Cellular Patterning of the Root Meristem: Genes and Signals

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I. Introduction

It is easy to see how an infant child relates to the parent. With respect to overall pattern, it is essentially the adult in miniature: 2 arms, 2 legs, 10 fingers, and 10 toes. The same is not true when one compares a newly emerged plant seedling to the adult. The adult plant body plan is often a highly elaborated structure with multiple lateral appendages and organs. These structures may be underdeveloped or completely lacking in the newly germinated seedling. This is because much of the adult plant body is patterned postembryonically through the action of coordinated groups of stem cells in the shoot and root apices: the shoot and root apical meristems (SAM and RAM, respectively). Postembryonic development of the adult body plan allows the plant the plasticity to adjust its overall structure, to optimize the acquisition of resources like light and water, and to respond appropriately to biotic and abiotic signals.

As the SAM and RAM collectively give rise to all of the tissues of the adult plant, much attention has been given to how they are structured, how they function, and how they form. In previous editions of this book, this chapter discussed the physical and mechanical properties of the RAM and provided an excellent comparative analysis of RAM form and function considering multiple different taxa. In this edition, I will focus on the regulation and maintenance of the RAMs of angiosperms. Particular attention is paid to Arabidopsis development as much of the molecular and genetic analysis of RAM formation and maintenance has been done in this system. When possible, comparisons are drawn to other systems. As there are separate chapters in this book that concentrate entirely on vascular patterning of the root and hormone signaling, these topics are given only modest coverage here, although they clearly play very important roles in the patterning of the root. Prior to discussing the RAM, I provide definitions of anatomical terms and a brief description of the SAM as a basis for comparison to the RAM.

II. Anatomical Coordinates

There is inconsistent use of anatomical terminology in the literature particularly with respect to defining relative directions of growth and cell division (Baluska et al. 2005). Therefore, these terms will be defined here. By convention the tip of the shoot and the tip of the root are referred to as the shoot and root “apex,” respectively (Figure 3.1A). Consequently in both the root and shoot, the term “apical” describes a location that, relative to the structure or feature being described, is toward the tip. For example, the epidermal cell labeled “a” in Figure 3.1B is apical to the cells labeled “b” and “c.” Conversely “basal” is...
away from the apex and toward the point where the root joins
the shoot (dotted line in Figure 3.1A; referred to by Dolan as the
collet zone in Arabidopsis). The terms “proximal” and “distal”
are also used to describe the position of a feature relative to the
junction of the root and the shoot (usually the root–hypocotyl
junction). “Proximal” indicates toward the root–shoot junction,
whereas away is “distal.” In Figure 3.1B, cell “c” is proximal to
“b.” Recently some have adopted the terms “root-ward” and
“shoot-ward” in favor of proximal and distal (Baskin et al. 2010).
In the root, shoot-ward is proximal, whereas in the shoot, proxi-
mal is root-ward.

With respect to cell division, the terms “anticlinal” and
“periclinal” (Figure 3.1D) are used to describe the orientation
of cell division relative to the surface of the organ, usually the
epidermis. Anticlinal cell divisions create a new cell wall that
is perpendicular (at a right angle) to the organ surface. In the
root, anticlinal cell divisions can occur in two orientations:
perpendicular or parallel to the axis of root elongation. The
divisions labeled “1” in Figure 3.1B and C have occurred perpen-
dicular to the epidermis and the primary axis of root growth.
The type of anticlinal cell division increases the number of cells
in the proximal–distal axis. In the Arabidopsis root, periclinal
cell divisions largely occur within the plane of cell elongation
and increase the number of cell layers in the organ. While the
terms periclinal and anticlinal are very helpful in describing
the orientation of cell divisions in the root meristem, they can
be confusing when referring to divisions when cells are not
cuboidal or when divisions are not clearly oriented parallel or
perpendicular to the organ surface. For example, the apical cell
in Azolla filiculoides Lam. (discussed in more detail later; see
Figure 3.3E and F) is a tetrahedral and most of the formative cell
divisions are oblique to the root surface. In this case, descriptors
other than periclinal and anticlinal are useful in explaining the
orientation of cell division, and the orientation may be described
relative to the axis of the cell.

III. Definition of the Term “Meristem”

The term meristem was coined in 1858 by Carl Wilhelm von
Nägeli to refer to populations of cells in the plant that are able
to give rise to entire organs, for example, the SAM and RAMs
(von Nägeli 1858). Part of the motivation for developing this new
term was to distinguish populations of cells that produced an
entire organ from those that added new tissues to an existing
organ, like those of the vascular and cork cambia. Therefore,
in its original conception, the term meristem would not have
been applied to the vascular and cork cambia (which add to
the vasculature and bark, respectively), although today both are
generally classified as types of meristems. In defining a more
modern definition of the term meristem, Esau suggested that the
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meristem is composed of a group of mitotically active cells that divide throughout the life of the plant to produce new cells, tissues, or organs. Esau points out that meristem cells are distinct from cells that divide occasionally within an organ to produce new cells or small clones of cells in that the meristem is maintained in a mitotically competent and relatively undifferentiated state throughout the life of the organ (Esau 1965). In this way, meristem cells are similar to stem cells in animals, which will be discussed later in this chapter.

