TECHNICAL ADVANCE

Marking cell lineages in living tissues

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Summary

We have generated a novel genetic system to visualize cell lineages in living tissues at high resolution. Heat shock was used to trigger the excision of a specific transposon and activation of a fluorescent marker gene. A histone-YFP marker was used to allow identification of cell lineages and easy counting of cells. Constitutive expression of a green fluorescent membrane protein was used to provide a precise outline of all surrounding cells. Marked lineages can be induced from specific cells within the organism by targeted laser irradiation, and the fate of the marked cells can be followed non-invasively. We have used the system to map cell lineages originating from the initials of primary and lateral roots in Arabidopsis. The lineage marking technique enabled us to measure the differential contribution of primary root pericycle cell files to developing lateral root primordia. The majority of cells in an emerging lateral root primordium derive from the central file of pericycle founder cells while off-centre founder cells contribute only a minor proliferation of tissue near the base of the root. The system shows great promise for the detailed study of cell division during morphogenesis.

Keywords: cell lineage, laser induction, root development, live cell, YFP, heat shock.

Introduction

Multicellular organisms develop via a coordinated pattern of cell division and cell differentiation. In plants such as *Arabidopsis thaliana*, cell division during embryogenesis is highly regular, resulting in a close correspondence between cell lineage and fate. However, in the adult plant, cell divisions are often less regular and it is clear that constant positional signals are exchanged between cells and their neighbours to mediate development (Sena *et al.*, 2004). To understand development it is important to have both genealogical information and an understanding of the influence of neighbouring cells.

Various methods have been employed to trace cell lineage in plants. The earliest observations were based on microscopical studies. In plants, these anatomical studies gave the first understanding of lineages but these were not entirely reliable because of the difficulty of identifying thinner, newly formed primary cell walls after cell division. Clonal analyses of genetic mosaics, generated as periclinal chimeras, that contain cells marked by polyploidy or albinism (Doring *et al.*, 1999; Marcotrigiano and Bernatzky, 1995; Pyke *et al.*, 2000; Szymkowiak and Sussex, 1996) allowed the generation of fate maps that have furthered the study of development. Additional techniques have been employed including X-ray-induced sectoring (Furner and Pumfrey, 1992, 1993; Irish and Sussex, 1992) and microinjection (Lusardi *et al.*, 1994). The use of DNA recombination to activate the expression of marker genes increased the ease of studying lineages (Bossinger and Smyth, 1996; Kilby *et al.*, 1993; Sieburth *et al.*, 1998). The Cre/*lox*P recombination system was used in mice and Arabidopsis (Dor *et al.*, 2004; Kilby *et al.*, 1993; Sieburth *et al.*, 1998), and FLP recombinase in tobacco, Arabidopsis (Kilby *et al.*, 1995, 2000) and *Drosophila* (Besse and Pret, 2003; Weigmann and Cohen, 1999). Natural transposons have provided markers for cell lineage in maize and *Antirrhinum majus* (Bossinger *et al.*, 1992; Carpenter and Coen, 1991) and the maize *Ac/Ds* transposition system

has been artificially introduced into Arabidopsis and used successfully to create mosaics (Balcells *et al.*, 1994; Dean *et al.*, 1992; Dolan *et al.*, 1994; Scheres *et al.*, 1994).

The use of inducible promoters has further enhanced the utility of recombinant systems; it allows the expression of the transgene to be induced at the desired time point in development. The most popular type of inducible promoter is the heat shock promoter from soybean in plants (Kilby *et al.*, 1995) and from *Drosophila* in animals (Halfon *et al.*, 1997; Stringham and Candido, 1993). By placing the *Ac* transposase under the control of a heat shock promoter from soybean, *Ac*-mediated transposition was induced at high temperatures (Balcells *et al.*, 1994).

This paper describes a novel transposon-based system in Arabidopsis which uses heat shock-induced transposase activity to switch on expression of the nuclear-localized yellow fluorescent protein (*YFP*) reporter gene. This reporter gene is normally transcriptionally silent due to the presence of a dissociator (*Ds*) element inserted between the promoter and reporter. Heat shock switches on expression of the Ac transposase which in turn excises the *Ds* element causing the reporter to be expressed in that particular cell and in all cells that derive from it (Figure 1).

