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Clonal analysis of the *Arabidopsis* root confirms that position, not lineage, determines cell fate

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Abstract. The cellular organization of the *Arabidopsis* thaliana (L.) Heynh. root meristem suggests that a regular pattern of cell divisions occurs in the root tip. Deviations from this pattern of division might be expected to disrupt the organization of cells and tissues in the root. A clonal analysis of the 3-d-old primary root meristem was carried out to determine if there is variability in division patterns, and if so to discover their effect on cellular organization in the root. Clones induced in the seedling meristem largely confirmed the predicted pattern of cell divisions. However, the cellular initials that normally give rise to the different cell files in the root were shown to exhibit some instability. For example, it was calculated that a lateral root cap/epidermal initial is displaced every 13 d. Furthermore, the existence of large marked clones that included more than two adjacent cell layers suggests that intrusive growth followed by cell division may occur at low frequency, perhaps in response to local cell deaths in the meristem. These findings support the view that even in plant organs with stereotypical cell division patterns, positional information is still the key determinant of cell fate.

Key words: *Arabidopsis* – Root – Clonal analysis – Invasions – Initials – Quiescent center

Introduction

The *Arabidopsis* root is an indeterminate structure, at the tip of which is a meristem covered and protected by a root cap. Clonal analysis indicates that the primary root tip of the seedling is derived from the uppermost cell of the suspensor and adjacent cells of the globular embryo (Scheres et al. 1994). The tip region includes four central cells that comprise the quiescent center, surrounded on all six sides by a single layer of cellular initials (Fig. 1). Cells derived from the lateral and proximal initials (that give rise to the lateral root cap/epidermis, cortex/endodermis and stele respectively) enter the meristematic zone of the seedling root and produce a root structure with a highly predictable cellular architecture (Fig. 1). Cells derived from the distal set of initials give rise to the columella, or central root cap. While the consistent spatial relationships between cells in the vicinity of the initials and quiescent center suggest that patterns of cell division are generally regular, it is unclear how variable these division patterns are.

Clonal analysis in shoots has shown that there is variation in the cell division pattern of cells in the tunica, which generally divide anticlinally (new cell wall forming perpendicular to the surface of the organ). Cells derived from these divisions are incorporated into the adjacent meristematic layer without disrupting tissue organisation or cell fate in the organ (Dermen 1947; Steeves and Sussex 1989). The invading cells respond to positional information and adopt the fate appropriate for their position in the organ. Similarly the initials of the shoot apical meristem are unstable and can be replaced by the adjacent cells that now occupy the position of initials. In the shoot an initial is 'the temporary inheritor of a permanent position' (Newman 1965).

While clonal analysis has been instructive in illustrating the dynamic role of initials in the shoot meristem, there have been few clonal analyses of root development. One of the most instructive was reported by Scheres et al. (1994) who used random excision of the *Ac* transposon from the β-glucusonidase (*GUS*) marker gene to characterize the embryonic origin of cell layers in the root. The distribution of clones showed that different regions of the seedling root are derived from discrete clonal compartments within the embryo (although there was marked variation in the location of clonal boundaries). To understand the cellular dynamics within the seedling root meristem, Brumfield (1943) generated

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Abbreviation: GUS = β-glucusonidase

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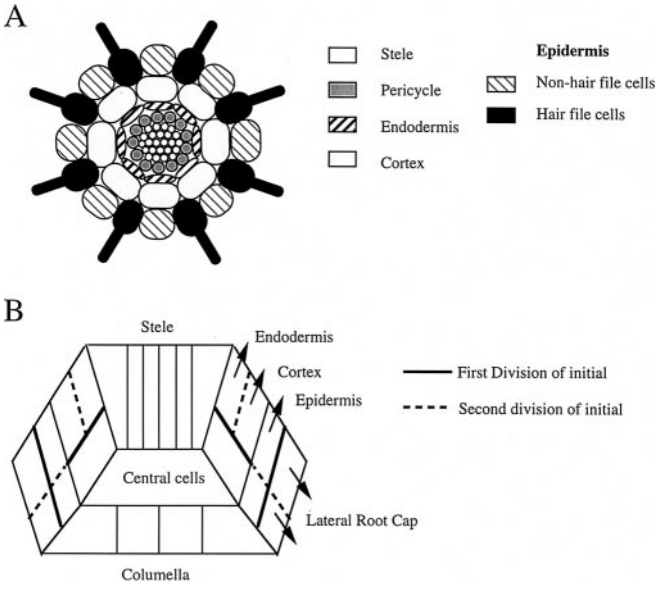

Fig. 1A,B. Anatomy of the *Arabidopsis* root. **A** Diagram of transverse section through the root showing arrangement of tissues. **B** Diagram of longitudinal section through the initials and central cells showing order of divisions in the endodermis/cortex initial and lateral root cap epidermal initials

chimeras using ionizing radiation in the young roots of *Vicia faba* seedlings. The number and organization of these clones suggested that there were four initials in the root. Further characterization of similar chimeras led to the conclusion that such a small number reflected not the number of initials themselves but rather the number of cells giving rise to a new meristem after radiation-induced destruction of the original meristem (Davidson 1958, 1961). Consequently, while we have a good understanding of the embryonic origins of cells that constitute the root meristem, we have little insight into the cellular dynamics of cells within preformed seedling root meristems.