IV. SAM

All of the aboveground tissues in the plant are produced through the action of the SAM. The SAM is located at the tip of the shoot axis and is generally a flat to dome-shaped structure that lies in between developing organ primordia (Esau 1965). Depending on the plant and the stage of development, the organs formed may be leaves, thorns, axillary, or floral meristems. The SAM of most angiosperms is organized into two to three distinct layers that are generally labeled the L1, L2, and L3, with the L1 being the outermost cell layer. In general the L1 and L2 layers are single sheets of cells, whereas the L3 layer is less organized and may contain multiple layers of cells. The L1 layer generally gives rise to the epidermis and the L3 layer, the vascular tissue (reviewed by Tooke and Battey 2003). However, this is not due to inherent developmental differences between the cells in these layers. Instead, experiments with cell ablations and L1–L2 chimeras have shown that cellular identity is neither predetermined nor invariant in the formation of lateral organs. Instead, cell fate is determined by its position. Indeed when examined, it appears as though most cell fate decisions in the plant are determined by position rather than lineage (reviewed by Szymkowiak and Sussex 1996).

Although cell fate is not invariant in the SAM, the formation of lateral organs and tissues generally proceeds in a predictable way. The regularity of SAM development has lead to the establishment of two models: the tunica-corpus model (originated by Schmidt 1924) and the meristem zonation model (from Foster 1943) that are widely used today to describe the organization and behavior of the SAM. In the tunica-corpus model, the meristem is divided, based upon the orientation of cell divisions, into two regions: the tunica and the corpus. In Arabidopsis the L1 and L2 layers of the SAM comprise the tunica (literally the tunic)—the outer covering of the meristem. The L3 comprises the corpus (literally the body). Cells in the tunica tend to divide anticlinally, and in doing so, they maintain single L1 and L2 cell layers. In contrast, the orientation of divisions in the corpus is irregular so a central mass of cells is created.

Overlaid upon the tunica-corpus organization are three different zones of cellular organization—the central zone (CZ), the peripheral zone (PZ), and the rib zone (RZ) (Medford 1992). These zones were originally based upon histological differences in gymnosperms (Foster 1943) but can be used to describe differences in cell division and gene expression in angiosperms. The central zone of the meristem extends over a portion of the cells in the tunica and corpus layers. The cells of the CZ were once thought to be largely quiescent, akin to the quiescent center cells found in the RAM of many species. However, it is now clear that the cells of the CZ do divide and in doing so displace their daughter cells toward the periphery of the CZ into the PZ, where organ initiation takes place. Cells in the peripheral zone divide more rapidly than their sister cells in the CZ. As these cells divide, their daughter cells are pushed further from the CZ and eventually differentiate as organ primordia. The RZ subordinates the central and peripheral zones and divides at a rate that is intermediate between the central and peripheral zones. Divisions in the RZ increase the height of the SAM and contribute cells primarily to the stem tissues (Kerstetter and Hake 1997). In this way, the height of the plant is increased and lateral appendages are produced at the flanks of the meristem (reviewed by Traas and Vernoux 2002; Tooke and Battey 2003).

V. Root System

In most plants, the first structure to emerge from the germinating seed is the radicle (primary root) (Clowes 1961). In dicots, the primary root is often long lived, forming a prominent tap-root that may continue to grow throughout the life of the plant. A primary root that continues to grow (or at least maintains the ability to grow) throughout the life of the plant is said to be indeterminate (Sinnott 1960). Truly indeterminate roots are probably rare (Shishkova et al. 2008). Lateral roots are generally smaller than the taproot and emerge from within the pericycle layer of the parent root (see Chapter 6). In monocots, the primary root is often short lived; it grows for a limited period of time and then growth ceases (determinate growth). Cessation of growth is often correlated with the emergence of multiple lateral and adventitious roots that form a fibrous root system composed of multiply branched lateral roots (Aloni et al. 2006). A prime example of this is rice. Upon germination the rice seminal (embryonic) root emerges. The seminal root is short lived, persisting only through the seedling stage of growth. Crown roots are prevalent in rice and emerge from the stem of the plant as opposed to root tissue and therefore are considered adventitious roots. Both the seminal and the crown roots generally give rise to lateral roots (both large and slender), some of which can themselves generate additional laterals (up to fifth-order branching is observed in rice root systems). As seminal, lateral, and crown roots have different developmental origins, there are specific mutations that inhibit formation of each type of root. For example, there are mutations that inhibit the formation of crown roots, but have little or no effect on the formation of the seminal root or the lateral roots (reviewed by Rebouillat et al. 2009). However independent of origin, once formed the growth of all of these roots relies on divisions in the root apical meristem (RAM).