Cell lineage patterns arising within the tissue initial zone of the primary root apical meristem (RAM) were analysed. Arabidopsis primary root tissue development is very regular and has been described from sectioned material. The origination of each of the tissues is well described (Baum *et al.*, 2002; Dolan *et al.*, 1993; Scheres *et al.*, 2002; Wenzel and Rost, 2001). Dolan *et al.* (1993) describe a model of main root meristem organization in Arabidopsis. In the model, initials of the different root tissues are located surrounding
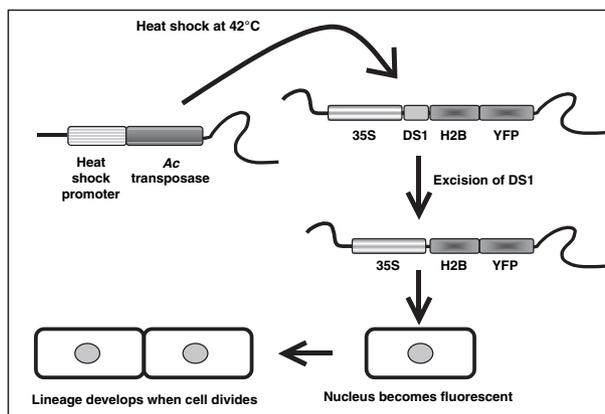

Figure 1. Schematic representation of the live-cell lineage marking system. The *Ac* transposase is under the control of the heat shock promoter. The other half of the system consists of the reporter gene, *H2B-YFP* (nuclear localized YFP) under the control of the 35S promoter. The *DS1* element is cloned between the promoter and reporter so that the reporter gene is not expressed. Upon heat shock at 42°C, the transposase excises *DS1* which allows the reporter gene to be switched on and the nucleus in that particular cell becomes fluorescent. All cells that derive from this cell also have a fluorescent nucleus.

the central cells of the quiescent centre; epidermis and lateral root cap (LRC) share a common initial as do cortex and endodermis. Pericycle, vascular tissue and columella files all derive from single initials. These findings were supported by clonal sector analysis in fixed tissues (Dolan *et al.*, 1994). Our live-cell lineage analysis supports the findings of these earlier reports and demonstrates the utility of the system.

Unlike the main RAM which is fairly easy to observe, lateral root initiation is difficult to study, especially in living roots as the new cell walls are very thin and occur within the primary root where stains, e.g. propidium iodide, do not penetrate. The best images of early developmental stages come from studies of cleared whole-mounts (Malamy and Benfey, 1997) and sectioned materials (Dubrovsky *et al.*, 2000, 2001). Lateral roots initiate in the pericycle layer of primary roots. Pericycle cell-cycle marking studies (Beeckman *et al.*, 2001; Himanen *et al.*, 2002) and cell type-specific marking (Malamy and Benfey, 1997), each utilizing GUS staining, showed the earliest indications of lateral root initiation. In addition, Malamy and Benfey (1997) produced a cell lineage map of the median longitudinal view of the emerging lateral root primordium (LRP). The relative contribution of different pericycle cell files to the LRP is not clear, however various studies placed the number of LRP founder cells at 6–11 (Casimiro *et al.*, 2003; Dubrovsky *et al.*, 2000; Laskowski *et al.*, 1995). Pericycle cells in longitudinal files in contact with the xylem differentiate as LRP founder cells. Generally, two files of pericycle cells are in direct contact with a xylem file but a minimum of three files of the pericycle are usually involved in LRP formation (Casimiro *et al.*, 2003). Xylem-contacting pericycle cells show the first signs of differentiation as LRP founders but adjacent files also contribute to the LRP. We have used our lineage marking system combined with a live-cell outlining marker to demonstrate uneven contribution of the LRP pericycle founder files to the emerging lateral root.

In addition, we show that the lineage marking system is laser-inducible to allow targeting of specific cell types as lineage progenitors.

Results

Heat shock induction can be used successfully to generate marked live-cell lineages

Arabidopsis plants carrying the *Ds1* construct (Ds-H2B-YFP) were genetically crossed with hs-Ac plants. F_1 seedlings from the crosses were analysed for the ability to induce activation of the H2B-YFP gene after heat shock. One of the tested lines (P6/7 × hs-Ac) showed a high level of transposition, the others had low to no transposition. Subsequent analyses were carried out with the progeny of this line (approximately 60% of these seedlings had one or more transposed sectors). Seedlings subjected to heat shock at 42°C showed

a minimum requirement of 20 min (data not shown) before any transposition was observed. Routinely, heat shock was applied to seedlings on media plates for 30 min.

Using the lineage marking system to analyse clonal relationships in the primary root

Before commencing the analysis, we checked that the reporter gene, H2B-YFP, was capable of being expressed at high levels in every cell type in the primary RAM. We analysed Arabidopsis seedlings carrying a 35S-H2B-YFP construct (Boisnard-Lorig *et al.*, 2001). Nuclei were fluorescent in every cell in every file of the RAM (not shown).