In the present study we examined the fate of cells in the 3-d-old primary root meristem of *Arabidopsis*. Three classes of clones were particularly instructive. First, clones induced in the central cells show that they can give rise to cells in almost any tissue of the root and therefore may act as a permanent reservoir of stem cells. Second, clones induced in initials indicate that these cells exhibit a degree of instability that could not be predicted from histological studies. Lastly, clones induced in a variety of cell layers indicate that there is variation in the pattern of cell divisions within lineages and that these give rise to invasion events whereby the daughter cells are located in different tissues from the mother cells.

Materials and methods

Production of lines. The two plasmids – a construct containing a heat-shock promoter driving *Ac transposase* expression (pYS8) and *Ds* inserted into the non-translated leader of a *GUS* gene under the control of a cauliflower mosaic virus (CaMV) 35S promoter (pYS3) were used to transform *Escherichia coli* strain DH5α. The constructs were introduced into *Agrobacteria* by triparental matings and *Arabidopsis thaliana* (L.) Heynh. Landsberg *erecta* plants were transformed via root transformation (Dean 1992). T3 plants homozygous for the transgenes were selected on kanamycin and crossed to generate the lines used for the analysis.

Production of clones. Seedlings were grown on MS medium as described in Dolan et al. (1993) for 3 d after germination. The plates were sealed with Nesco film and floated on a water bath heated to the given temperature for the given time. The plates were dried, the length of root marked on the back of the plates, and returned to the growth room for 48 h. Seedlings were then fixed with 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 1 h at 4 °C. This limits diffusion of the GUS stain. The plants were then GUS-stained as described in Dolan et al. (1993). Seedlings were incubated in the substrate either at 37 °C or at room temperature overnight. Plants were examined using a ×40 long-working-distance lens on a Nikon Microphot SA microscope. The number of cells making up the clone was determined using a ×100 objective. The distance of the basalmost point of the clone from the central cells was measured from photographic slides. Seedlings containing clones in the primary root were fixed individually in microtiter plates overnight in 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). To aid identification, every fifth root was stained with safranin (0.03%). Seedlings were aligned in groups of five on a thin layer of agarose, upon which a drop of molten agarose was placed and allowed to solidify. The agarose block containing the embedded roots was fixed overnight at 4 °C. The block was trimmed of excess agarose, washed in water and dehydrated through an ethanol series. The blocks were infiltrated with Historesin (Leica) and polymerized at room temperature overnight. Serial sections of 5, 10, 15 or 20 μm were cut with a glass knife on a Leica Microtome and dried onto multiwell slides. The slides were stained with 0.05% ruthenium red for 10 min at room temperature. The sections were examined on a Nikon Microphot SA with a ×60 oil immersion objective to determine the radial extent of the clone and confirm the distance of the clone from the central cells.

Calculation of meristem size. The size of the meristem is calculated from the average number of meristematic cells per file and the average number of cell files of each tissue in the root (Table 1). Fifteen medial longitudinal sections of 3 d-old root tips were used to estimate the number of meristematic cells per file. The number of cells was counted in each file between the initial cell and the point at which no more cell divisions were observed and cell length began to increase consistently. This gave an average and standard error of the mean of the meristematic cells per file for each tissue type. The average number of files per tissue was calculated from transverse sections through 50 roots. Transverse sections through 20 root cap regions were used to give the average number of files of the columella and lateral root cap.

Results

Heat-shock-induced GUS expression as a marker for clonal analysis. To study the patterns of cell division in the seedling root, cells in the 3-d-old root meristem were genetically marked, and the resultant clones in the roots were then examined 2 d later. These clones represent a sample of the products of the cell divisions that occurred in the root at the time of heat shock. A transgene expressing a nuclear-localized *GUS* under a 35 S promoter (Fig. 2) was used as a heritable cell-autonomous marker. The 3′ untranslated leader of the *GUS* is disrupted by a *Ds* transposon, preventing *GUS* expression except in cells which have inherited an active *GUS*, from which the *Ds* has been excised in the mother cell.

Table 1. The number of cells in the primary root meristem

Tissue	Meristematic cells per file (SE)	Number of files (SE)	Meristematic cells per root
Columella	1 (0)	12 (0)	12
Lateral root cap[a]	2.75 (0.13), 6.12 (0.31)	17 (0.4), 27 (1.2)	213
Hair file cells	35.8 (1.10)	8 (0)	286
Non-hair file cells	18 (1.22)	12.22 (0.41)	220
Cortex	26.3 (1.7)	7.96 (0.04)	209
Endodermis	27.5 (2.1)	8.06 (0.03)	221
Pericycle	18 (0.8)	13.16 (0.22)	237
Stele	13 (1.09)	25.02 (0.44)	325
Shared Initials[b]			24
Total			1747

[a] The meristem of the lateral root cap is composed of the cells of the first and second layer after the epidermis
[b] The shared initials of the epidermis/lateral root cap (16) and cortex/endodermis (8) are not included in the calculations for the individual cell layers