Unlike the SAM, the RAM is a subapical structure that is covered at its apex by protective layers of cells that comprise the root cap (false colored in green in Figure 3.2). The RAM makes no lateral organs; instead, when cells in the RAM...
divide, their daughter cells are displaced either apically to contribute to the root cap or basally to contribute to the body of the root (Clowes 1961). As cells are displaced basally into the body of the root, they generally divide one to several times (similar to the behavior of cells in the peripheral zone of the SAM) before eventually exiting the root meristem and entering into the root elongation zone, where rapid cell expansion occurs. Basal to the elongation zone is the cell differentiation zone, which is the region in which cells begin to adopt their respective developmental fates. For example, root hairs emerge from the epidermis (Figure 3.2) in the differentiation zone and the Casparian strips form in the endodermis. Cells in the root apex that are displaced apically during cell division enter the root cap. In Arabidopsis, these cells do not undergo a series of divisions before differentiation. Instead, they immediately differentiate as columella. As new columella cells are added through divisions in the RAM, the most apical cells of the root cap are sloughed off, resulting in the maintenance of a relatively constant number of root cap cells. The root then is comprised of a continuum of cells in which the least mature cells occupy a subapical position below the root cap with more mature cells situated both proximally in the root body and distally in the root cap/columella (Dolan et al. 1993; Baum and Rost 1996; Groot et al. 2003; Rost 2010).
cells exists at the tip of these roots, similar to the CZ population of cells in the SAM (Clowes 1961; Barlow 1976). Hanstein labeled these progenitor cells histogens and proposed that three distinct histogens exist: the dermatogen, the periblem, and the plerome. These histogens provided a continuous source of new cells that produced the epidermis, cortex, and stele tissues, respectively (Hanstein 1870). In conception, the histogens were similar to the three primary germ layers in animals. Later Janczewski added a fourth histogen, the calyptrogens (Janczewski 1874; Clowes 1961). In cases where the root cap and the epidermis were clonally related, a calyptro-dermatogen was proposed. As illustrated in the following example (Figure 3.3A and B), a calyptro-dermatogen is common in dicots like *Arabidopsis*.

Implicit in Hanstein's theory of histogenesis was the concept that cell lineage determines cell fate. While a strict relationship between cell lineage and cell identity has not been experimentally borne out, in many roots a localized region in the apex has been identified that maintains the cell layers of the growing root. In the absence of perturbation, these cells divide in a predictable way to give rise to a constant number of cell files with predictable positions within the organ and predictable cell identities (Figure 3.3; Clowes 1961). These cells are now generally referred to as initials (Esau 1965). The initial cells have been variously defined based upon both position in the root and behavior. The definition that will be used here is that of Scheres et al. (1996) as it combines essential ideas from Esau (1965), Seago (1969), and Dolan et al. (1993). Scheres et al. define the root meristem initials as the cells at the end points of the linear cell files that with each asymmetric cell division add one cell to the plant body or the root cap, while the initial cell retains its position between the stele and the root cap. The daughter that is displaced from the center of the meristem may itself also divide asymmetrically (in Körper T divisions) to generate additional cell layers or divide symmetrically in transit-amplifying divisions that increase the length of the root. The behavior of the initial cells in *Arabidopsis thaliana* (L.) Heynh., *Oryza sativa* L. (rice), and *Azolla filiculoides* (water fern) provide good examples of how the cortex, epidermis, and root cap cells are generated through the asymmetric divisions of the initial cells and their daughters (Figures 3.3 and 3.4).

Upon germination the primary root of *Arabidopsis thaliana* is composed of single concentric layers of epidermis (p), cortex (c), endodermis (e), and pericycle. These four cell layers surround a central cylinder of vascular tissues, which along with the pericycle comprise the stele (S). At the root apex is the root cap, which is composed of the columnella and the lateral root cap (LRC).
The cellular pattern of the root is generated during embryogenesis (Scheres et al. 1994) and then maintained postembryonically through a series of stereotypical cell divisions that occur in the root apex (Dolan et al. 1993; Scheres et al. 1994, 1996; Baum and Rost 1996; Benfey and Scheres 2000; Baum et al. 2002). The cells primarily responsible for maintaining the pattern of the root are the initial cells and their immediate daughters (Figure 3.3A and B). The initial cells are arranged into three tiers that are located between the stele and the root cap and encircle the quiescent center (QC; as defined by Dolan et al. 1993). Periclinal and anticlinal divisions of dedicated initial cells in the upper and lower tiers of initials give rise to the columella and stele cell files, respectively. In contrast, common initials in the second and third tier give rise to the cortex and endodermis and the epidermis and the lateral root cap. Following these formative cell divisions, divisions (anticlinal) in the transition amplifying cells transiently increase the number of cells in the meristematic zone.

In longitudinal cross sections through the root tip, the cortical endodermal initials (CEIs) are visible on the flanks of the QC (Figures 3.3A and B and 3.4A and B). The CEIs are the ultimate source of the cortex and endodermis. Generally each CII divides once anticlinally to produce a daughter cell, the CED (cortical endodermal daughter), which is displaced proximally from the QC and divides periclinaly (a Körper T division) to produce separate endodermal and cortical cell layers. Once formed, cells in the endodermis and cortex will continue to divide transversely and symmetrically in the meristem until they are displaced into the elongation zone. In some ecotypes, there can be a delay before the CED cells divide periclinaly so that there are two or more undivided daughters in the cell file. However, in other ecotypes, the CEI itself may divide periclinaly to produce separate initials for endodermis and cortex (Figures 3.3B and 3.4B; Dolan et al. 1993).