In hs-Ac/Ds-H2B-YFP lines, cell lineages demonstrating the origin of every cell type in the RAM were observed. Examples of tissue initiation are shown in Figure 2(a–d). In each case, a progenitor cell and its derivatives were marked with fluorescence. The LRC and epidermis derive from a single initial cell (Figure 2a,b). Subsequent divisions of this cell and its daughter cells resulted, on
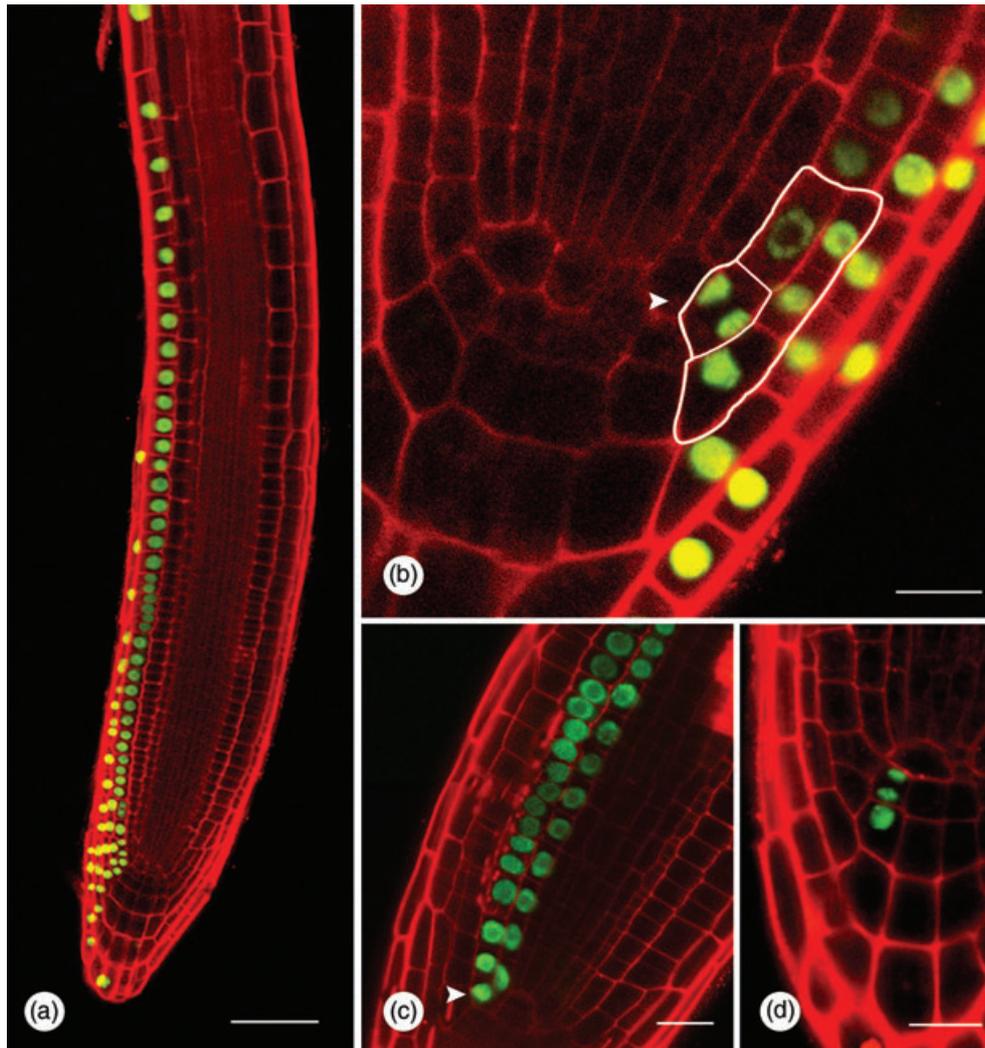

Figure 2. Main root live-cell lineages.
Nuclei marked by H2B-YFP (green) represent cells derived from single progenitors that were marked by a heat shock-induced transposition event. Root morphology is revealed by counterstaining with propidium iodide (red).
(a) An epidermal/lateral root cap lineage in which >60 epidermal cells were marked (42 epidermal cells shown). Note that it is not until lateral root cap cells are lost from the root, about half way up in the figure, that epidermal cells form the outer layer. Bar = 50 μm.
(b) Derivation of the epidermis/lateral root cap (as in a). The initial cell (inner outlined region) divides periclinally and then anticlinal divisions of the daughter cells produce lateral root cap progenitors on the outer side and the epidermis on the inner side of the root (outer outlined group). The apical daughter cell of the inner anticlinal division (arrowhead) remains in place as the initial for both tissues. Bar = 10 μm.
(c) Cortex (outer marked layer) and endodermis (inner marked layer) also derive from a common initial cell (arrowhead) during early stages of root development. Bar = 15 μm.
(d) Columella files arise independently from an initial cell that is in contact with one of the large central cells of the quiescent centre. Bar = 10 μm.

occasion, in marked epidermal cell files >60 cells long (42 marked epidermal cells are shown in Figure 2a). The LRC/epidermis initial first divides periclinally (Figure 2b, inner outlined group). Subsequent anticlinal divisions within the two resultant layers produce the LRC to the outside and the epidermis to the inside (Figure 2b, larger outlined group). The LRC/epidermis initial cell is regenerated as the distal (towards root tip) cell in the inner layer. Root cortex and endodermal layers also derive from a single initial cell, the basal (away from root tip) daughter of which divides periclinally to form the endodermis towards the inside and the cortex towards the outside (Figure 2c). Uniseriate files of root cap columella cells (Figure 2d) and stele cells (not shown) derive from a single initial cell in each file. Fluorescence was rarely observed in the central cells of the quiescent centre and no consistent lineage patterns were observed after such an event. Often, a low level of YFP fluorescence was observed in the columella cells at the root tip. This fluorescence was very dim compared to that resulting from *Ds1* transposition.

