

Short-range control of cell differentiation in the *Arabidopsis* root meristem

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Meristems are distinctive regions of plants that have capacity for continuous growth. Their developmental activity generates the majority of plant organs¹. It is currently unknown how cell division and cell differentiation are orchestrated in meristems, although genetic studies have demonstrated the relevance of a proper balance between the two processes^{2–6}. Root meristems contain a distinct central region of mitotically inactive cells, the quiescent centre⁷, the function of which has remained elusive until now. Here we present laser ablation and genetic data that show that in *Arabidopsis thaliana* the quiescent centre inhibits differentiation of surrounding cells. Differentiation regulation occurs

within the range of a single cell, in a manner strikingly similar to examples in animal development, such as during delamination of *Drosophila* neuroblasts⁸. Our data indicate that pattern formation in the root meristem is controlled by a balance between short-range signals inhibiting differentiation and signals that reinforce cell fate decisions⁹.

Plants develop and maintain groups of stem cells called meristems. Meristems control the development of plant organs through balanced cell proliferation and differentiation. In the *Arabidopsis thaliana* root, files of all different cell types originate from the meristem (Fig. 1a). Each cell file begins in an initial cell (low colour intensity), which performs stem-cell like divisions to generate a new initial and a daughter cell. The daughters undergo a small number of divisions and differentiate further¹⁰. There is a strong correlation between cell type and embryonic lineage, but cell fate requires continuous positional signalling⁹. The initial cells surround the four mitotically inactive cells of the quiescent centre (QC; grey in Fig. 1a)⁷. We have exploited the well-defined location and the small size of the QC in the *Arabidopsis* root meristem to analyse its function through laser ablation and genetic studies. The QC has been proposed to function as a stem cell reservoir to replace damaged cells¹¹ or as an ‘organizer’ of cellular pattern^{12,13}. Laser ablation of initial cells has shown that many cells can replace the ablated cells⁹ and the QC therefore is not a unique stem cell population. Follow-