Genetic analysis of root patterning has shown that two related GRAS family transcription factors, SHORT-ROOT (SHR) and SCARECROW (SCR), promote the asymmetric divisions of the CEDs (Figure 3.4A and B; Di Laurenzio et al. 1996; Helariutta et al. 2000). The SHR protein is also required independently for specification of the endodermis (discussed in more detail Section XIV; Helariutta et al. 2000; Nakajima et al. 2001). SCHIZORIZA (SCZ) (Figure 3.4C), a protein with similarity to heat shock transcription factors, is required for specifying the cortex. Cortex specification prevents cells in the outer ground tissue layer from differentiating as epidermis (Doerner 2010; Pernas et al. 2010; ten Hove et al. 2010). In the absence of SCZ, root hairs emerge from inside of the root, dramatically tearing through the epidermis (Mylona et al. 2002). A role for small RNAs in radial patterning has also been suggested as mutations in ARGONAUTE 1 (AGO1) or HYL1 cause extra ground tissue layers in the root (Miyashima et al. 2009).

In Arabidopsis the root cap is composed of both the columella and the lateral root cap. The columella cells are produced by the transverse divisions of initial cells (CIs) that are immediately distal to the QC (Figures 3.3B and 3.4D and E) (Dolan et al. 1993; Baum and Rost 1996). Following the division of the

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**FIGURE 3.4** (See color insert.) Proteins involved in Arabidopsis root patterning. (A) SHR (yellow) and SCR (green) as SHR is transcribed in the stele and moves into the endodermis; the yellow dots in the endodermis indicate SHR movement. (B) The asymmetric division (dotted line) shown in the cortical endodermal daughter (CED) requires both SHR and SCR activity. (C) SCZ expression is highest in the QC and initials (dark orange) and present throughout the stele and ground tissue (lighter orange). (D) Expression of FEZ (magenta) and SMB (blue). (E) FEZ expression in the initials promotes periclinal cell division, whereas SMB inhibits periclinal division. E/LRC, epidermis lateral root cap initial; CI, columella initial; CEI, cortical endodermal initial.
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Cl, the daughter cells that are in contact with the QC remain as initial cells, whereas the apically displaced daughter cells differentiate as columella. This is evidenced by the accumulation of starch granules in these cells. There are no transit-amplifying divisions in the production of the columella in Arabidopsis (Dolan et al. 1993; Willemsen et al. 2008); however, in other species, there are proliferative divisions in the root cap. For example, in both Helianthus annuus L. and Zea mays L., there is a gradation of cell divisions in the columella region of the root cap with the lowest rate of division generally near the QC, which suggests that the columella initials are not the only source of new columella cells in the root caps of these species (Clowes 1981).

In contrast to the dedicated initials that produce the columella, the cells that give rise to the lateral root cap (the epidermal lateral root cap initials; E/LRCIs) produce both the lateral root cap and the epidermis (Figures 3.3 and 3.4). The E/LRCI cells are apical to the QC and encircle the CI. For the most part, periclinal cell divisions of the E/LRCI cells produce the LRC, whereas anticlinal divisions produce the epidermis (Dolan et al. 1993; Baum and Rost 1996). However, the timing of these divisions is not invariant. During time-lapse imaging periclinal cell divisions in cells one would have predicted to develop as epidermis resulted in the formation of new LRC cells (Campilho et al. 2006). There is therefore some flexibility in the sequence of divisions that leads to the formation of these two cell types. Once formed, transit-amplifying divisions occur in the LRC and the epidermis to increase the number of cells in these files. With continued division, cells in the epidermis will eventually be displaced into the elongation zone, whereas cells of the root cap will be lost as the root grows through the soil. Interestingly although the LRC and the COL are not clonally related, they share regulatory pathways. Willemsen et al. (2008) showed that two NAC domain–containing transcription factors, FEZ and SOMBRERO (SMB) (Figure 3.4D and E), regulate divisions in both the columella and E/LRC initials. The FEZ protein is expressed in both the columella and E/LRC initials and promotes periclinal cell division. SMB is expressed specifically in the CI and E/LRC daughter cells. The primary function of SMB appears to be inhibition of FEZ in cells outside of the initials, as ectopic expression of FEZ or loss of SMB activity results in ectopic periclinal cell divisions and extra cell layers in the columella and LRC. SMB along with two other NAC-domain proteins, BEARSkin1 and BEARSkin2, also controls the maturation of the root cap (Bennett et al. 2010). In contrast, FEZ appears to not play a direct role in cell identity (Willemsen et al. 2008).

Similar to the Arabidopsis root, rice roots are also organized into rings of cells that surround the vascular cylinder (Figure 3.3C and D). In rice however, there are multiple layers of cortex (the number of cortex layers varies between the different root types), and the ontogeny of the cortical, epidermal, and root cap cells differs between Arabidopsis and rice as it does between most dicots and monocots (Clowes 2000). In rice, the cortex and epidermal cell layers are clonally related, and the root cap is entirely separate from all other cell layers of the root (Clowes 1994).

