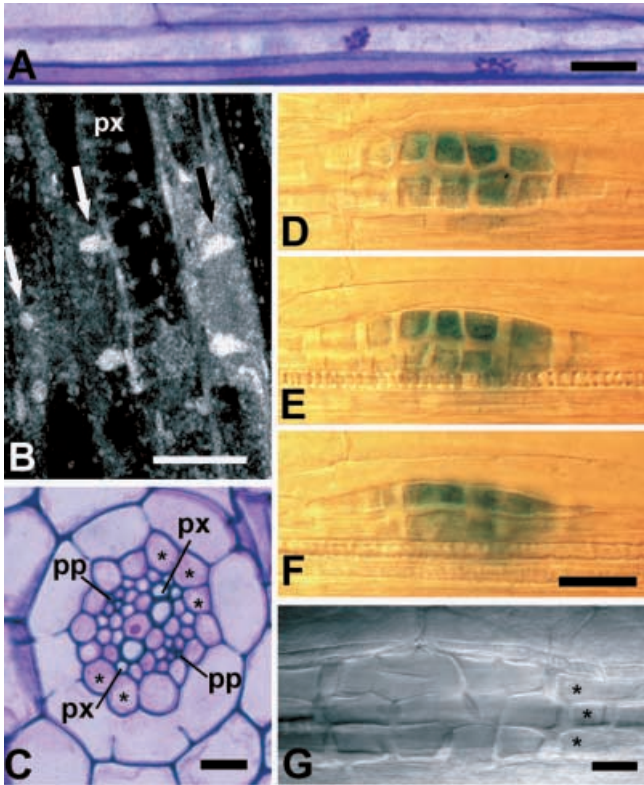


Fig. 3A–G Analysis of the minimum number of founder cells. **A** Synchronous metaphases on a section of two adjacent pericycle cells in the protoxylem radius 6–8 mm from the *A. thaliana* root tip. **B** Synchronous mitotic figures (arrows) in three adjacent pericycle cells in the protoxylem radius 4 mm from the root tip; from the left to the right, late telophase, early telophase, late anaphase. Whole mount of the root stained by Feulgen and observed in the confocal microscope; eight successive optical sections were superimposed. **C** Transverse section of the root 6 mm from the tip. The protoxylem cells are in contact with two or three cells of the pericycle (asterisks). **D–F** Optical sections through the same primordium of a *CycB1;1-GUS* line stained for GUS and cleared. The root is lying in a protoxylem plane. Each of the three layers represents descendants of a founder pericycle cell. **G** Optical section through a primordium of a wild-type seedling root lying in the protophloem plane. Three cell files of the pericycle (asterisks) are involved in LRP formation. **A, C** Bright-field microscopy. **D–G** Nomarski optics. *pp* Protophloem, *px* protoxylem. Bars = 10 μ m (**A–C, G**), 20 μ m (**D–F**)

tissues. Therefore, the simplest model for a signal pathway controlling LRP initiation would be that an inductive signal to determine the longitudinal position of a primordium simultaneously affects prospective founder cell formation and/or division in up to three, adjacent pericycle cell files. We propose that those founder cells with convex walls at both ends (Fig. 2A) likely had perceived the positional signal at birth, whereas those with convex walls at only one end had perceived the signal just prior to founder cell formation. Such cells were likely formed as a result of developmentally asymmetric divisions. In order to generate a discrete primordium this model would also have further requirements. To prevent adjacent pericycle cells from participating in LRP formation, a signaling pathway

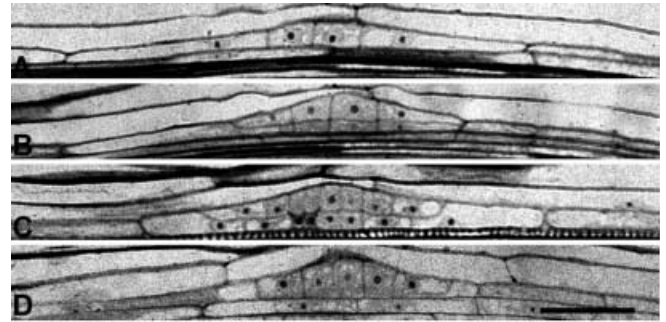


Fig. 4A–D Serial histological sections of the primordium depicted in Fig. 2C showing the number of pericycle cell layers participating in the primordium formation. **A** A section 12.5 μ m above the median section. **B** A section 7.5 μ m above the median section. **C** Median section through the primordium. **D** A section 12.5 μ m below the median section. Bar = 40 μ m