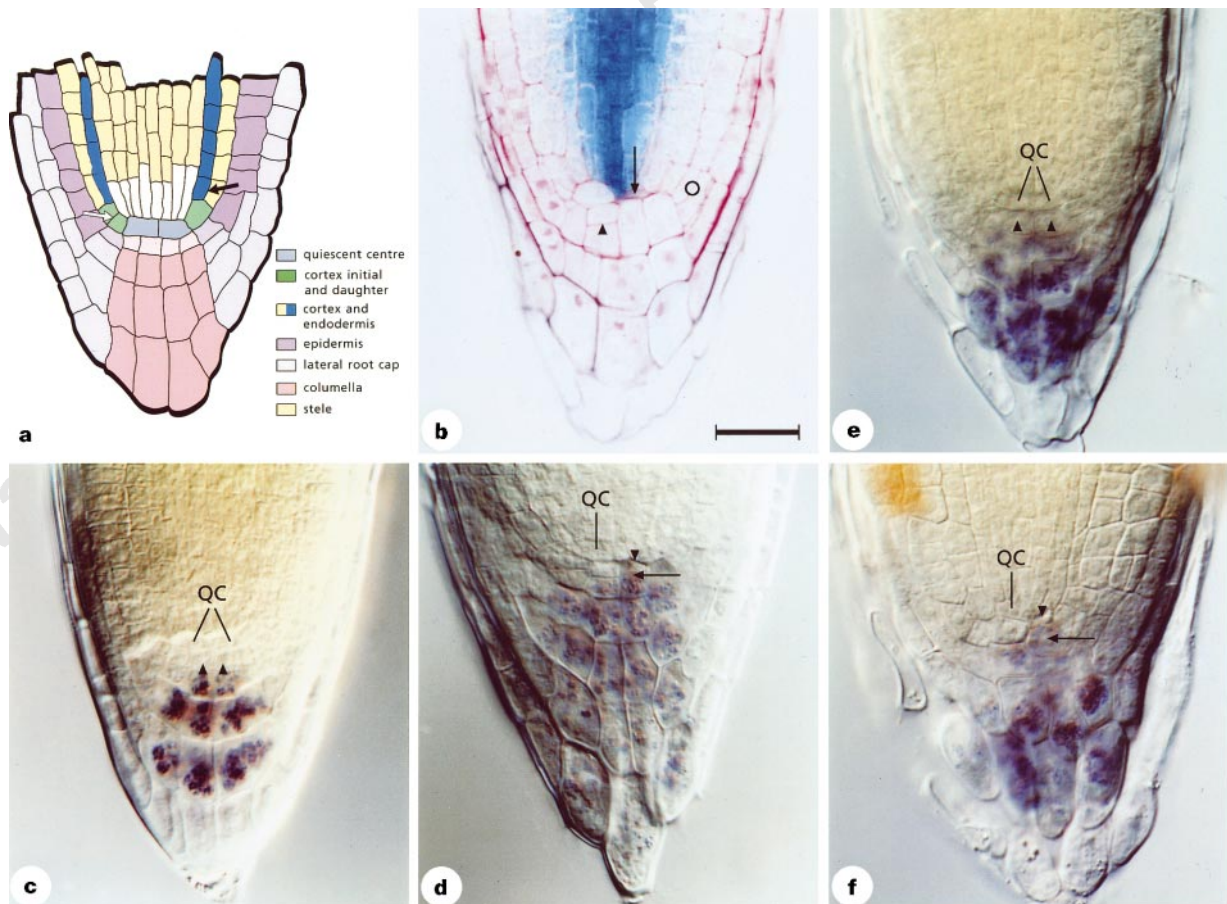


Figure 1 QC cells inhibit differentiation of contacting columella initials. **a**, Schematic representation of the cell types present in the *Arabidopsis thaliana* root meristem. The QC contacts initials (low colour intensity) of all cell types. White arrow: cortical initial divides into a daughter cell. Black arrow: cortical daughter forms cortex and endodermis. **b**, Ablation of 1 QC cell (arrow) results in cessation of division of the contacting columella initial (94.8%, $n = 77$). The columella initial underlying the intact QC cell still divides (arrowhead). Circle, division of epidermal initial generating lateral root cap (89.6%, $n = 48$). **c**, Starch

granules in wild-type *Arabidopsis* columella cells. The columella initials (arrowheads) lack starch. **d**, Progression of differentiation of contacting columella initials (arrow) after QC ablation (arrowhead). **e**, Post-embryonic cell division mutants have a wild-type pattern of starch granule distribution (arrowheads: columella initials). **f**, One QC cell ablation (arrowhead) in post-embryonic cell division mutants results in progression of differentiation of contacting columella initials (arrow; Table 1). Scale bar, 25 μm .

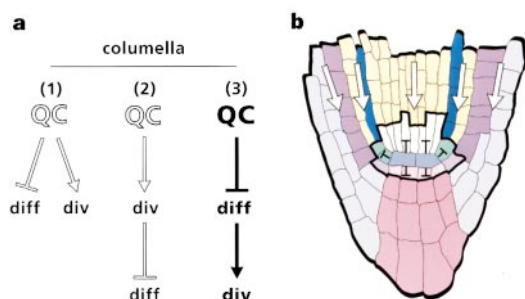


Figure 2 Models for the regulation of cell differentiation and cell division of columella initials by the QC (a), and a generalized model for the control of differentiation of cells in the root meristem (b). a, The QC is either involved in regulating both differentiation (diff.) and division (div.) independently (1), in promoting division which prevents differentiation (2) or in inhibiting differentiation which allows division (3). Our data support model 3. b, We suggest a simple model where signals guiding position-dependent differentiation originate from more mature cells and reach the initial⁹ (arrows). Initial cells differentiation is inhibited through their contact or close proximity to the QC (inhibitory signals). Upon division of the initials, daughter cells no longer contact the QC and no longer receive the QC signals. They now respond to signals from their more mature daughters that guide further differentiation into the corresponding cell type.

