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The quiescent center in roots of maize: initiation, maintenance and role in organization of the root apical meristem

Nancy Kerk* and **L. Feldman**

Department of Plant Biology, University of California, Berkeley, California

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Summary. Using roots of maize, we tested the hypothesis that the origin and maintenance of the quiescent center (QC) are a consequence of polar auxin supply. Exposing roots to the polar auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA), or to low temperature (4 °C, with subsequent return to 24 °C), enhances mitotic frequency within the QC. In both treatments, the QC most typically is activated at its distal face, and the protoderm/dermatogen undergoes several periclinal divisions. As a result, the root body penetrates and ruptures the root cap junction and the characteristic "closed" apical organization changes to "open". A QC persists during these changes in apical organization, but it is diminished in size. The data from the TIBA-treated roots suggest a role for auxin in the origin and maintenance of the QC, and further, that alterations in QC dimensions are a consequence of polar auxin supply. We hypothesize that the root cap, and specifically the root cap initials, are important in regulating polar auxin movements towards the root apex, and hence are important in determining the status of the QC.

Keywords: Auxin; Meristem (root); Quiescent center; Root cap; *Zea mays*.

Abbreviations: QC quiescent center; TIBA 2,3,5-triiodobenzoic acid.

Introduction

The origin and maintenance of a quiescent center (QC) is a topic of considerable speculation and discussion (Barlow 1976, Clowes 1978, Feldman 1975, Torrey 1972). During embryogenesis, the QC originates concomitant with or slightly after the root cap meristem begins to function (Clowes 1978, 1984). As a consequence, Clowes (1978, 1984) and others (Barlow 1976) suggest that quiescence is a state which is imposed on specific cells as a result of the establishment of distinctive apical cell patterns and activities (Clowes 1978, 1982). Exactly how this "imposition" gives rise to the QC is unknown, although physical constraints, mainly those generated by the root cap, have been proposed to result in the establishment and perpetuation of the QC (Barlow and Adam 1989, Clowes 1984). If the cap is excised, as can be done in certain monocot roots, the QC activates and gives rise to a new cap initial layer which produces a new root cap. Coincident with the differentiation of new root cap initials and a root cap, the QC is re-established to a size approximately the same as before excision of the cap. These experiments indicate a role for the cap in the establishment and maintenance of the QC.

In addition to surgical manipulations, the QC can be activated by exposing roots to various sorts of environmental extremes, including low temperature, and X- or γ-irradiation (Clowes and Stewart 1967). The subsequent activation of the QC is believed to occur as a consequence of damage to the surrounding meristematic cap initial cells. Because root cap initials cycle rapidly, they are postulated to be hypersensitive to treatments which affect the cell cycle (e.g., cold) (Clowes and Stewart 1967). Hence, the activation of the QC at its distal surface, and the regeneration of a new root cap, is believed to occur as a consequence of environmental damage to the original root cap initials (Barlow and Rathfelder 1985).

Implicit in these experimental approaches is the notion that some form of communication must exist between

* Correspondence and reprints: Department of Plant Biology, University of California, Berkeley, CA 94720, U.S.A.

the root cap or root cap initials and the QC. Damage to the cap or its components thus leads to an activation of the QC as a result of interrupting or modifying signaling between the QC and the adjacent root cap or root cap initials. The nature of these hypothesized signals is obscure, but Barlow and Adam (1989) suggest that because of their mobility within the root, hormones may represent one class of postulated signals. The mechanism by which hormones could alter QC function is not known.

Here we use both low temperature and an inhibitor of polar auxin transport, 2,3,5-triiodobenzoic acid (TIBA) (Katekar and Geissler 1980), as a means of probing interactions between the QC and adjacent meristematic portions of the root apex. Our data favor the view that:

1. the establishment and maintenance of a QC is a consequence of polar auxin transport;
2. this transport is influenced by the root cap and/or root cap initial layer;
3. the distinctive apical organization of roots is dependent on the establishment and maintenance of a QC.

Materials and methods

Materials

Caryopses of *Zea mays*, cv. Merit (Asgrow Seed Co., Kalamazoo, MI) were imbibed for 2 h in rapidly running water and then transferred to petri plates lined with moistened filter paper. The petri plates were then placed in a light-tight cannister in a darkened 24 °C chamber.