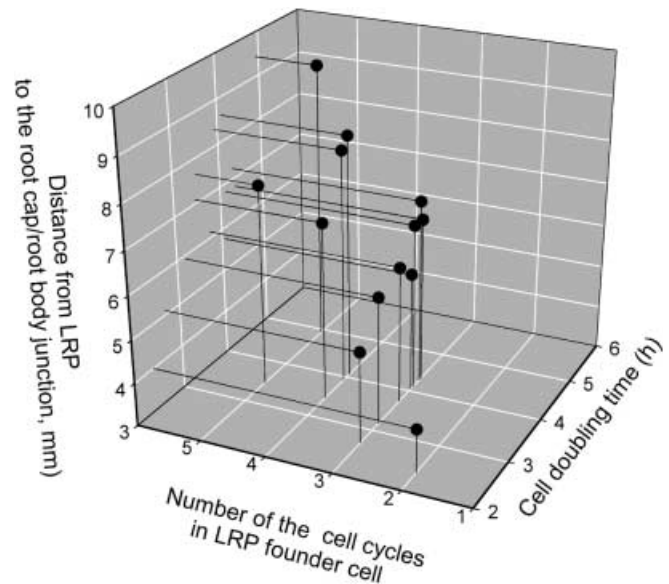


Fig. 5 Characteristics of the young LRPs of the uni-cellular longitudinal type of initiation by their location in the *A. thaliana* primary root, by the number of cell cycles in LRP founder cells, and by the estimated cell-doubling time. Each primordium, marked on the graph by a point, was analyzed in an individual root. Cell-doubling time was estimated based on the rate of root growth and the location of the most distal mitoses in the pericycle found in the sample, which was equal to 2,205 μ m

should be established between founder cells and their neighbors. This would then be followed by a lateral inhibition, in which the established group of founder cells prevents its neighbors from progressing down the same developmental pathway.

Three founder cells from adjacent pericycle files can give rise to a primordium

We used Nomarski optics and serial histological sections of young LRPs to address the question of the minimum

number of cells sufficient for LRP formation. This analysis, together with the observation of first mitoses proceeding simultaneously in three cells (Fig. 3B) showed that three cells are sufficient to give rise to a primordium.

The length of the pericycle cells in the protoxylem radius is on average half of those in the protophloem radius (Laskowski et al. 1995; Sussex et al. 1995; Dubrovsky et al. 2000). Roots grown under the same conditions as in the present study had an average pericycle cell length of cells adjacent to the protoxylem poles of 90 μm , 4–6 mm from the tip (Dubrovsky et al. 2000). Founder cells can be significantly longer, for example cells in Fig. 2A, C found 6–8 mm from the tip were 170 and 169 μm long, respectively. Their size is comparable to the diameter of the base of the lateral root (not shown). Laskowski et al. (1995) determined that the number of pericycle cells in the longitudinal dimension that give rise to a lateral root was equal to 2.4 cells in *A. thaliana* grown in a medium supplemented with auxin. The discrepancy with our results is likely due to the different experimental conditions. By making a projection of the lateral root base to the surface of the pericycle, they estimated the number of founder cells to be 11 (Laskowski et al. 1995). The size of the primordium could increase, however, because of the activity of descendants of founder cells. Thus, the method of comparison of sizes of the root base and number of pericycle cells below the root base gives only a hypothetical maximum number of founder cells. We estimated the minimum number of founder cells needed to give rise to an LRP in our growth condition to be three. We cannot exclude some involvement of other pericycle cells in LRP formation, but they probably would not have a significant impact on the histogenesis of a lateral root. For example, when the first set of periclinal divisions in pericycle founder cells takes place, differential gene expression was demonstrated in two cell layers of the primordium produced (Malamy and Benfey 1997a). In our case, though periclinal divisions had taken place (Fig. 4B, C) or would soon take place (Fig. 4D) in founder cells of a primordium, additional cells (Fig. 4A) did not grow radially and only anticlinal division occurred.

The determined minimum number of cells represents the most probable number. Because we have observed that rarely only one (not shown) and more frequently two or three (Fig. 3C) pericycle cells are located at the appropriate position adjacent to the protoxylem, we cannot exclude that one or two founder cells could give rise to a primordium if its initiation were of the longitudinal uni-cellular type. The minimum number of founder cells should be doubled when primordia initiation is of the longitudinal bi-cellular type. If few founder cells give rise to an LRP, then the lateral root will be of a clonal nature and clonal analysis (Poethig 1987) would be necessary to independently confirm these findings.