Table 1 Columella initials differentiate upon QC ablation

| | Initials | Columella Daughters |
|-------------------|----------|---------------------|
| wt control | 0% | 100% (n = 40) |
| wt QC ablation | 100% | 100% (n = 33) |
| c6044 control | 0% | 100% (n = 30) |
| c6044 QC ablation | 100% | 100% (n = 19) |
| c4761 control | 14.1% | 100% (n = 106) |
| c4761 QC ablation | 100% | 100% (n = 17) |
| c4912 | 6.7% | 100% (n = 15) |
| c4976 | 17.2% | 100% (n = 29) |

Accumulation of starch granules in columella cells. In control plants (both wild-type and 4 mutants without post-embryonic cell division) starch is present in columella daughters and more basal columella cells but absent in initials. Upon QC ablation, initial cells differentiate as indicated by starch accumulation. c4761, c4912 and c4976 express starch at low frequency. Therefore we cannot exclude minor contributions of cell division to differentiation status.

ing complete QC ablation, the QC is rapidly restored by cells of the stele⁹, thus preventing analysis of its function. We therefore analysed the effects of ablating only one or two QC cells, which results in slower replacement (see Methods).

Ablation of one QC cell results in cessation of cell division of columella initials (dull pink cells in Fig. 1a) which are in direct contact with the ablated cell (Fig. 1b). This effect is specific, as non-QC cell ablation next to columella initials (for example, columella daughters (pink in Fig. 1a)) does not result in mitotic arrest. After ablation of one QC cell, the columella initials contacting the remaining intact QC cells still perform their normal asymmetric division (Fig. 1b, arrowhead). This demonstrates that the influence of QC cells does not extend to non-contacting columella cells. We concluded that individual QC cells promote columella cell division within a single cell range.

We followed the fate of the mitotically arrested columella cells through the analysis of a columella-specific marker. Mature columella cells contain starch granules that are not found in initial cells (Fig. 1c; Table 1). After QC ablation, starch granules appear in the underlying elongated initials (Fig. 1d, arrow; Table 1). This shows that the differentiation state of columella initials progresses upon QC ablation. Therefore, the QC can arrest differentiation thus allowing division, can promote division thus preventing differentiation, or can regulate both (Fig. 2a).

To test whether cell division is required to prevent differentiation of columella initials, we analysed the QC activity in mutants lacking post-embryonic root meristematic cell divisions. We characterized four independent *Arabidopsis* mutants (c4761, c4912, c4976 and c6044) defining at least three genetic loci. These mutants have a wild-type cellular architecture of the root meristem but lack post-embryonic cell divisions. Root cell number counts¹⁴ reveal that the embryonic cell number did not increase in the mutants (not shown). As in wild type, starch is absent in the columella initial cells but is present in the daughters (Fig. 1e; Table 1), showing that the initial status is independent of cell division. QC ablation in these

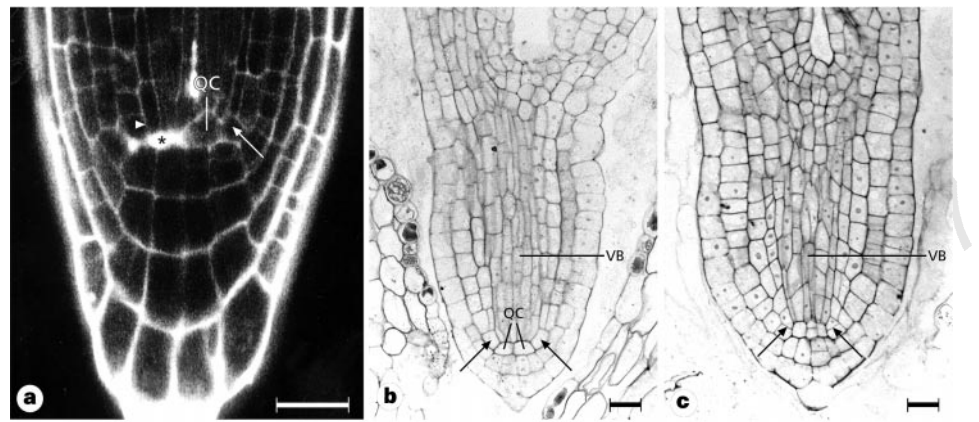
mutants results in starch accumulation in columella initials as observed in wild-type roots (Fig. 1f, arrow; Table 1). The QC thus controls the differentiation state of columella initials in the absence of cell division. These results show that columella cell division is not a prerequisite for inhibition of differentiation (Fig. 2, model 2).