Analysis of lateral root lineages

Use of the EGFP-LTI6b plasma membrane marker enhanced visualization of cellular architecture within initiating LRP where traditional stains (e.g. propidium iodide) do not penetrate. Heat shock-induced transposition of *Ds1* resulted in lineage marking within LRP. Of particular interest was the situation in which a single file of pericycle cells was marked in the primary root (Figure 3a–d). Two outcomes resulted,

Figure 3. Lateral root and stomatal lineages.
Nuclei marked by H2B-YFP (red) represent cells derived from a single progenitor that was marked by a heat shock-induced transposition event. Root anatomy is revealed by expression of the plasma membrane marker EGFP-LTI6b (green). In Figure 2(a–d), a single file of pericycle cells is marked in the main roots from which the lateral root primordia are emerging.
(a) When the marked axial file of pericycle cells is the central file in the lateral root primordium (LRP), all cells in the median section of the inner and outer layers of the developing LRP are marked.
(b) Ultimately, cells of the entire lateral root derive from the single marked file of pericycle cells in the situation described in Figure 2(a).
(c) When the axial file of marked pericycle cells is adjacent to the central file of the pericycle that contributes to the LRP (*zy*-plane), the proliferating cells in the median section (*xy*-plane) are not marked.
(d) The situation of Figure 2(c) results in a small group of marked cells that flank the emerging lateral root base.
(e) Random heat shock transposition events within proliferating cells can result in marked lineages of various sizes within the LRP.
(f) The live-cell lineage marking system works in all plant tissues studied. Stomatal lineages result from successive asymmetric cell divisions of a single, marked meristemoid progenitor and a final symmetric cell division to produce the guard cell pair in young cotyledons and leaves. Bars = 10 μm.

either almost all cells within the LRP were marked (Figure 3a,b), or marking was confined to a basal group of cells displaced from the centre axis of the lateral root (Figure 3c,d). The first visible sign of LRP formation occurred in three axially adjacent pericycle files when several transverse divisions in single or in axial pairs of pericycle cells are followed by tangential periclinal divisions of an inner pair of derivatives resulting in the inner layer (IL) and outer layer (OL) of LRP cells (Figure 3a). When the marked parent pericycle file was central in the three axially adjacent files that contribute to LRP formation, virtually the entire primordium was marked (Figure 3b). Figure 3(c), however, illustrates the situation in which the marked parent pericycle file is not central. In the median longitudinal section (seen in *xy*-plane), IL and OL cells have formed but cell marking is confined to an adjacent longitudinal file (seen in *zy*-plane) that has not proliferated to the same extent. Proliferation of cells within the marked file results in only a basal group of marked LRP cells (Figure 3d). Occasionally, small, spontaneously arising marked sectors that did not proliferate into the LRP were also observed (Figure 3e).

Analysis of cell lineages in other tissues

The cell lineage marking system was observed to work in aerial parts of Arabidopsis seedlings as well as in roots. Lineage sectors were marked in hypocotyls, stems, cotyledons and leaves. Figure 3(f) shows a stomatal lineage formed by successive asymmetric divisions of a single, marked meristemoid cell and a final symmetric division to produce a guard cell pair.

Laser induction of the live-cell lineage marker

Laser induction of lineage marking is possible. Figure 4 shows a time series. A control image of the main root tip expressing EGFP-LTI6b was taken immediately before laser-induced heat shock (Figure 4a). No H2B-YFP was detected in the root tip. The vascular initials were targeted and heat shocked (Figure 4a, outlined region). Figure 4(b) shows the root tip after pulsed-laser heat shocking. The EGFP-LTI6b has photo bleached in the targeted region. At 6 h after heat shock, *Ds1* transposition had occurred and triggered the production of H2B-YFP to mark a four-cell lineage in one axial file of vascular tissue and a single marked cell in a non-adjacent file (Figure 4c). The most distal (youngest) cells in the marked file remained in the cell division zone of the meristem as the root grew and, at 29 h post-induction, the original marked file had increased via transverse cell divisions to include 14 fluorescent nuclei (Figure 4d, 8 of 14 nuclei shown in the original file). The original single marked cell did not divide and is no longer visible in Figure 4(d). During the same period, one of the earlier marked cells had divided longitudinally to produce a second, adjacent file of

marked cells. Four of the fluorescent nuclei in the marked adjacent file are visible in Figure 4(d).