The initial cells in rice are arranged in three tiers that surround the QC. The cortical epidermal cell initial (common endodermis epidermis initial, CEII) occupies the same tier as the QC cells. To form the epidermis and cortex, the CEII divides anticlinally to produce a daughter cell that divides periclinally to generate the epidermal and the endodermal precursor cells. The resulting cell in the epidermal cell file divides anticlinally in a series of transit-amplifying cell divisions to produce the epidermal cell lineage. In contrast, the cell occupying the endodermal position divides periclinally several times to produce all of the specialized cortical cell layers of the root: exodermis (x), sclerenchyma (s), mesodermis (cortex, which will generally differentiate into aerenchyma in all roots with the exception of some laterals), and endodermis. These cell layers help the root to survive in water-saturated soils (Coudert et al. 2010). It is unclear how many initial cells are required to sustain the stelar cell population or how these divisions are oriented. However, some work has been done to describe how various cell types arise in the vascular cylinder (reviewed by Rebuillat et al. 2009). In the mature rice roots, there appear to separate initial cells for the LRC and columella cells.

In contrast to Arabidopsis and rice, all of the cell types (epidermis, cortex, and stelar) of the A. filiculoides (water fern) root are derived from a single initial cell (Figure 3.3E and F; Gifford and Polito 1981a,b). At its apex, the A. filiculoides root is covered by a root cap. Proximal to the root cap are the root cap initial cells and a single pyramidal-shaped "apical cell" that gives rise to all cells of the plant body, except the root cap. It is thought that early in the development of the root, the apical cell divides along its distal face to produce the root cap initials so that for most of its growth, the A. filiculoides root has a closed meristem. However, ferns of the genus Marsilea have an open meristem; there is no separate group of initials for the root cap. Instead, the apical cell divides continuously throughout the growth of the root along its apical face to maintain a steady population of root cap cells (Clowes 1961). Therefore, all cells in these roots are clonally related—produced by the regular divisions of a single initial cell.

The cells of the A. filiculoides root body are produced by divisions of the apical cell in which the new cell walls are oriented parallel to the three proximal faces of the mother cell. These formative cell divisions produce what are referred to as merophytes (essentially packets of cells) that divide periclinally several times (T divisions) to increase the number of cell files and then ultimately divide anticlinally in a series of transit-amplifying divisions to increase the number of cells in the file. The first division of the apical daughter cell (dark green in Figure 3.3E and F) produces an inner and an outer cell. The inner cell will give rise to the vascular tissue including the pericycle, the endodermis, and the inner cortex. The outer cell will give rise to the root hairs, the epidermis, and the outer cortex (Gunning et al. 1978; Gifford and Polito 1981b). Based upon experiments in other root systems that have a single apical cell at the root tip, some have contended that the apical cell of Azolla is not the source of all of the cells of the root body, but is instead more akin to...
the QC cells of rice and Arabidopsis (reviewed by Gifford 1983). The thought was that the large apical cell of A. filiculoides was largely quiescent and the first merophytes were the actual initials. Experiments by Clowes (1956a, 1958b) and Nitayangkura et al. (1980) however have shown this to be incorrect. The apical cell is mitotically active and provides all of the cells of the adult root. This has also been shown for the apical cells in Equisetum scirpoides Michx (Gifford and Kurth 1982) and Marsilea vestita Hook and Grev. (Kurth 1981). Azolla roots therefore lack a QC.

VII. Significance of an Open versus a Closed Meristem

The classification of roots based upon the organization of their meristem as either open or closed conveys information on how the cells in the meristem are arranged and potentially on how they divide to contribute cells to the root body and the root cap. The initial cells in closed meristems are often arranged in discrete tiers of cells; whereas the organization of open meristems is less well defined, and there is not a clear separation between the root body and cap. Likewise in roots with an open meristem, the quiescent center is often not as well defined as it is in roots with a closed meristem, and there is often a gradation of quiescence (Clowes 1961, 1981). However, these differences do not appear to correlate with any differences in growth capacity (Clowes 1961). So what then is the advantage of one configuration versus the other? Hamamoto et al. (2006) suggest that it may be the ability to produce border cells, individual free-living cells that are derived from the root cap through the activity of cell-wall-degrading enzymes. Roots with open meristems produce and shed considerably (7.5–200 times) more live border cells into the environment than do roots with a closed meristem. Bingham et al. (1993, 1995) has shown in pea that upon release from the root, border cells change their pattern of gene expression to produce antibiotics and even aluminum binding mucilage. It has been suggested that one of the functions of border cells is to act as guards in the soil—protecting the intact root from damage as it grows (Hawes et al. 1998). Roots with open meristems therefore may have an advantage to those with closed meristems in terms of resistance to biotic and abiotic attack. Interestingly one of the additional observations that Clowes (1981) made in examining Helianthus and maize roots was that due to differences in the distribution of mitotically active cells in their respective RAMs, Helianthus is expected to replace its root cap approximately every 1.5 days, whereas in maize this process would take 3 days. This may contribute to a larger pool of potential border cells in Helianthus as compared to maize.