To determine whether the QC cells also control the differentiation of other initial cells, we examined the differentiation of cortical initials (green; Fig. 1a) after QC ablation. Cortical initials normally generate cortical daughters. These generate an inner layer of endodermal and an outer layer of cortical cells (blue and yellow, respectively, Fig. 1a) by an asymmetric division of daughters but not initials (Fig. 1a, arrows; see Methods). When one or two QC cells are ablated, directly contacting cortical initials behave as cortical daughters and divide asymmetrically into cortical and endodermal cells (Fig. 3a, arrowhead). Cortical initials contacting intact QC cells divide normally into cortical daughters (Fig. 3a, arrow). Removing the QC thus results in the progression of differentiation of contacting cortical initials. We conclude that the QC inhibits differentiation of contacting cortical initial cells.

Genetic evidence corroborating the effect of the QC on cortical initials comes from mutations in the *HOBBIT* (*HBT*) gene. In *hbt* mutant embryos, the progenitor cell of the QC and columella undergoes aberrant divisions. As a consequence, an aberrant QC is formed and no functional columella is present (Fig. 3c) (V.W. and B.S., manuscript submitted). In these mutants, all cortical cells divide into cortex and endodermis during embryogenesis (Fig. 3c, arrows; compare with Fig. 3b), inferring that without a functional QC the initial status cannot be maintained.

We questioned whether the QC functions primarily as a negative regulator of cell differentiation independent of cell division (Fig. 2a, model 1), or whether it regulates cell division through differentiation (Fig. 2a, model 3). We investigated this by examining cell divisions after QC ablation. In wild-type roots, divisions in the columella initials and the epidermal initials are always in a similar order: columella initials always divide before epidermal initials (100%; n = 56). After ablation of one QC cell, contacting columella initials no longer divide, whereas both epidermal initials contacting the dead QC cell (Fig. 1b, circle) and columella and epidermal initials contacting the intact QC cells (Figs. 1b, 3a) divide normally. Second, we observed new cell walls in cortical initials (see above, Fig. 3a). Third, ³H-thymidine incorporation in QC-ablated roots shows that cells surrounding the QC passed S-phase at rates similar to those in non-ablated roots (not shown). Together, this shows that all surrounding cells except columella cells are still able to divide at rates indistinguishable from wild type. It is noteworthy that in wild type, only the columella daughters lack division whereas in all other cell types daughters undergo further divisions while differentiating. Therefore, it is only in the columella that a progression of the initial stage into the next stage of differentiation infers the cessation of division. We conclude from these experiments that it is most likely that the QC does not directly regulate cell division in the columella but primarily arrests cell differentiation (Fig. 2a, model 3).

Figure 3 The QC arrests differentiation of cortical initials. **a**, Upon ablation of 1 QC cell (asterisk), contacting cortical initials divide in a cortical daughter-specific manner (arrowhead) indicating progression of differentiation ($n = 184$) (arrow: normal cortical initial). **b**, Wild-type torpedo-stage embryo (arrows: cortical initials, VB: vascular bundle). **c**, *hobbit* torpedo-stage embryo lacks a typical QC. Cortical cells have divided into cortex and endodermis (arrows; $n = 104$). Cortical initials are recognized because they are always located next to the vascular bundle (VB; compare Figs 1a and 3b). Scale bars, 25 μm .



In the *Arabidopsis* root meristem, positional signals for proper differentiation appear to derive from more mature cells to guide cell fate of initials of the same cell type⁹. We show here that contact with QC cells keeps cells in an initial-specific, less differentiated state. A balance between these two antagonistic signals could be the mechanistic basis for orchestrating pattern formation in the root meristem (Fig. 2b).