Discussion

Heat shock-induced marking of cell lineages

We describe a new system to mark and follow cell lineages in living tissues. Use of a live visual reporter gene circumvents the problem of destructive assays and allows one to follow the lineage of a given cell through time without the dilution of marker signal that occurs in other live-cell marking systems, e.g. photo-activation of fluorescent proteins or fluorescent tracer injection. It also overcomes the limitations of the availability or generation of periclinal chimeras and the low frequency of chimerism and associated cell damage generated by X-ray irradiation.

There are several advantages to using a heat shock promoter for triggering the formation of marked clones. The promoters are characterized by quick response times coupled with high induction levels (Lyznik *et al.*, 1995), and heat shock treatment can be adjusted to titrate the frequency of sectoring, and to control the timing of clone formation during development. In addition, localized induction of heat shock using a laser can be used to follow the lineage of a particular cell over several days.

Lineage marking of the Arabidopsis primary RAM

The origin of tissues in the Arabidopsis RAM has been described from sectioned root material (Dolan *et al.*, 1993; Wenzel and Rost, 2001). Live-cell lineages resulting from *Ds1* transposition in tissue initial cells generally support the observations made using sectioned material. The LRC and epidermis arise from asymmetric T-division of a single initial (Dolan *et al.*, 1993; Wenzel and Rost, 2001) to produce a commonly observed wedge-shaped lineage in median section view. Marked epidermal cell files >60 cells long represent at least four to five divisions of the LRC/epidermal initial cell and many daughter cell divisions with no reduction in the brightness of marking over time. Perpetual marking is not possible in other live-cell lineage analysis systems as the marker is diluted by subsequent rounds of cell division. Cortex and endodermal layers were observed to arise both from a common initial and from separate, adjacent initials. This observation is due to alternation in the orientation of the cell division plane in the initial. A previous report (Baum *et al.*, 2002) described the transition to separate initials as the root aged. Columella, pericycle and vascular tissue cell files were all observed to arise from *Ds1* transposition in single initials. *Ds1* transposition to mark the central cells of the quiescent centre was infrequently observed. Very often, the majority of cells in the columella display a dim YFP fluorescence that may have resulted from low levels of leaky
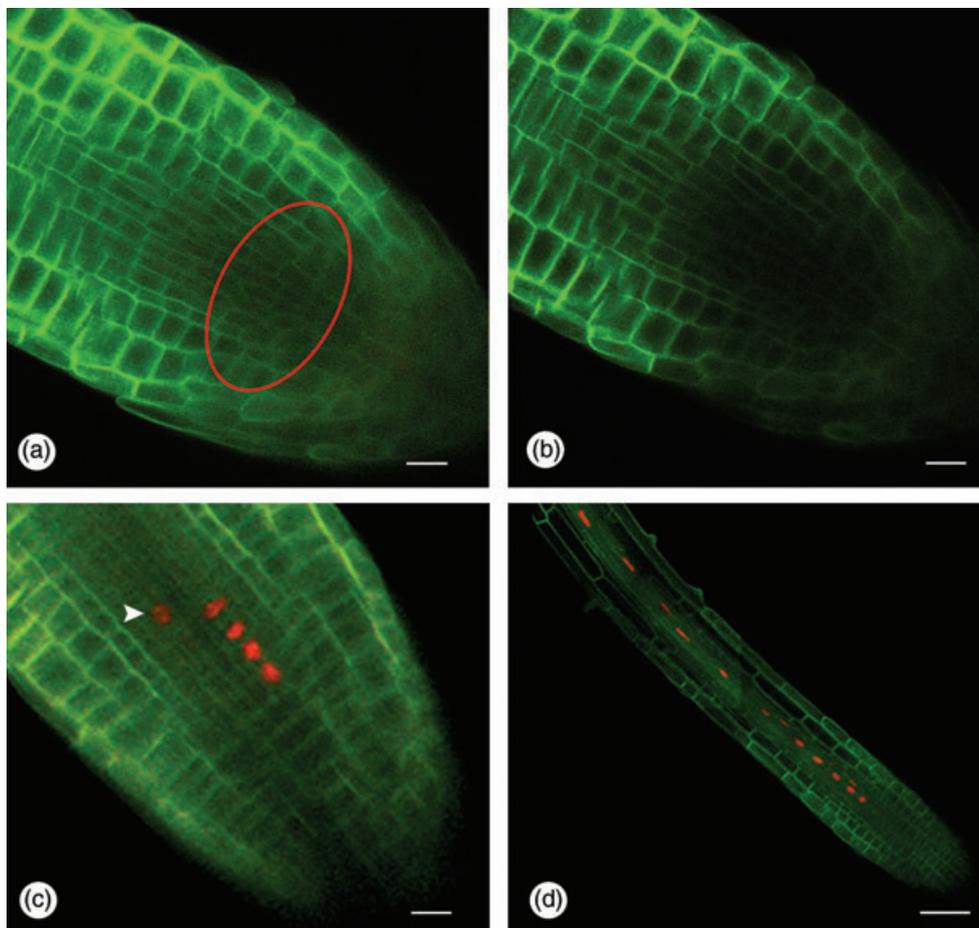
Figure 4. Laser induction of main root lineages.
A time series showing a main root tip growing in nutrient agar. Nuclei marked by H2B-YFP (red) represent cells derived from a single progenitor and root morphology is revealed by expression of the plasma membrane marker EGFP-LTI6b (green).
(a) Pre-laser induction. The zone of vascular initials that was targeted by the laser is shown outlined. No nuclear marking was seen prior to heat shocking.
(b) Immediately post-laser induction. The EGFP-LTI6b has been photobleached in the targeted zone.
(c) Six-hour post-laser induction. Two cell lineages have been marked. One has undergone two rounds of cell division to produce a four-cell lineage and the nearly adjacent marked cell (arrowhead) has not divided.
(d) Twenty-nine-hour post-laser induction. The original four-cell lineage has continued to grow by transverse cell divisions and now includes 14 cells (eight shown). During this period, a longitudinal division has occurred in one of the marked cells resulting in a second, adjacent vascular tissue lineage (four cells visible). Scale bars: (a–c) 10 µm, (d) 50 µm.