Low temperature treatment

After 60 h the cannisters were transferred to a 4 °C refrigerator for 7 days. Following the low temperature treatment, roots were returned to 24 °C and then at approximately 24 h time intervals the terminal centimeter of the root was excised and cultured for 8 h in a 0.5 × solution of MS medium (Murashige and Skoog 1962) supplemented with 2% sucrose and 2.5 μCi/ml ^{3}H-thymidine (specific activity 47 Ci/mM; Amersham) and agitated gently. At the end of the 8 h labeling period, the roots were fixed.

TIBA-treatment

For TIBA-treatments, seeds were germinated for 60 h in the dark and then seedlings with roots approximately 3 cm in length were selected. These seedlings were attached to styrofoam boards covered with moistened filter paper, by inserting a pin through the kernel. A 1 cm wide 1% agar collar (± 10^{-4} M TIBA) (Hinchee and Rost 1992) was then placed over and under the root at its proximal end according the protocol of Hinchee and Rost (1992). (Hence, the terminal 2 cm of the root did not have any direct contact with the TIBA.) Roots were returned to a moistened chamber in the dark (24 °C) and maintained in a horizontal position. At approximately 24 h intervals, root tips were excised and fixed, or the tips were incubated 8 h in MS medium containing ^{3}H-thymidine (as above), and then fixed.

All tissues were fixed in FAA (Jensen 1962), dehydrated in the cold (4 °C) in a graded series of ethanol, and embedded in either paraffin of JB-4 plastic (Polysciences, Inc.). Roots were sectioned at either 8–10 μm (paraffin-embedded) or at 3 μm (plastic-embedded). For autoradiography sectioned roots were prepared as described elsewhere (Feldman and Torrey 1975). After developing the emulsion, sections were stained in 0.05% aqueous toluidine blue, dehydrated in an alcohol/xylene series and mounted. For histological examination, plastic-sectioned material was stained in 0.05% toluidine blue in a borate buffer while heated briefly to 60 °C.