VIII. Control of Meristem Size

The root apex is divided into the meristematic, elongation, and differentiation zones, with a region of transition between the meristem and elongation zones (Figure 3.5B; Baluska et al. 1990, 2010). For the size of each of these individual zones to remain constant, the rate of cell division in the meristem must equal the rate at which cells “enter” each of the other two zones. Often this is not the case. (B) (C) 

**FIGURE 3.5** Regulation of meristem size. (A) The relative actions of cytokinin and auxin control the size of the meristem (MZ) in (B). In the transition zone (TZ) where cytokinin predominates cells are predisposed toward differentiation and auxin signaling is suppressed. In the MZ, auxin promotes cell division and inhibits cytokinin. Early in the growth of the meristem, high levels of gibberellins inhibit cytokinin signals, and the size of the meristem increases. Later in root growth, gibberellin levels fall off, and the size of the meristem is kept relatively constant. (C) In the elongation zone, a gradient of \( H_2O_2 \) (with levels increasing basally) promotes differentiation. A mobile protein, UPB1, facilitates this gradient by limiting \( O_2^- \) levels in the transition zone. An \( O_2^- \) gradient in the meristem promotes cell division. The overlap between these two ROS gradients defines the transition zone and the switch from cell division to cell elongation.
case, and the relative sizes of each of the regions change throughout the development of the root. For example, in examining the meristematic and differentiation zones of the pea root, which has an open meristem, Rost and Baum (1988) found that early in the development of the root when divisions were frequent, the height of the meristem (the distance between the root cap and the elongation zone) increased from 1.9 to 2.7 mm. Later in development as the mitotic index decreased, the meristem height returned to 2.0 mm. Interestingly, the height of the meristem was inversely correlated with the distance to the differentiation zone so that the differentiation zone was closer to the tip in slowly dividing roots. That is, as the rate of cell division decreased in the meristem, the differentiation zone moved apically toward the root tip. The apical movement of the differentiation zone was not directly equivalent to the decrease in the height of the meristem; instead, for every 0.19 mm change in the height of the meristem, they found a 1 mm change in the position of the differentiation zone. This effect could be exaggerated by treating the roots with an inhibitor of both cell division and cell elongation, indicating that the size of the meristem is dynamic and that mitotic activity in the tip may delay differentiation.

In Arabidopsis the size of the meristem is generally measured by counting the number of unexpanded cortex or epidermal cells on one side of a medial longitudinal cross section through the root tip. Therefore, factors that either promote divisions in the meristem or slow the progression of cells into the expansion zone increase the size of the meristem. The primary regulators of meristem size in Arabidopsis are the phytohormones, in particular auxin, cytokinin, and gibberellin (Figure 3.5A; reviewed by Galinha et al. 2009; Durbak et al. 2012). In the meristem, high levels of auxin and low levels of cytokinin promote cell division. In contrast, outside of the meristem, in the transition zone high levels of cytokinin promote cell differentiation and inhibit cell division. Both auxin and cytokinin signal through SHORT HYPOCOTYL2 (SHY2), a member of the Aux/IAA family of auxin-induced genes, which inhibits the expression of PIN proteins and consequently polar auxin transport. Cytokinin promotes expression of SHY2 (through the ARR1 and ARR12 transcription factors), whereas auxin inhibits SHY2 expression. Gibberellin feeds into this pathway by promoting the degradation of the repressors of gibberellic acid (RGAs), which in turn results in decreased SHY2 expression and decreased expression of cyclin-dependent kinase inhibitors (Dello Ioio et al. 2007; Achard et al. 2009). During the first few days of growth, gibberellin levels are high in the root, and cell division predominates over cell differentiation. However, by day 5 after germination, gibberellin levels begin to fall, and SHY2 expression reaches its maximum (Dello Ioio et al. 2007). At this stage in root development, cell division matches cell differentiation, and the size of the meristem is largely stable. However, later in the growth of the root, cell divisions decrease, and the meristem shrinks in size. This often precedes a complete cessation of growth (Zhu et al. 1998a, b; Shishkova et al. 2008).

In Arabidopsis there is a natural variation between different ecotypes in the size of the root meristem. Mouchel et al. (2004) were able to exploit this natural variation, identifying a single locus that accounts for approximately 80% of the differences in root lengths between the individuals examined. They named this locus BREVIS RADIX (BRX). Homozygosity for the brx allele reduces the size of the elongation zone and the meristem by approximately 50% and 70%, respectively, when compared to plants with the BRX allele. The BRX gene was shown to function in the biosynthesis of brassinosteroids through upregulation of the CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARF (CPD) gene. Consistent with this finding, application of brassinolide to brx roots was able to partially rescue root length; auxin was not. However, auxin-responsive gene expression (upon application of exogenous auxin) was almost entirely lost in the brx roots, an effect that was mimicked by treating BRX roots with brassinosteroid inhibitors. These results suggest that BRX activity is required for auxin signaling. Indeed, the production of brassinosteroids through BRX appears to be the “rate-limiting step” in auxin-induced gene expression. Auxin also increases the expression of BRX (Mouchel et al. 2006) and promotes its translocation into the nucleus, which is required for its function (Scacchi et al. 2009). In contrast, BRX expression is negatively regulated (albeit moderately) by the application of brassinolide and by auxin-induced degradation of BRX in the nucleus (Scacchi et al. 2009), indicating that BRX is at the center of a feedback loop between auxins and brassinosteroids, which have both positive and negative effects on BRX activity (Mouchel et al. 2004; Briggs et al. 2006; Scacchi et al. 2009).