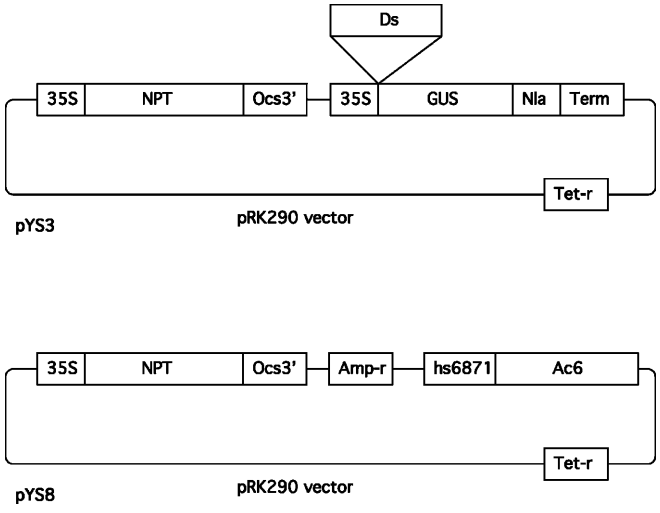

Fig. 2. Two plasmids that were used to transform *Arabidopsis* in the generation of a line to be used as a heat-shock-inducible lineage marker. The transcription of *Ac transposase* is regulated by a soybean heat-shock promoter (hs6781) on pYS8. Insertion of the *Ds* transposon into the untranslated leader of the *GUS* gene blocks its expression on pYS3. The transposase induces excision of the *Ds* from the *GUS* gene, thereby restoring marker expression. Excision of *Ds* occurs at a low frequency during or just after DNA synthesis

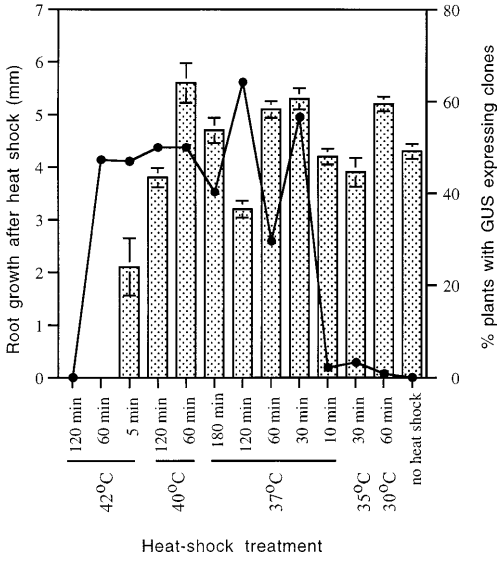

Fig. 3. The effect of heat shock on root growth and clone induction. Plants were germinated at 21 °C. After 3 d the extent of root growth was marked and the seedlings transferred to 30, 37, 40 or 42 °C for 5, 10, 30, 60, 120 or 180 min before being returned to 21 °C for 48 h. The error bars show the SE. Numbers of plants per treatment varied from 50 (42 °C 120 min) to 370 (no heat shock)

To ensure that all the clones observed were induced in the post-embryonic root meristem the excision of the *Ds* is under the control of a heat-shock-inducible *Ac* transposase (*HS:Ac*) (Fig. 2) Exposure to elevated temperature (heat shock) at 3 d after germination results in transient transcription of the transposase which catalyses the excision of the *Ds* in a small proportion of cells, resulting in a heritable active *GUS* gene.

Lines homozygous for *HS:Ac* were crossed to lines homozygous for the *GUS-Ds* transgene. F2 and F3 generations from the crosses were used for the clonal analysis. Incubation at 37 °C for 30 min was sufficient to induce clones of *GUS* expression in some part of the plant (root, hypocotyl, cotyledon, leaf) in approximately 50% of seedlings. These conditions were used for induction of all the clones analyzed here. Over the course of these studies, about 4% of seedlings produced clones of *GUS*-expressing cells in the root.

To establish the effect of elevated temperature on root growth, seedlings were grown for 3 d at 21 °C and then transferred to a range of temperatures for various lengths of time. Root length was measured (in mm) 48 h after heat shock (Fig. 3). Temperatures over 40 °C for periods greater than 1 h resulted in an inhibition of root growth while roots grown for up to 3 h between 30 °C and 37 °C exhibited no detectable heat-induced growth defects. This suggests that incubation at 37 °C for 30 min does not affect root growth. This fluctuation in temperature is within the normal range of conditions for plants such as *Arabidopsis thaliana* that grow in exposed areas and have shallow root systems. We therefore consider the cell division patterns determined using a

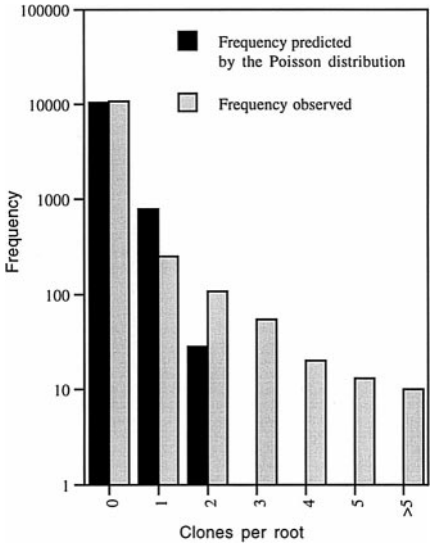

Fig. 4. The observed distribution of clones per *Arabidopsis* root (*grey bars*) compared to the expected distribution (*black bars*) based on the Poisson distribution. The observed and expected distributions differ significantly ($\chi^2 > 5000$) indicating that the excision of *Ds* is not an independent random event

30-min heat shock to be a reasonably accurate reflection of the activity of the true meristem.