H2B-YFP expression amplified by endoreduplication in these cells (Kidner *et al.*, 2000). Nuclear fluorescence in marked columella lineages was an order of magnitude brighter.

Roots were sometimes observed in which cells were marked in adjacent tissue layers. This may have resulted from intrusive growth from a marked tissue into an adjacent tissue layer with a subsequent switch in cell fate, so that the intruding cells matched other cells in the same file (Kidner *et al.*, 2000). Observations of this type can also arise from independent, closely adjacent transposition events, although only about 25% of primary root tips observed had more than one cell lineage marked. Our live-cell lineage marking system will allow direct observation to establish the frequency of invasive growth and positional fate switching during normal growth.

Lateral root initiation

Unlike the primary root primordium which is enclosed within the seed, newly initiated lateral root primordia (LRP) are ideal organs for study of *de novo* patterning as they are accessible to microscope and laser. Unfortunately, LRP initiation is difficult to study in living roots because of the internal location of the first pericycle divisions in the parent root. Stages of LRP development have been described in cleared whole-mounts using histochemical markers of reporter gene activity obtained by wide-field microscopy (Dubrovsky *et al.*, 2001; Malamy and Benfey, 1997). However, due to limited resolution, it was only possible to map the very earliest cell divisions in the non-emergent LRP in median longitudinal view. Our method for outlining cells

with EGFP-LTl6b while marking cell lineages allows clear visualization of cell arrangements throughout the developing primordia, as well as direct visualization of actual division patterns. We have confirmed the order of cell divisions described previously (Malamy and Benfey, 1997) and have provided new insight regarding the contribution of adjacent pericycle files of the parent root to the developing lateral root. Cells in three axial files of the pericycle in contact with the primary root protoxylem undergo a series of transverse divisions to produce the lateral root founder cell population. The number of cells in this founder population is variously put at 6–11 (Casimiro *et al.*, 2003; Laskowski *et al.*, 1995). This number will remain contentious as it depends on how founder cells are defined. Most cells in an emerging LRP, however, derive from the centre file of parent root pericycle founder cells. When the central file of pericycle progenitors is marked by *Ds1* transposition, all cells in the LRP apical meristem region are marked and, ultimately, all cells in the lateral root excepting a few cells at the base are marked (Figures 3b and 5a). When one of the two flanking pericycle files is lineage marked initially, only a small population of cells at the base of the lateral root is ultimately marked (Figures 3d and 5b). This developmental pattern can be studied in a single parent root with a marked pericycle file because while the marked file is central in one LRP it will be

Figure 5. Lateral root primordium initiation.
Schematic of the primary root with outer tissues (green) transparent revealing the pericycle (blue). Cells in three axial files of the pericycle begin to divide signalling lateral root primordium (LRP) initiation. Xylem (red).
(a) When the central file of pericycle cells (yellow nuclei) in the LRP are lineage marked in the primary root, almost all of the cells in the emerging lateral root will be marked indicating their origin from the single, central file.
(b) If the lineage-marked pericycle file is off-centre, only a small basal group of cells is ultimately marked in the emerging lateral root.

flanking in others. The possible function of the small proliferation of cells at the base of the lateral root that derive from an off-centre progenitor file was not determined.