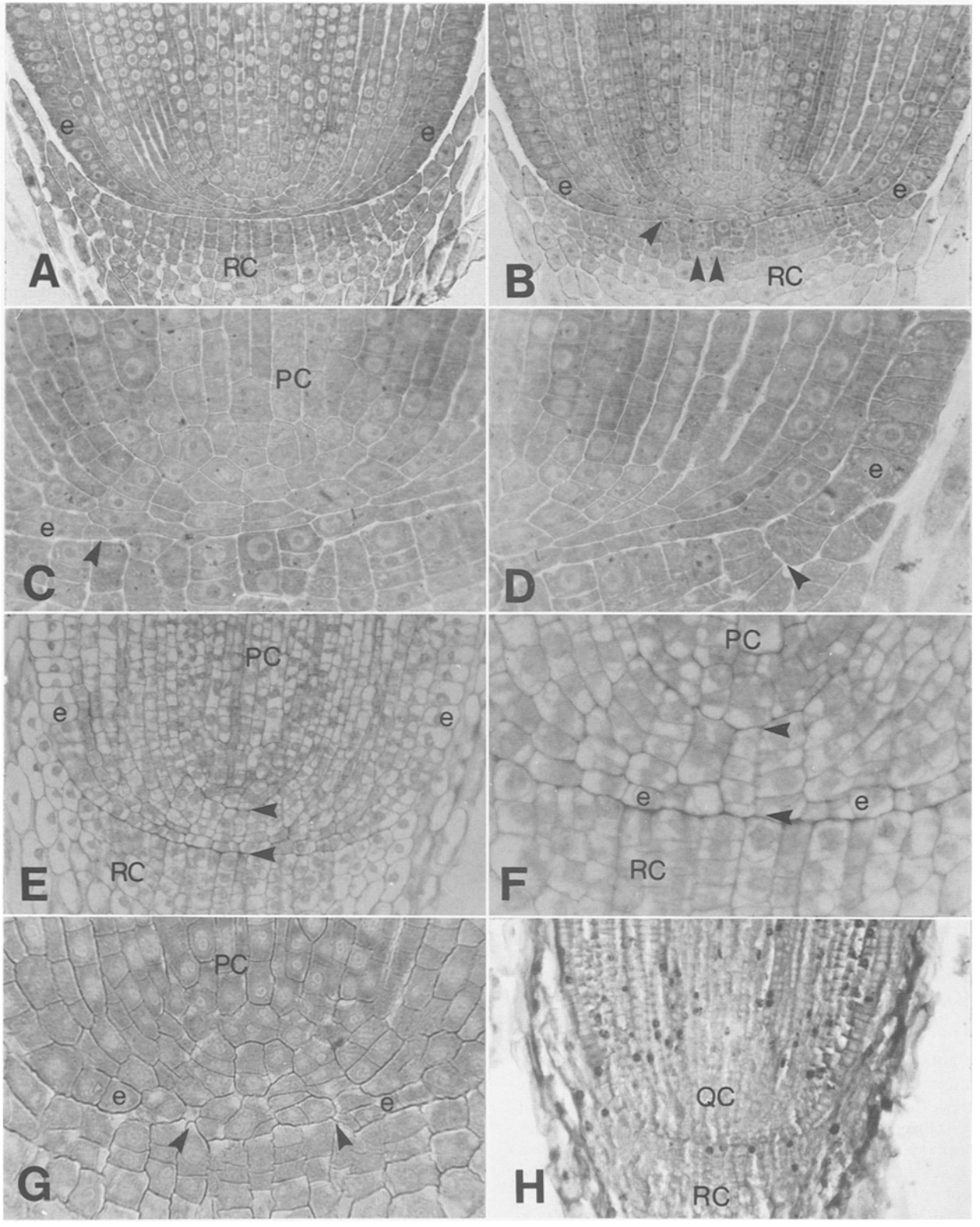
Results

Maize root apices maintain a clear boundary between the cap and the root body, and hence are said to show "closed" apical organization. This type of meristem is contrasted with the "open" meristem in which there is no distinct boundary between the root proper and the root cap. In roots showing a closed meristem, the root cap initiates from a distinct layer of cells, the calyptrogen, whereas the main body of the root arises from adjacent, more proximal layers (histogens); the dermatogen (equivalent to the protoderm), the periblem and plerome, giving rise, respectively, to the epidermis, the cortex, and the vascular tissues. In maize, a distinct single protoderm layer abuts the root cap (Fig. 1 A).

TIBA

Treating intact roots with 10^{-4} M TIBA results in marked developmental changes at the apex. Most commonly, periclinal divisions occur just distal to the pole of the procambial cylinder, resulting in a penetration of the root proper through the root cap junction with a concomitant loss of the characteristic closed apical organization. Subsequent periclinal divisions in lateral (more proximal) epidermal cells result in the production and interposition of additional cells between the root cap and the root proper (Fig. 1 A–D). These added cells are identical to those produced in roots exposed to low temperature. In a few TIBA-treated roots, the epidermis does not divide and remains continuous, and repeated periclinal cell divisions occur in the periblem initials between the tip of the procambial cylinder and the epidermis, resulting in a multi-layered zone (Fig. 1 E, F). Because of a relatively early cessation of cell divisions, apices of TIBA-treated roots never develop a completely open organization (Fig. 1 G).

TIBA-treated roots were exposed to ^{3}H-thymidine in order to monitor the status of the QC. At least during the first few days of exposure to TIBA, a QC of normal size is evident (as in Fig. 2 G). With increased time of exposure to TIBA, an apparently much enlarged QC appears (Fig. 1 H). Such abnormally large QCs are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likely artifacts, probably arising as a consequence of the requirement for auxin or some auxin-mediated process necessary for continued mitoses.