In a population of 11,214 seedlings subjected to a 30-min heat shock over the course of this study, 849 clones were found on primary roots of 453 plants. The large number of clones induced in some seedlings, in combination with the absence of clones in 10,761 plants, suggests that induction of clones is not a random event. The expected frequencies (based on a Poisson distribution – assuming random induction of clones) and observed frequencies are presented in Fig. 4. While the "0 clones/root" class was the most frequent, the observed and expected frequencies differ greatly (chi-squared $P < 0.001$, 6 d.f.). This suggests that there may be a sub-population of plants with elevated levels of transposase activity, as has previously been reported for plants carrying *HS:Ac* (Keller et al. 1992).

Frequency of clone induction is high in the initials and central cells. The cell type distribution of the 849 clones is shown in Table 2. Many of the clones (128) are present in more than one cell layer. These clones include those induced in initials that gave rise to cells located in more than one tissue type (cortex/endodermis, lateral root cap/epidermis), clones that include more than one unrelated tissue (clones found in tissues that are not derived from a common initial cell), and clones that include central cells and other tissues.

Table 2. The distribution of clones between cell layers

Tissue	Clones
Columella	127
LRC	118
Hair file cells	64
Both hair and non-hair files	13
Non-hair file cells	64
Cortex	87
Endodermis	59
Pericycle	118
Stele	71
Others[a]	128
Total	849

[a]Clones including more than one cell layer

To interpret these clones we must consider the manner in which they were induced. Clones are induced when *Ds* excises from *35S:GUS* producing a functional *GUS* gene. Transposition of *Ds* largely takes place during or just after DNA replication with only one of the two daughter cells inheriting the excision which results in active *GUS* (Greenblatt 1974). Therefore, half the clones induced will display GUS activity in the mother cell only, while in the other half, the functional *GUS* will be inherited by the daughter cell (and the mother cell will not be included in the clone). For most cell divisions, both daughter cells have the same fate, i.e. both daughters of a cortex cell generally become cortex cells themselves. However, the two daughter cells of the initials can have different fates. The lateral root cap/epidermis initials undergo a periclinal division to produce an inner cell (initial) and an outer cell (lateral root cap daughter) (Fig. 1B). If the functional *GUS* is inherited by the lateral root cap daughter, the clone produced will be indistinguishable from a clone induced in the lateral root cap. If on the other hand, an excision event were inherited by the newly produced initial, it would go on to form a clone that includes both epidermis and lateral root cap. If we assume that the probability of inheriting the excision (active *GUS* gene) is equal for each daughter cell, the total number of clones induced in the lateral root cap/epidermis initial would be twice the number of clones observed that include both cell layers ($2 \times 90 = 180$) and 90 of the 118 clones which included only lateral root cap cells were actually induced in the initial. The remaining 28 clones were induced in the rest of the lateral root cap.

The situation in the cortex/endodermis is different since the two tissues are not produced by division of the initial but by an asymmetric division in the daughter of the initial cell. The initial cell divides to produce one cell that is still in contact with the central cells and an upper cell. The lower cell, in contact with the central cells, remains the initial and the upper cell divides periclinaly to produce an inner (endodermis) and an outer (cortex) cell Fig. 1B. Clones induced in the upper daughter cell will give rise to clones in either the endodermis or the cortex but not both. Therefore, all the clones induced in the initial, will result in a clone that includes both endodermis and cortex. Twenty three such clones were observed.

One hundred and twenty seven clones were found in the columella. Only the columella initials divide and endoreduplication occurs in the third cell from the initials (Dolan et al. 1993). Single cell clones three or more cells from the initial are presumed to result from excision during endoreduplication. All other clones in the columella represent clones induced in initial cells (80 clones).

The number of clones induced in the initials is surprisingly high: of a total of 849 clones 283 were initial clones. Initial cells divide at a 3-fold lower rate than the rest of the meristem (Fujie et al. 1993; Dolan et al. 1993). Those of the lateral root cap/epidermis, endodermis/cortex and columella (for which initial clones can be readily identified), comprise 2.06% of the meristem (36 cells out of a total estimated 1747 cells in the meristem; see Table 1). The total number of clones induced in the meristem (excluding initials and central cells) is 556 [849 − 180 (clones induced in initial cells of the lateral root cap/epidermis) −23 (clones induced in the initials of the endodermis/cortex) −80 (clones induced in the initials of the columella) −10 (clones induced in the central cells)]. Therefore, $(556 \times 0.0206)/3 = 3.82$ is the expected number of initial clones, and 283 clones represents approximately 74 (283/3.82) times the number predicted.

Daughter cells of a central cell division displayed a variety of potential fates. The clones including central cells found in this population suggest that replacement of adjacent initials is more common than generation of an additional central cell. Five clones in this population included the central cells. This represents half the number of clones induced in central cells, since in 50% of excision events the functional *GUS* gene is inherited by the daughter cell that no longer occupies the position of a central cell. The number of clones induced in the central cells is therefore approximately 10.