Laser-induced live-cell lineage marking

Confocal microscope systems allow precise targeting of laser output at individual cells. Laser induction of heat shock promoters has been used previously in *Caenorhabditis elegans* (Harris *et al.*, 1996; Stringham and Candido, 1993) and in *Drosophila* (Halfon *et al.*, 1997) but not for live-cell marking. We have used repeated scanning of the 488 nm line from an argon laser to activate the heat shock promoter in specific regions of the Arabidopsis RAM. In this example, when the vascular tissue initials were targeted two separate cells were activated (Figure 4a–d). One of these was in the cell division zone (closer to the root tip) and a 14-cell lineage developed in a single file over the next 29 h. During the 6–29 h after heat shock, a longitudinal division occurred in one of the marked cells resulting in an adjacent file of marked cells. Thus, radial proliferation of the vascular tissue begins within the cell division zone of the root. The second originally activated cell did not divide.

Development of the heat shock protocol required optimization of the laser treatment. Continuous scanning of the root tip resulted in high levels of *Ds1* transposition. Precise targeting was possible only if the heat generated from continuous scanning did not heat the media and surrounding cells. To this end, discontinuous scanning was used so that heat would not build up and propagate to non-targeted tissues. The best results were obtained by scanning 50–100 times and allowing 2.5–5 sec to elapse between scans. With this level of scanning, not all nuclei within the region-of-interest were activated. Single-cell targeting should be possible, i.e. for activation of a central cell or a specific initial, if scan number is increased and the inter-scan time lapse is sufficient. Attenuation of laser output or the use of longer wavelengths as in multiphoton laser scanning microscopy might help to control the precision of targeting even further.

This live-cell marking system is ideal for tracking cell division patterns during plant growth. In addition to confirmation of established lineage patterns in the main root, we have observed the unequal contribution of adjacent pericycle files into the LRP. The situation we describe is that of the entire radial organization of the emerging LRP deriving from a few cells in a single file of pericycle progenitors. Lateral root tissues do not derive, as we might have expected, equitably from three adjacent pericycle files. This lineage marking technique will allow further study of LRP development, in particular, in traverse section view so that the origin of radial symmetry from a single file of progenitors can be described.

Lineage patterns are also easily observed in stems and leaves and this system will greatly simplify the study of processes such as stomatal ontogeny. Lineage marking
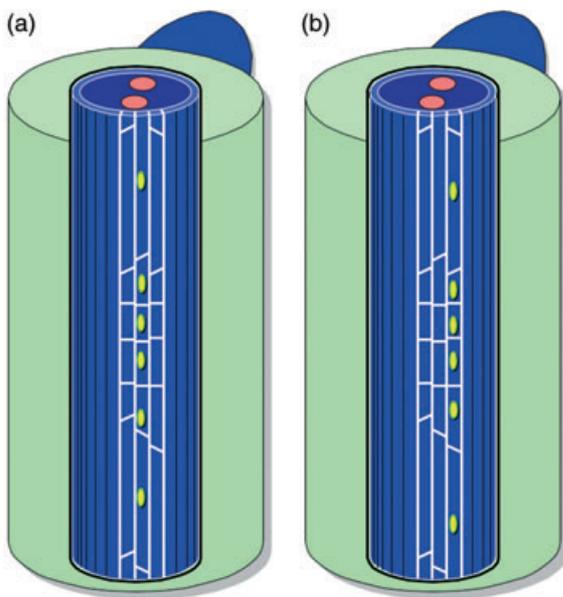
techniques are vital for the study of whole organism development. The components of this genetic system are not even limited to plants. The maize Activator (*Ac*) element belongs to the *hAT* family of transposons that have been characterized in animals including humans (Kempken and Windhofer, 2001). Heat shock promoters are known for a number of different systems (Balcells *et al.*, 1994; Kilby *et al.*, 1995) and modified fluorescent proteins are used widely. In principle, construction of a similar lineage marking system in other plant species and organisms would be straightforward.

Experimental procedures

Plant material and growth conditions

Arabidopsis thaliana plants, ecotype Landsberg *erecta* were used in the experiments described. Seeds for plants (hs-Ac) carrying the soybean heat shock promoter (*Gmhsp 17.3-B*) upstream of the maize *Ac* transposase gene (Balcells *et al.*, 1994; Schoffl *et al.*, 1984) were a generous gift from George Coupland's laboratory (Max-Planck-Institute for Plant Breeding Research, Köln, Germany). A gene fusion between an *Arabidopsis* histone 2b and yellow fluorescent protein (H2B-YFP) was constructed in our laboratory and used to generate transgenic Arabidopsis lines (Boisnard-Lorig *et al.*, 2001). Seeds of Arabidopsis line N84726 (see Plasmids used) were obtained from the Nottingham Arabidopsis Stock Centre. Seeds were surface sterilized in 20% (v/v) Parazone (commercial bleach) for 15 min, washed twice with water and plated on media plates containing 1/2 strength MS basal salts + Gamborg's B5 vitamins (Sigma M-0404), 0.5 g l^{-1} MES, 0.7% agar (Sigma A-1296), pH 5.6 and sealed with gas permeable tape. Seedlings were grown under continuous light at 18–22°C. If required, seedlings were transferred to soil and grown in the greenhouse. Seeds were harvested and allowed to dry at room temperature for at least a week prior to use.