Early work by Sachs (reviewed by Peters and Tomos 1996) suggested that some tissues exert more control over the development of the plant or organ than others. Sachs’s findings may result from differential mechanical signals or constraints on growth between different tissues that are imposed by the rigid network of cell walls that encase all cells within the plant. For example, the localized application or expression in the outer layers of the SAM of EXPANSINS, which reduces tension between cells by promoting cell-wall “loosening” is sufficient to induce leaf formation in tomato and tobacco plants (Fleming et al. 1997, 1999; Pien et al. 2001), suggesting that the L1 and L2 layers may constrain growth. Alternatively the primacy of a particular tissue in the development of an organ may be the result of the cell-to-cell transport or perception of tissue-specific growth factors. In Arabidopsis the dwarf phenotype of the cpd and brassinosteroid-insensitive 1 (bril) (brassinosteroid receptor deficient) mutants is rescued by the cell autonomous restoration of brassinosteroid perception in the epidermis (but not in the ground tissue), suggesting that the epidermis is the primary tissue regulating brassinosteroid-dependent above ground organ growth (Fleming et al. 1997, 1999; Pien et al. 2001). Similar experiments have been conducted to determine the primary tissues that regulate meristem growth in the root. In analyzing tissue-specific requirements for BRX, Mouchel et al. (2006) concluded that expression throughout the root vasculature was sufficient for rescue of the brx roots. In contrast, signal perception appears to be regulated by the epidermis as epidermal-specific restoration of the BRII receptor is sufficient to rescue bril roots.
IX. Changes in the Patterning of the Meristem

Not only does the size of the meristem change with development but also the patterning of the meristem. Many have argued that all roots in their mature embryonic form have a closed meristem and that the open arrangement is generated after germination. Heimsch and Seago convincingly dispute this contention citing multiple examples of roots that indeed start out with an open meristem configuration (Heimsch and Seago 2008). What this argument highlights however is that the organization of the RAM is not static; instead, it changes in response to both the environment and developmental signals. In examining twelve different species of roots, Armstrong and Heimsch (1976) found frequent changes in meristem organization during root growth, with examples of both apparently open meristems changing to closed and more frequently closed meristems becoming open. The conversion of a closed meristem into an open arrangement was often correlated with periclinal divisions in the cortex cell layers giving rise to columella cells so that the cortex and cap cells had a common cell lineage. It was unclear what changes led to the conversion of an open meristem into a closed one (Clowes 1981).

Experiments by Chapman et al. (2003) suggest that changes in the organization of the meristem, particularly from a closed to an open conformation, may indicate a change in the growth potential of the root and signal the eventual differentiation of the meristem. Using a labeling scheme that distinguishes between two different types of open meristem—basic open and intermediate open (Groot et al. 2004)—Chapman et al. (2003) showed that in five different species (Clarkia unguiculata Lindl., Oxalis corniculata L., Dianthus caryophyllus L., Blumenbachia hieronymi Urb., and Salvia farinacea Epling) with initially a closed meristem, the organization changed to an intermediate open prior to cessation of root growth. What they observed in all five species was an initial burst of growth upon germination that was then followed by a gradual deceleration of growth that preceded root determinacy. In all species, there was a wide-ranging shift in the organization of the meristem in which the size of the root cap generally decreased, cell shapes became irregular, cells became less densely cytoplasmic, and the clear distinction between the root cap and the root body was lost. This transformation took between 14 and 41 days depending upon the root species. These results indicate a connection between an open meristem organization and a limited growth potential but only in roots that started out with a closed meristem. Therefore an acquired open meristem may signal a switch to determinate root growth.

Examination of multiple different species indicates that the organization of the meristem and the potential for root growth is under both endogenous and environmental control. Shishkova et al. (2008) point out that for most roots, there is an initial phase of development where root growth is rapid and “indeterminate.” During this phase, cell divisions within the meristem are able to replenish the cells that are lost to the elongation and the differentiation zones, and overall root growth is maintained. This indeterminate phase may then be followed by a period in which cell divisions in the meristem and growth cease either due to genetic programming or due to unfavorable growth conditions. In some cases, the cessation of growth correlates with root “dormancy” (as used by Clowes et al. 1967) in which the cells in the apex are still meristematic but simply arrested until conditions or programming signals reactivation. In contrast, cessation of growth may indicate determinate root growth and the eventual differentiation of the root apex. In this context, Shishkova et al. (2008) defined two types of determinate root growth: constitutive and nonconstitutive. For constitutively determinate roots, the loss of meristematic potential “is a natural part of root development” that occurs independently of the environment. Nonconstitutive “determinacy is induced usually by an environmental factor.” However, even for constitutively determinate roots like those of pea, growth conditions can accelerate the loss of meristematic activity.