Given that central cells divide at 10.9% the rate of meristematic cells (Fujie et al. 1993) and comprise only 0.23% of the meristem (4 cells of an estimated total of 1747 – see Table 2), we expected to find 0.14 ($556 \times 0.0023 \times 0.109$) clones originating in central cells given that 556 clones were induced in the meristematic cells. The number of clones induced in the central cells of this population (approximately 10) therefore represents a 70-fold excess over the number expected. This excess of central cell clones is similar to the excess number of initial clones that were found.

Central cells can divide to replace initial cells of the columella, pericycle or stele. Serial sections through all of the clones induced in this study revealed that five included central cells. These clones demonstrate that the central cells of *Arabidopsis* divide and that they can replace the adjacent initials. Three serial sections through pericycle and stele initials, central cell and columella initial zones, and diagrams showing the longitudinal extent of each clone are presented in Fig. 5. The interpretation of these clones is as follows below.

Clone A9.2 includes a single central cell, 5 stele initials above and extends into the meristematic stele. The formation of A9.2 may have involved the transverse division of a central cell with the upper daughter cell subsequently undergoing longitudinal divisions to generate five smaller stele initial cells. Each new stele initial went on to give rise to a file of approximately 40 cells. This was the largest clone in the population.

Clone G17.1 includes a single central cell, the underlying columella initial and one cell in the tier below the initial. The formation of this clone may have involved a transverse division in a central cell producing a daughter cell that replaced the columella initial below. The daughter then divided once as a columella initial.

Clone I3.1 includes a single central cell, two columella initial cells, two cells in one columella file and a single cell in an adjacent file. The formation of clone I3.1 may have involved a transverse division in a central

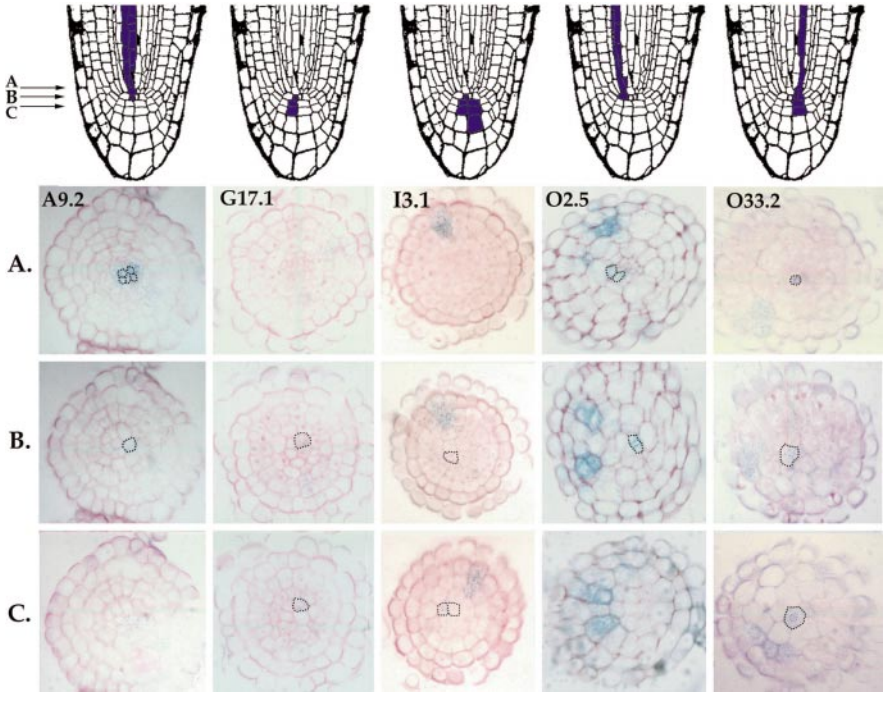

Fig. 5. Clones including the central cells. Serial transverse (5 μm) sections through the pericycle and stele (*A*), central cells (*B*) and columella initials (*C*) of roots containing central cell clones. The GUS-expressing cells that are part of the clone are ringed by a black dotted line. Lateral root cap/epidermis clones are also visible in each section. The longitudinal extent of clones that include the central cells is indicated in the diagrams above the sections. These clones indicate that the daughter cells of central cells (resulting from division in the four cells of the quiescent centre) can replace the initials of the stele, pericycle and columella

cell, the basal derivative becoming a columella initial, which then divided longitudinally to form another initial. One initial then underwent a further round of cell division to form a single daughter cell, while the other underwent two rounds of cell division to form two daughter cells.

Clone O2.5 includes a central cell and two files of pericycle above it. A central cell divided transversely, the upper cell then divided longitudinally, forming initials of the pericycle and stele. Each initial gave rise to a file of 25 cells.

Clone O33.2 includes a central cell, the columella initial below it and a single columella cell below the initial. The clone extends above the central cells including two initial cells and continues into the stele. The development of this clone may have involved a longitudinal division of a central cell to form a marked pair. One of these central cells then divided transversely to form a columella initial that divided once more to produce a columella cell. The other central cell divided transversely to form a stele initial. This initial subsequently formed a packet of cells that extended into the stele.