Cell cycle progression is drastically accelerated after a formative cell division

The average cell cycle duration in pericycle cells within the meristem in roots grown under the same conditions was 13.6 h (Dubrovsky et al. 2000). From that work, we estimated that proliferative divisions detected 3–4 mm from the tip occurred 9.5 to 12.3 h after the cells left the meristem. In dividing pericycle cells that did not produce a primordium, cell division accelerated slightly outside the meristem. However, in those pericycle cells that initiated primordia, the cell-division cycle was dramatically accelerated (Fig. 5). In young developing primordia, the cell-doubling time was 2.7–4.9 h. In young primordia, as in this case, cell-doubling time is equal to the duration of the cell cycle (Thompson and MacLeod 1981, 1983).

Such a short duration of the cell cycle is unusual for vegetative tissues in plants and resembles animal embryogenesis. The most rapidly dividing cells in mammals are cells of the primitive streak that divide every 3–3.5 h during rat gastrulation (Auley et al. 1993). In zebrafish, during the mid-blastula transition, the cell division cycle can be concluded in only 20 min (Kane et al. 1992) and during early *Xenopus* embryogenesis it takes only 30 min (Murakami and Vande Woude 1998). Previous reports have also highlighted rapid proliferation in plants, during early stages of LRP formation. The shortest previously reported cycle time was 2.7 h in embryonic LRPs of *Cucumis sativus* (Dubrovsky 1986). It was 5–6 h in developing root primordia of the radish (Laskowski et al. 1995), 4 h in *Vigna angularis* (Mitsuhashi-Kato et al. 1978), 2.9 h in *Pisum sativum*, and 4.5 h in *Zea mays* (MacLeod and Thompson 1979). Acceleration of cycling can in general be accomplished by truncation or elimination of G1, S, and G2 (Auley et al. 1993; Edgar et al. 1994), by decreasing replicon size (Walter and Newport 1997), or by other mechanisms (Van't Hof 1985). Notably, during the early stages of LRP development, new cells formed as a result of founder cell divisions only and had very limited scope for growth as all cell descendants were enclosed in a cell packet of a founder cell (Fig. 2C). Such divisions resemble cell cleavage during plant (Pollock and Jensen 1964) and animal embryogenesis. If lateral inhibition plays a role during LRP formation, short cell cycles would serve to rapidly establish a primordium whose cells could prevent adjacent cells from adopting the same fate. Further studies are required to understand the basis for cell division acceleration during the establishment of LRPs, especially because of their potential relevance for other phases of plant development during which rapid cycles are likely to occur, for example during embryogenesis.

Acknowledgements We thank the Mexican Council for Science and Technology (CONACyT) for support of J.G. Dubrovsky during his sabbatical leave. This work was supported by a UCMEXUS grant from University of California and by the exchange program between the Royal Society and the Mexican Academy of Sciences. Work in the P. Doerner laboratory was supported in part by

USDA grant 95-37304-2228 and A. Colón-Carmona was supported by NSF Postdoctoral Fellowship BIR-9510821. Thanks also to Natalia Doktor for her help with preparation of the illustrations.