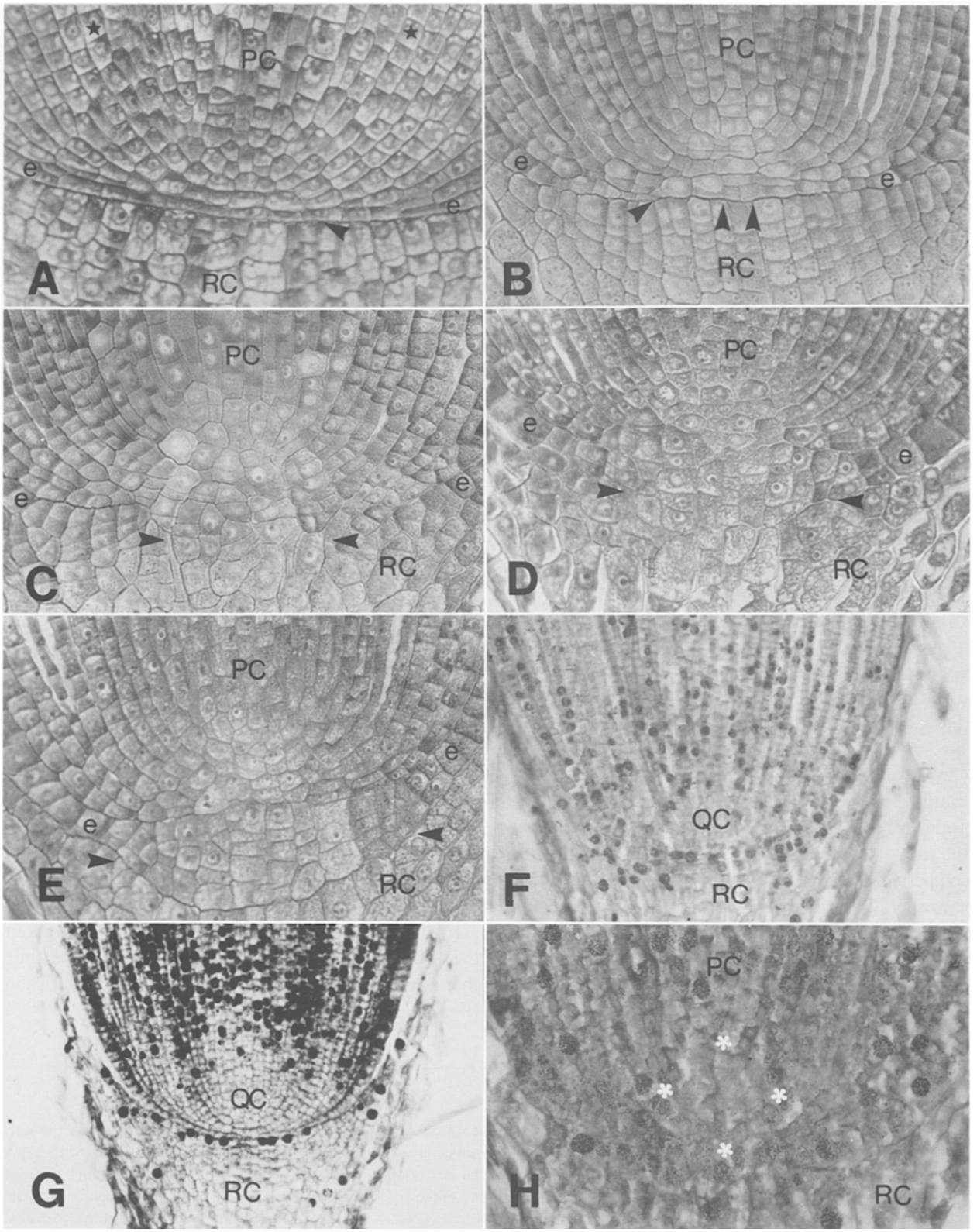
As noted, prolonged treatment of roots with TIBA typically causes the root apex to begin losing its "closed" organization. However, because the TIBA is so effective at inhibiting the polar transport of physiologically significant amounts of auxin (e.g., treated roots become ageotropic), the root soon stops growing and cells in the apex cannot divide further. Hence the apex never shows fully "open" apical organization as seen with low temperature treatments.

Low temperature

In apices subjected to 7 days at low temperature (4 °C), cell lineage patterns are distinct and files demarcating the procambial cylinder are continuous from one side of the root to the other (as viewed in median longitudinal section) (Fig. 2 A). Usually two layers of cells (the periblem and dermatogen) are interposed between the tip of the procambial cylinder and the root cap junction (Fig. 2 A). The root cap is distinct and the meristem shows "closed" organization typical of monocot roots. Apices of these roots do not differ from those in 60-h-old roots just prior to being placed in the cold. After 32 h of growth following removal from 4 °C, the first alterations in apical organization are observed. As a result of periclinal divisions in the dermatogen, a new layer of cells is interposed between the tip of the procambial cylinder and the root cap junction and the protoderm becomes discontinuous, usually just lateral to the tip of the procambial cylinder (Fig. 2 B). Continued periclinal divisions result in a penetration by the root proper through the root cap junction, into the root cap, so that by 48 h the demarcation between root cap and root is less distinct. By 72 h the root cap junction is obscured as a result of the continued periclinal divisions of epidermal derivatives just distal to the procambial cylinder (Fig. 2 C). The root no longer shows closed organization, but rather is open with no distinct separation between the cap and the root body. By 84 h the apex shows maximum alterations with files of cells extending from the procambial cylinder well into the root cap, and hence is reminiscent of open apical organization (Fig. 2 D). Between 84 and 100 h, in the region between the discontinuous epidermis, files of cells organize in line and continuous with the original epidermis, eventually forming a distinctive grouping of cells interposed between the end of the procambial cylinder and the original root cap (Fig. 2 E). Within this region a new root cap initial layer develops and the root cap again becomes distinct from the main body of the root. By 110–120 h after removal of roots from low temperature, the epidermis is now continuous and unbroken, and a closed type of organization is once again evident.