These clones indicate that cells derived from central cells can give rise to stele, pericycle and columella cells. Our small sample size means that it is not possible to say if the division of central cells can also give rise to cells in the lateral root cap/epidermis and cortex/endodermis lineages.

Initial cells are replaced. The lifetime of initials can be estimated from clones including initial cells. As described above, only half of the clones induced in initial cells will include the initial cell. The displacement of these clones from their original position reveals replacement of initial cells. As the functional GUS gene can be inherited by either daughter cell, only half of the clones induced in the lateral root cap/epidermal initial include the initial. These can be recognised as they include both tissues. Any clone including both lateral root cap and epidermis that does not include the current initial must be the result of an initial cell displacement event. Of the 90 identified lateral root cap/epidermis initial clones, 63 include both cell types but do not include the initial. Therefore 70% (63/90) of the lateral root cap/epidermal initials in which clones were induced were displaced by replacement of the initial. This suggests that of the 16 lateral root cap/epidermis initials in a root, about 11.2 (0.7×16) are replaced during the 48 h following heat shock.

Since cell division is restricted to the initial cell of the columella (Dolan et al. 1993) all clones are induced in the initial. If initials were being displaced, then more than the predicted 50% of clones induced in the initial would be displaced. The deviation from 50% displacement provides a measure of initial instability. Of a total of 80 columella clones, 55 do not include the initial (this is three standard deviations greater the expected number (40), $P < 0.0025$). The estimated number of displaced initials is 15 (55–40), therefore 37.5% (15/40) of the columella initials that inherited active *GUS* were displaced. Of the 12 columella initials, approximately 4.5 (0.375×12) are replaced during the 48 h growth between heat shock and GUS staining.

In the endodermal/cortex lineage of a total of 23 clones that were induced in initial cells, 14 clones did not include the initial. In half of the excision events the functional GUS is inherited by the daughter that does not remain the initial; therefore, 11.5 (50% of 23) is the expected number of clones which would not include the initial. The observed number of clones not including the initial is 14, a deviation of 2.5. This is just over one standard deviation from the expected value ($P = 0.3174$) and consequently not significant. A small proportion of cortex/endodermis initials may be replaced but the numbers of initial clones in this population is not large enough to allow detection.

From this population of clones it appears that the lateral root cap/epidermis initial and columella initials are unstable but there is no evidence that the endodermis/cortex initials are replaced. The frequency of replacement of the lateral root cap/epidermis and columella initials appears high: 70% and 37.5% of initials, respectively, were replaced in the 48 h between heat-shock and GUS staining. However, the large numbers of clones induced in the initials and the central cells in this population may reflect an increase in the division rates in cells of heat-shock-treated roots. This may lead to increased replacement of initials by adjacent initial and central cells. Approximately 70 times more initial and central cell clones were observed than were expected on the basis of previous estimates of relative rates of cell division in the root. If this reflects a similar increase in cell division rates and a similar increase in the rate of replacement of initials, the rates of replacement observed here would indicate 0.16 ($1/70 \times 11.2$) lateral root cap/epidermis initials replaced per 48 h (or one over 13 d) and 0.064 ($1/70 \times 4.5$) columella initials replaced per 48 h (or about one over 31 d). Thus, a number of lateral root cap/epidermal initials might be replaced over the life of a meristem. The columella and cortical/endodermal initials are more stable and probably exhibit little turnover in the life of an individual meristem. Therefore, the initials of different cell types are replaced at different rates.

Successive invasions occur in some lineages of the root. Cell layer invasions occur when a daughter cell occupies a different cell layer from the mother cell. A subset of such invasions could be identified in this population as clones that included two or more cell types that did not share a common initial. Potential invasions can be divided into two distinct classes – those involving a single invasion event (small invasions) and those clones that span more than two tissue layers (large invasions). Three clones were found to include pericycle and adjacent stele cells. This suggests that these two cell types may be derived from the same mother cells. Three further small invasions were observed but will not be discussed further because their low frequency does not allow us to distinguish between genuine invasions and chance *Ds* excisions in adjacent cells.

Large invasions represent an unusual pattern of cell division that may be due to a specific underlying event. Five clones that span more than two cell layers were found among the population of 849 clones. It is unlikely that these represent five multiple adjacent excision events. Transverse sections through the clones are shown in Fig. 6 and schematic representations of both the radial and longitudinal extent of the clones are shown in Fig. 7. The clones have three features in common, suggesting that each is due to a similar event: (i) 48 h after induction, all are located outside the meristem (displacement from the central cells ranges from 525 to 2105 μm; (ii) all clones are about the same length, ranging from 10 to 13 cells long; and (iii) all probably start in the endodermis or cortex since the basal region of the clones includes endodermis and cortex. A striking feature of these large invasions is that there is neither a concomitant increase in cell file number per cell layer nor is gross cellular disorganization observed in regions of the root occupied by such clones. This is particularly striking in clones N23.1 and A30.3.