The shoot meristem also contains a central region of cells with reduced division rates¹. Genetic analyses in *Arabidopsis* suggest that this region regulates the rate of either cell division or cell differentiation^{4–6}. *clavata* (*clv*) mutants display overproliferation of the central zone of the shoot meristem^{15,16}. The *CLV1* gene encodes a putative serine-threonine receptor kinase, inferring that receptor–ligand interactions are either required to promote cell differentiation or to inhibit cell division in meristems¹⁷. We show here that in root meristems, central cells control cell differentiation rather than cell division. Furthermore, we show that inhibition of differentiation is within single-cell range and possibly cell-contact dependent. This provides a striking analogy to controls of cell differentiation in *Drosophila*, such as the NOTCH–DELTA receptor–ligand interaction that represses the differentiation of neuroblasts by contact-dependent mutant inhibition⁸. □

Methods

Cell ablation experiments and analyses. Cell ablation experiments and sectioning were done as described^{9,10}. Ablation of the whole QC (4 cells) results in a rapid replacement by the stele (percentage of replacement: 0%, 50.7%, 75% and 82.4% at 1, 2, 3 and 4 days after ablation, respectively). Ablation of 1 or 2 QC cells results in a slower replacement (0%, 10.3%, 43.3% and 46.2% at 1, 2, 3 and 4 days after ablation, respectively). Only roots where replacement had not started were further examined. The effects we observed were specific for the QC because ablation of all vascular initials above 1 QC cell did not result in any defects. This shows that the QC ablation effects are not the result of a longer-range apical–basal-directed signal. Furthermore, the effects we observed are not likely to be a wounding effect because, following QC ablation, we observed specific cell divisions (periclinal division of epidermal initials), whereas columella initials cease division.

To analyse cortical initial divisions in both the wild-type control and QC-ablated plants, transverse sections were made using a confocal laser scanning microscope⁹. Divisions of each individual cortical cell were counted before ablation and 2, 3 or 4 days after ablation. In wild-type control roots (Col-0), these divisions ranged from 3.1% (start of experiment) to 12.0% (end of experiment) ($n = 584$ cells). In Ler and C24, similarly low numbers of cortical divisions were observed (11.6%, $n = 112$; 19.4%, $n = 72$). NO–O was an exceptional ecotype, as the percentage was much higher (86.5%, $n = 96$). In cortical cells not contacting the ablated QC, cell divisions were similar to control plants (1.4% at the start of the experiment to 11.6% at the end; $n = 552$) showing that the ablation has no effect on divisions of non-contacting cortical initials. In cortical initials contacting the ablated cells, a significantly

higher percentage divided (2.2% start to 47.8% end, $\chi^2 P < 0.05$; $n = 184$ using SPSS 6.1).

Starch was visualized in whole roots by incubation for 3 min in 1% Lugol (Merck). Roots were mounted in 75% chloralhydrate (Merck) and examined with Nomarsky optics. After QC ablation, roots were grown for 4 days on agar plates containing 2 $\mu\text{Ci m}^{-1}$ ³H-thymidine (TRK.418, Amersham).

Analysis of mutants. Mutants lacking post-embryonic cell divisions were isolated in a genetic screen using EMS-mutagenized Col-0 seeds¹⁸. Complementation was observed between *c4912* and *c4761*, between *c4912* and *c6044* and between *c4761* and *c6044*, establishing 3 genetic loci. Isolation and characterization of *hobbit* mutants will be described elsewhere (V.W. and B.S., manuscript submitted). Asymmetric periclinal cortical divisions in wild-type embryos (from heart stage onwards) (2.2%, $n = 44$) were compared to strong *hobbit* (2311: 80%, $n = 30$; 5859: 66.7% $n = 18$; GVII-24/1: 62%, $n = 34$) and a weak *hobbit* allele (*e56*: 86% $n = 22$).

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