Plasmids used

The *Ds1* transposable element was PCR amplified from the *Adh1-Fm335* allele in maize (generous gift from Liz Dennis, CSIRO Plant Industry, Canberra, Australia) using the following oligonucleotides: forward 5′-GGCAGATCTGGGACTGATAGGGATGAAAACGG-3′ and reverse 5′-GGCAGATCTCCTCAGTCCCTAGGGATGAAAGTGGTAA-TCCG-3′ (Sutton *et al.*, 1984). The amplified *Ds1* sequence was cloned into the vector pBIN35S-H2B-YFP (Boisnard-Lorig *et al.*, 2001) between the CaMV 35S promoter and the H2B-YFP reporter gene, using the *Bam*HI site downstream of the 35S promoter to make Ds-H2B-YFP. The cell membrane marker EGFP-LTI6b was PCR-amplified from genomic DNA of line N84726 (Cutler *et al.*, 2000) using the following oligonucleotides: forward 5′-GGCGGAA-TCCAACAATGGTGAGCAAGGGCGAG-3′ and reverse 5′- GGCGA-GCTCTCAAAAGGTGATGATATA-3′ and the PCR fragment was cloned into the pGEM-T-Easy vector (Promega, Madison, WI, USA), sequenced and cloned downstream (*Bam*HI and *Sac*I sites) of the CaMV 35S promoter into the pBIB binary vector carrying a hygromycin resistance selection gene to yield pBIB-35S-EGFP-LTI6b. All binary vectors were electroporated into the *Agrobacterium tumefaciens* strain GV3101 (Koncz and Schell, 1986). Arabidopsis plants were transformed with these vectors using the floral dip method (Clough and Bent, 1998). Primary transformants were selected on agar media containing kanamycin (50 μg ml^{-1}) or hygromycin (40 μg ml^{-1}). Plants from the T_2 generation, characterized by resistance to selection agent, were used for crossing.

Heat shock conditions

hs-Ac/Ds-H2B-YFP seedlings growing on agar media were heat shocked at 42°C for 30 min unless stated otherwise. After heat shock, seedlings were transferred to 18–22°C.

Analysis of lineage patterns

Seedlings were analysed initially for YFP fluorescence using a Leica MZFLIII epi-fluorescence stereomicroscope (Filter sets - GFPPlus, excitation 480/40 nm, barrier 510 nm and YFP, excitation 510/20 nm, barrier 560/40 nm). Further analysis of marked lineages was done using a Leica TCS-SP confocal microscope (Leica, Milton Keynes, UK). In seedlings without the EGFP-LTI6b membrane marker, cells were outlined by staining with 5 μg ml^{-1} propidium iodide (Sigma, St. Louis, MO, USA) for 1.5 min, rinsed and mounted in water. To prevent seedling movement during high magnification confocal microscopy, seedlings were mounted between a microscope slide and a no. 0 coverslip in warm (35°C) 0.7% low melting point agarose, which was then allowed to set. EGFP was excited using the 488 nm line of an argon laser and propidium iodide and YFP were excited using the 514 nm line. Fluorescence emission was collected between 505–530 nm for EGFP, 527–553 nm for YFP, and 606–635 nm for propidium iodide. Specimens containing both EGFP and YFP fluorescence were imaged by sequential frame scanning.

Laser induction of Ds1 transposition

Seedlings were grown in Nunc coverglass-bottomed two-cell chambers (Fisher Scientific, Loughborough, UK) containing agar media as described above. Chambers were placed inside larger Petri dishes to prevent desiccation and seedlings grown in continuous light at 18–22°C in a growth room. At 3–5 days post-germination, roots had grown through the agarose to the bottom of the chamber and then continued to grow along the coverglass. Observation and heat shock treatment were performed with a Zeiss LSM 510 confocal microscope system equipped with an Axiovert 100M inverted microscope (Zeiss, Welwyn Garden City, UK). Prior to heat shock, primary root tips (*n* = 25) were imaged as a control using EGFP and YFP settings similar to those described above. Heating of specific cells within the root tip was accomplished by repeated scanning of the 488 nm argon ion laser line within a region-of-interest drawn over the meristem initials. Precise activation of the H2B-YFP marker within the region-of-interest occurred when a 100× 1.4 NA oil-immersion objective lens was used and the 25 mW laser was set to maximum output power. One hundred scans were performed at 2.5 sec intervals using the time-series function of the Zeiss LSM 510 software. Immediately after heat shock, the laser power was reduced and EGFP and YFP fluorescence was imaged. Subsequent images of the heat-shocked root tip were taken periodically during the next several minutes after adjustment of the microscope stage to compensate for root growth. Chambers containing heat-shocked seedlings were then returned to the growth room. Root tips of heat-shocked plants were imaged periodically during the following 72 h.

Acknowledgements

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