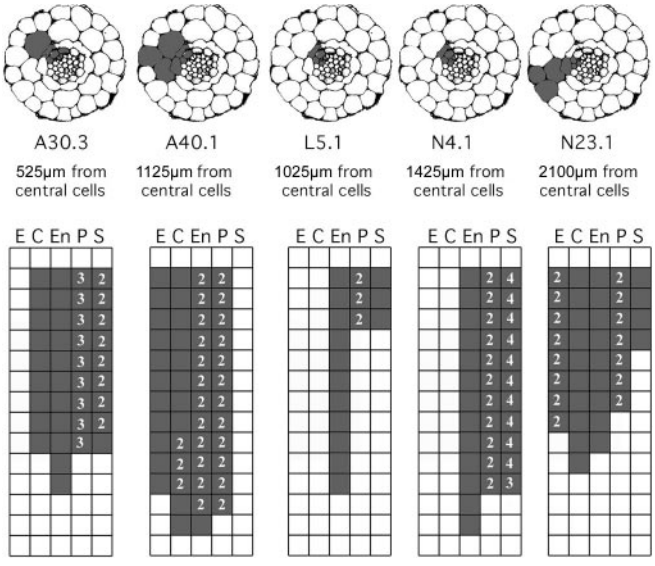

Fig. 7. Representations of the radial and longitudinal extent of the clones (*shaded*) including successive invasion events. The *upper diagrams* show the radial extent of the clones, and the distance of the basal-most cell of the clone from the central cells is indicated. The *lower schematic* shows the longitudinal extent of the clones in each cell layer: *E*, epidermis; *C*, cortex; *En*, endodermis; *P*, pericycle; *S*, stele. Each box corresponds to one cell. If the clone includes more than one cell per tissue layer, the number of cells in the tissue layer is indicated in the boxes

Discussion

Results presented here demonstrate that the central cells of *Arabidopsis* can act as stem cells for the root. Like the shoot initials the root initials are unstable, demonstrating that even in a highly organized meristem, variation in cell division patterns does occur.

Instability in the root meristem. The size and distribution of clones described in this study show that some of the root initials are unstable. Shoot meristem initials are also unstable (Furner and Pumfrey 1992), with about 10% of initial clones lost from the shoot apical meristem each plastochron. The plastochron in *Arabidopsis* is 1–2 d (Pyke et al. 1991), indicating that the lifetime for shoot initials is about 10–20 d. This can be compared to lifetimes of approximately 31 d for columella initials and 13 d for lateral root cap/epidermal initials.

Reports on the effect of environmental factors on initials suggest that the lifespan of initials is variable. In maize the initials of the root cap are replaced in response to changes in temperature (Clowes and Wadekar 1989). In *Vicia faba*, cells from the quiescent center (QC) replace all the initials after exposure to radiation (Clowes 1961; Davidson 1961). Results reported here suggest that a heat shock of as little as 37 °C for 30 min

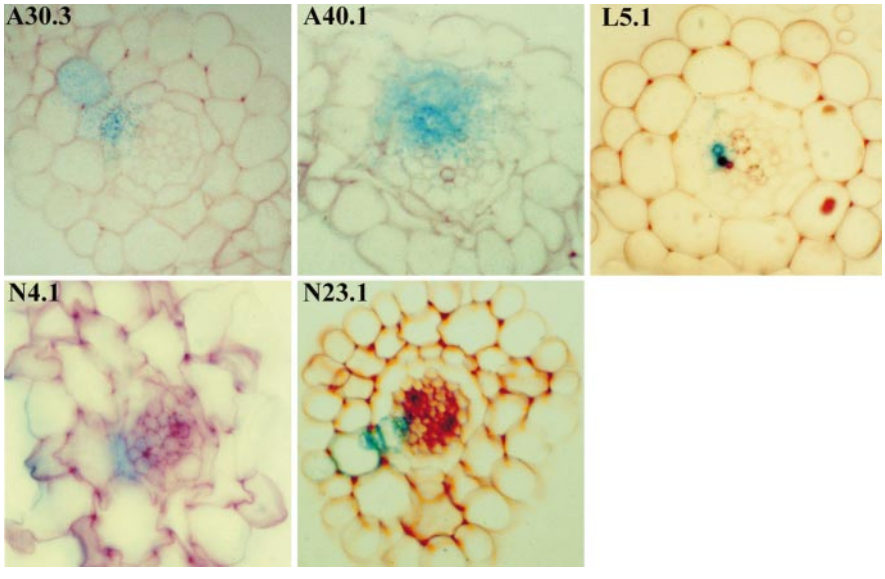

Fig. 6. Clones including successive invasion events. Transverse sections (20 μm thick) through the clones A30.3, A40.1, L5.1, N4.1 and N23.1 show the radial extent of clones

may increase the division rate and the rate of replacement of initials. In maize and *Vicia* the initials are replaced by the daughter cells of the QC. Clones described in this paper show that this can also happen in the *Arabidopsis* root. As suggested by Barlow (1978) the QC could act as the ultimate source of cells for the whole root though their actual contribution may vary depending on the environment.