References

- Auley AM, Werb Z, Mirkes PE (1993) Characterization of the unusually rapid cell cycles during rat gastrulation. *Development* 117:873–883
- Barlow PW (1983) Cell packets and cell kinetics in the root meristem of *Zea mays*. In: Böhm W, Kutschera L, Lichtenegger E (eds) *Wurzelökologie und ihre Nutzenanwendung. Ein Beitrag zur Erforschung der Gesamtpflanze*. Verlag Bundesanstalt für alpenländische Landwirtschaft, Irnding Austria, pp 711–720
- Blakely LM, Durham M, Evans TA, Blakely RM (1982) Experimental studies on lateral root formation in radish seedling roots. I. General methods, developmental stages, spontaneous formation of laterals. *Bot Gaz* 143:341–352
- Casero PJ, Casimiro I, Rodríguez-Gallardo L, Martín-Partido G, Lloret PG (1993) Lateral root initiation by means of asymmetric transversal divisions of the pericycle cells in adventitious roots of *Allium cepa*. *Protoplasma* 176:138–144
- Casero PJ, Casimiro I, Lloret PG (1995) Lateral root initiation by asymmetrical transverse divisions of pericycle cells in four plant species: *Raphanus sativus*, *Helianthus annuus*, *Zea mays*, and *Daucus carota*. *Protoplasma* 188:49–58
- Colón-Carmona A, You R, Haimovitch-Gal T, Doerner P (1999) Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. *Plant J* 20:503–508
- Dawe RK, Freeling M (1991) Cell lineage and its consequences in higher plants. *Plant J* 1:3–8
- Demchenko NP (1987) Changes in population structure of epidermal, endodermal, and pericycle cells in the course of their development in the wheat root (in Russian). *Tsitologia* 29:174–181
- Demchenko KN (1999) Cell proliferation during lateral root initiation (in Russian). PhD thesis, V.L. Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg
- De Veylder L, de Almeida Engler J, Burssens S, Manevski A, Lescure B, Van Montagu M, Engler G, Inzé, D. (1999) A new D-type cyclin of *Arabidopsis thaliana* expressed during lateral root primordia formation. *Planta* 208:453–462
- Dubrovsky JG (1986) Dynamics of cell reproduction and cell complexes (cell packets) in the embryonic lateral root primordium of the cucumber. (English translation from Russian appeared in *Sov J Dev Biol*, New York, N.Y.: Consultant Bureau 17:337–344.) *Ontogenez* 17:525–534
- Dubrovsky JG, Doerner P, Colón-Carmona A, Rost TL (2000) Pericycle cell proliferation and lateral root initiation in *Arabidopsis thaliana*. *Plant Physiol* 124:1648–1657
- Edgar BA, Sprenger F, Duronio RJ, Leopold P, O'Farrell PH (1994) Distinct molecular mechanisms regulate cell cycle timing at successive stages of *Drosophila* embryogenesis. *Genes Dev* 8:440–452
- Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, Inzé D (1993) *cdc2a* expression in *Arabidopsis* is linked with competence for cell division. *Plant Cell* 5:1711–1723
- Ivanov VB (1981) Cellular basis of root growth. *Sov Sci Rev D2*:365–392
- Kane DA, Warga RM, Kimmel CB (1992) Mitotic domains in the early embryo of the zebrafish. *Nature* 360:735–737
- Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two-stage process. *Development* 121:3303–3310
- MacLeod RD, Thompson A (1979) Development of lateral root primordia in *Vicia faba*, *Pisum sativum*, *Zea mays* and *Phaseolus vulgaris*: rates of primordium formation and cell doubling times. *Ann Bot* 44:435–449
- Malamy JE, Benfey PN (1997a) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124:33–44
- Malamy JE, Benfey PN (1997b) Down and out in *Arabidopsis*: the formation of lateral roots. *Trends Plant Sci* 2:390–396
- Mitsuhashi-Kato M, Shibaoka H, Shimokoriyama M (1978) Anatomical and physiological aspects of developmental processes of adventitious root formation in *Azuki* cuttings. *Plant Cell Physiol* 19:393–400
- Murakami MS, Vande Woude GF (1998) Analysis of the early embryonic cell cycles of *Xenopus*; regulation of cell cycle length by *Xe-wee1* and *Mos*. *Development* 125:237–248
- Poethig RS (1987). Clonal analysis of cell lineage patterns in plant development. *Am J Bot* 74:581–594
- Pollock EG, Jensen WA (1964) Cell development during early embryogenesis in *Capsella* and *Gossypium*. *Am J Bot* 51:915–921
- Scheres B, Benfey PN (1999) Asymmetric cell division in plants. *Annu Rev Plant Physiol Plant Mol Biol* 50:505–537
- Sussex IM, Godoy JA, Kerk NM, Laskowski MJ, Nusbaum HC, Welsch JA, Williams ME (1995) Cellular and molecular events in newly organizing lateral root meristem. *Phil Trans R Soc London Ser B* 350:39–43
- Thompson A, MacLeod RD (1981) Increase in size and cell number of lateral root primordia in the primary of intact plants and in excised roots of *Pisum sativum* and *Vicia faba*. *Am J Bot* 68:955–964
- Thompson A, MacLeod RD (1983) Change in size and cell number during the development of lateral root primordia in *Zea mays* L. *Ann Bot* 52:777–780
- Van't Hof J (1985). Control points within the cell cycle. In: Bryant JA, Francis D (eds) *The cell division cycle in plants*. Cambridge University Press, Cambridge, pp 1–13
- Walter J, Newport JW (1997) Regulation of replicon size in *Xenopus* egg extracts. *Science* 275:993–995