The presence of a quiescent center was determined by supplying ^{3}H-thymidine to roots at varying intervals of time after removal from low temperature. In roots exposed to low temperature followed by 24 h at 24 °C (cold + 24 h), the QC was usually reduced in size as a consequence of the activation of cells in the most distal region of the QC, bordering on the root cap (Fig. 2 F). This contrasts with the well-developed QC in roots just prior to subjecting them to low temperature (Fig. 2 G). In those cold + 24 h-old roots showing a reduced QC, cells that have not yet activated are usually located just proximal to the tip of the procambial cylinder, with cells most generally activated located in peripheral regions (epidermis and cortex) of the former (enlarged) QC. From this, one would conclude that a reduction in QC size proceeds from the outside of the QC inwards, with the most internally located cells least likely to activate. In general, a small QC is present throughout the time an apex is reorganizing, including the time that the apex moves from a closed to an open meristem (Fig. 2 H).

Fig. 1 A–H. Median longitudinal sections through *Zea mays* primary root apices from roots treated with 10^{-4} M TIBA. **A** Root at the start of the TIBA-treatment. Note the discrete root cap (*RC*) and continuous single-layered epidermis (*e*). × 200. **B–G** Roots treated with TIBA for 48–72 h. **B** Periclinal divisions immediately distal to the procambial cylinder result in the protrusion of the root proper through the root cap junction, into the root cap (double arrowhead). Note the discontinuity in the epidermis (single arrowhead). × 200. **C** High power view of **B** showing periclinal divisions just distal to the tip of the procambial cylinder (*PC*). × 320. **D** High power of **B** showing multiple periclinal divisions (arrowhead) of the epidermis (*e*) resulting in new cell formation × 320. **E** Apices from some roots produce multiple layers between the distal end of the procambial cylinder (*PC*) and the root cap (*RC*) (demarcated by arrows). × 200. **F** As in **E** but higher magnification, × 320. **G** View of an apex in which there is almost a complete loss of closed apical organization. The original epidermis (*e*) is here discontinuous. Located between the two arrows are cells arising from mitoses just distal to the procambial cylinder (*PC*). × 320. **H** Autoradiograph of a root approximately 72 h after beginning TIBA treatment. Note the highly enlarged quiescent center (*QC*). × 155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Discussion

We have hypothesized that polar auxin transport is important in the establishment and maintenance of a QC, and, as a consequence, in the development of distinctive apical patterns. In order to test this hypothesis, we used TIBA to determine if we could experimentally modify both the QC and apical patterns in maize roots. TIBA application initiates an activation of the QC at its distal surface, in the protoderm abutting the root cap. As a result of repeated periclinal divisions, the typically single-layered, discrete protoderm becomes multilayered protruding through the root cap junction into the cap.

Because TIBA-treatment causes a reduction of polar auxin transport into the root, significant physiological changes occur in the root, including a gradual decrease in DNA synthesis and an eventual cessation of cell divisions. By 48 h ^{3}H-thymidine incorporation is so reduced as to make difficult an assessment of the status of the QC. Moreover, because cell division ceases 36–48 h after beginning TIBA treatments, only early stages in apical reorganization can occur. However, because these early stages of apical reorganization are essentially identical in low temperature-treated and in TIBA-treated roots (compare Figs. 1 B and 2 E), this suggested that low temperature and TIBA may be acting through a similar mechanism to modify apical organization and QC activity. This observation provided the rationale for following apical reorganization in relation to QC activity using low temperature-treated roots.

Using low temperature, we experimentally modified maize root apical organization and the dimensions of the QC. As a result of the activation of cells at its distal end (cells comprising the dermatogen and periblem), the QC diminishes in size. However, a QC never totally disappears, and 72–84 h after returning roots to 24 °C the QC begins to re-enlarge. This places the re-enlargement of a QC nearly coincident with the time (72–84 h) of maximal development of "open" apical organization (Fig. 2 D). Fully reorganized apices, with characteristic closed organization, are not observed until 110–120 h after transfer of roots from the low temperature to ambient. Hence, even though the QC is diminished in size, it is present throughout the time low temperature-induced apical reorganization is occurring.