The central cells are stem cells for the root. Results presented here show that the initial cells of the root are periodically replaced – probably by the daughter cells of a central cell division. The central cells of the root are therefore analogous to the initials of the shoot meristem – few in number and acting as stem cells. The criteria by which stem cells are defined vary but commonly include: mitotic quiescence, self renewal, asymmetric divisions producing one initial daughter and one daughter that remains a stem cell, and the ability to clonally regenerate the entire adult tissue of the organ to which they belong (Barlow 1978). The four central cells of *Arabidopsis* have been shown to be mitotically quiescent (Dolan et al. 1993; Fujie et al. 1993). Clone O33.2 (Figs. 5, 6) illustrates self renewal; two daughter cells were produced, both of which remained central cells. The divisions of the central cells are mainly asymmetrical, producing one daughter that replaces an initial and another daughter that retains the properties of a stem cell, remaining part of the quiescent center. The clones illustrate that dividing central cells can replace the adjacent initials of at least the columella, the stele and the pericycle. As the sample consisted of only five clones, replacement of the cortical/endodermal or lateral root cap/epidermal initials cannot be ruled out. It is possible that the central cells of *Arabidopsis* can re-populate the entire meristem, as observed in the quiescent centers of maize (Feldman and Torrey 1976) and *Vicia faba* (Davidson 1961). The role of the central cells may include the replacement of initial cells after environmental damage or at the end of a limited life span as initials.

Positional information. The instability of initials, and the variation in the pattern of cell divisions of both meristematic and central cells, suggests that the fate of a root cell is independent of its lineage. If this were not the case then these rare "aberrant" divisions would distort the pattern of cell differentiation. There is no evidence for this being the case since the morphology of cells in all the clones including invasions is normal. This is in agreement with the demonstration of a role for positional information in the specification of cell fate in roots, i.e. it is the position of a cell relative to its neighbors that directs fate (van den Berg et al. 1995, 1997; Berger et al. 1998). Cell divisions in the meristem may be seen as adding to a population of cells that respond to a pre-existing homeostatic pattern originally set up in the embryo. A similar plasticity is maintained in shoots (Pouteau et al. 1997). The adoption of five different cell fates by descendants of a single cell (clone A30.3, N23.1, Fig. 7) reflects an extreme, though not unexpected plasticity. What is unexpected is that the descendants of a single cell find themselves in such a wide range of positions in the root without disrupting the stereotyped cell file pattern.

These clones may be the result of extensive reorganization of the initial region with derivatives of a single cell replacing several initial cells in adjacent cell layers. The initial clones indicate that initial replacement is rare and no evidence was found for replacement of the endodermis/cortex initial. Endodermis or cortex cells are included in all these clones so this suggests that their formation was not part of a normal round of initial replacement but a singular occurrence, perhaps in response to heat shock. Alternatively, a possible mechanism for the formation of large invasions is one of intrusive growth.

Intrusive growth. The large invasions described demand that a mechanism must exist in the root allowing cells to invade adjacent tissue. Such invasions may result from a form of intrusive growth similar to that described for developing fibre cells. The fusiform initials that give rise to fibres are shorter than the mature cells and this difference is due largely to growth that takes place at the tips of the growing cells. Since many adjacent cells develop as fibres it can be concluded that they must each grow past each other as opposed to sliding (Sinnott and Bloch 1939). Intrusive growth is a process in which new wall deposition is localized to a particular region of the cell surface and results in the insertion of a growing cell between two adjacent cells (Wenham and Cusick 1975). It is accompanied by the separation of adjacent cells walls of neighboring cells. The formation of the 'large invasion' class of clones could be due to intrusive growth of a cell into a neighboring cell layer, accompanied by cell division in which the new cell plate is aligned to recreate the original invading cell and a new daughter in the next cell layer.

The population of clones represents about 8000 cell divisions occurring between 3 and 5 d after germination; 1/1600 of these divisions gave rise to large invasions. This suggests large invasions were relatively frequent in this population of roots. The trigger for a cell to undergo intrusive growth may be the sudden release of wall pressure that would accompany damage to or death of a neighboring cell. The invading cell would then occupy the space previously occupied by the corpse, and this would explain the lack of cell pattern disruption that characterizes the invasions described here. It has been shown that the artificially induced death of a cell (by laser ablation) is accompanied by the invasion of the vacated space by an underlying cell which undergoes a cell division immediately afterwards (van den Berg et al. 1995; Berger et al. 1998).

If this explanation for invasions were correct, invasion frequency would then reflect the frequency of cell deaths that occur in the root, which may be higher in heat-shocked roots. This explanation does not take into account the fact that large sectors are present in more than two tissue layers. It is possible either that large clones are formed when cell death occurs in two adjacent cells layers or that invasion of one layer (in response to a

death) is followed directly by the invasion of an adjacent layer.

Conclusion. Clowes (1961) describes root apical meristems as divided into two classes – 'open' and 'closed'. Open meristems are those in which the apex of the root contains no clearly defined layers of initials, and cell division patterns appear random. In 'closed' meristems a clear pattern of cell divisions is visible with each tissue layer terminating in a discrete set of initials surrounding the QC. The *Arabidopsis* root is an example of a closed meristem – with a clear set of initials for each tissue type and a minimally sized QC of four central cells. With each tissue layer composed of a single layer of cells any changes in the pattern of divisions would clearly disturb the pattern of tissues and may disrupt root function. However, evidence presented in this paper shows that even in *Arabidopsis*, variations in cell division patterns do occur, and when they occur they do not disrupt the root anatomy. Maybe this is to be expected as plants are limited in their ability to avoid environmental damage so must be able to repair damage as it occurs. That changes to the typical division patterns did not cause disruption of the tissues is testimony to the presence of a robust positional patterning system in the root meristem.

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