Because apical organization can be perturbed by both low temperature and TIBA, resulting in similar anatomical changes, we hypothesize that both treatments may affect common elements. We suggest that this common element is the root cap initials. Because root cap initials are the most rapidly cycling cells in the root, it has long been assumed that they are highly susceptible to low temperature and hence replaced when roots are returned to ambient temperatures (Clowes and Stewart 1967, Clowes and Wadekar 1989). Our results confirm this view, because when low temperature-treated roots are returned to ambient conditions, the root cap initials are replaced from the activated QC. We conclude that this replacement is indicative of some damage to the original root cap initials. In what manner might this presumed damage to these initials cause an activation of the QC and the attendant loss of the distinctive closed apical organization? We and others have hypothesized that the root cap, or only its initials, produce a "message" associated with the onset and perpetuation of quiescence (Barlow 1976, Barlow and Adam 1989, Feldman 1975). Here we suggest that that "message" is auxin and that activation of the QC results from a diminution in polar auxin transport to the root apex. We have previously shown that the root cap or its initials are a site of synthesis for a small amount of auxin, and that this cap-produced auxin influences the amount of auxin moving towards the root tip from the shoot (Feldman 1981). If the cap is removed, polar auxin transport towards the root apex is reduced, but

Fig. 2 A–H. Median longitudinal sections through *Zea mays* primary root apices subjected to low temperature (4 °C) for 7 days and then returned to ambient temperatures for the times indicated. **A** Apex of root just after removal from low temperature. Stars denote the outermost layer of the procambial cylinder. Note that the epidermis (*e*) is intact and continuous. *PC* Procambial cylinder, *RC* root cap. × 275. **B** Apex from root returned to ambient temperature for 32 h. Note that the epidermis (*e*) is discontinuous (single arrow) and has divided periclinally just distal to the procambial cylinder (double arrow). × 250. **C** Apex from a root returned to ambient temperature for 72 h. Note that the root body (region between arrows) has protruded through the root cap junction into the root cap. The epidermis (*e*) on the left has divided periclinally forming additional cells. × 250. **D** Apex from a root returned to ambient temperature for 84 h. The typical closed apical organization is lost and this root now shows an open apical meristem × 250. **E** Apex from a root returned to ambient temperature for 100 h. Note the enlarged distinctive grouping of new cells (between arrows) in line with the original epidermis (*e*) and continuous from one side of the root to the other. × 250. **F** Autoradiograph of an apex from a root returned to ambient temperature for 24 h. Note that a distinct quiescent center (*QC*) is evident. × 155. **G** Autoradiograph of a root apex from a root showing the quiescent center (*QC*) as it would appear just before the root was placed in low temperature. × 155. **H** Autoradiograph of an apex from a root returned to ambient temperature for 72–84 h, equivalent to apices in **C** and **D**. A quiescent center is evident and is circumscribed by 4 white stars. × 250

can be restored by substituting exogenous auxin for the excised cap (Feldman 1981). We hypothesize that the nature of the communication between the cap and the QC involves auxin, and that activation of the QC can be a consequence of damage to, or removal of, the cap initials resulting in a reduction in polar auxin transport towards the root tip. This hypothesis is supported by experiments showing that treatment of intact roots with TIBA results in a loss of apical organization. Hence, our work suggests that auxin transported into the root has a role in the establishment and maintenance of the QC. Thus, perturbing auxin transport into the root (via low temperatures or TIBA) affects the status of the QC and, indirectly, apical organization as a consequence of QC activation. Hinchee and Rost (1992) have previously shown that TIBA blocks lateral root organization, supporting our hypothesis that polar auxin transport is needed for the establishment and maintenance of distinctive root apical organization.

Hence, we conclude that polar auxin transport underlies organization in the root meristem. Moreover, because modifications in apical organization occur without an attendant loss of the QC, we conclude that a quiescent center is not a consequence of the establishment of particular distinctive types of apical patterning. Rather, the development of a quiescent center, before characteristic apical organization, suggests the reverse; namely that the formation of a QC is important in the establishment of characteristic root apical organization. Thus the mechanism by which auxin influences apical organization likely involves auxin's ability to regulate factors that control the rate of division of cells in the quiescent center.

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