The root of the desert cactus, Stenocereus gummosus (Engelm.), provides a particularly interesting example of constitutively determinate root growth. The primary root of S. gummosus has an open meristem; cells in the apex divide to produce the root cap and root body. Upon germination, the body of the root is composed of an epidermal cell layer, 4–5 ground tissue layers, and a central stele. The initial cells are present at the base of the stele, and the meristem lacks any obvious QC. As the root grows, the size of the meristem decreases; the initial cells become enlarged and vacuolated. During this process, the size of the root cap also decreases until it is eventually lost. As a consequence of the loss of the meristem, the root tip differentiates, and root hairs form at the apex (Rodríguez-Rodríguez et al. 2003). This process is recapitulated in roots of S. gummosus that are generated from callus tissue, indicating a strong genetic component to determinate growth in this species (Shishkova et al. 2007).

When grown on rich medium in tissue culture, the primary root of Arabidopsis is maintained for more than 1 month with no obvious changes in the organization of the meristem. When grown in sand, the RAM of Arabidopsis seedlings switches from closed to open at about 28 days, which coincides with a cessation of root growth (Zhu et al. 1998a,b). Growing Arabidopsis roots on low-phosphorus medium can further accelerate this process (Sanchez-Calderon et al. 2005). By day 12, the meristems of Arabidopsis roots, grown on low-phosphate medium, were disorganized, the cells were highly vacuolated, there was no visible
QC, and root hairs had formed close to the tip, indicating a loss of meristematic activity and an apical migration of the differentiation zone similar to what was observed in pea (Rost and Baum 1988). Examination of mitotic activity in the low-phosphate plants using the CycB1;1:uidA marker revealed a significant decrease in the rate of cell division by day 4 after germination. By day 14, no expression of CycB1;1:uidA was observed. If plants were shifted back to high-phosphate medium at days 6 or 7, when roots still had between 11 and 13 CycB1;1:uidA-expressing cells in the meristem, root growth could be recovered. However, if roots were shifted to high-phosphate medium at day 8, when approximately 10 cells still expressed CycB1;1:uidA, no recovery was observed (Sanchez-Calderon et al. 2005), perhaps indicating that a critical number of cells are required in the meristem to maintain root growth.

One way to understand the factors that control root growth is to look for roots that have precocious determinate growth. Null alleles of either SHR or SCR result in roots whose meristems become disorganized (Di Laurenzio et al. 1996; Helariutta et al. 2000). The shr and scr mutants initially have a closed meristem. However, as they grow, the division of the initial cells becomes irregular, the QC is lost, and differentiation proceeds to the root tip. This process takes 3–5 days in shr-2 mutants and 5–7 days in scr-4 mutants when grown on standard plant growth medium. The most other obvious defect in shr-2 and scr-4 mutants, which is apparent at the time of germination, is the presence of only one ground tissue layer instead of two in between the pericycle and the epidermis (Di Laurenzio et al. 1996; Helariutta et al. 2000). In the experiments of Rost and Baum (1988), the diameter of the pea root also decreased as the meristematic activity and meristem height decreased, perhaps indicating a decrease in the number of ground tissue layers. Rodriguez-Rodriguez et al. (2003) noted a decrease in the number of cortex cell layers from four to one, in S. gummosus so that a single endodermal and cortical cell layer comprised the ground tissue at the time that root growth ceased. As SHR and SCR are found in many plant species (Bolle 2004), perhaps changes in the levels of SHR and/or SCR play a role in regulating the switch to determinate growth in desert cactus and/or pea.

One consequence of determinate root growth in the desert cactus, the low-phosphate-grown Arabidopsis, and the shr and scr mutants is the emergence of lateral and adventitious roots (Rodríguez-Rodriguez et al. 2003; Sanchez-Calderon et al. 2005; Lucas et al. 2011). These results suggest that determinate growth causes a release of apical dominance, akin to cutting off the root tip (Zhang and Hasenstein 1999; Aloni et al. 2006). Therefore, constitutive determinacy (as in the case of S. gummosus) may be a timing mechanism that allows for the establishment of the primary root, which terminates early so that lateral roots are produced close to the soil surface for better access to water. In the case of Arabidopsis roots grown on low phosphate, determinate root growth may divert resources away from the primary root to allow the lateral roots to explore and potentially access more favorable soils. In plants like rice, which have short-lived determinate primary roots, the production of lateral and adventitious roots may create a multiply branched root that mechanically supports the plant better in wet soils (Rebouillat et al. 2009; Coudert et al. 2010). For shr and scr mutants, the emergence of laterals may help support plant growth in the presence of a smaller primary root (Lucas et al. 2011).

Extreme examples of mutations that cause precocious determinate root growth are root meristemless (rml1 and rml2) of Arabidopsis (Cheng et al. 1995). Upon germination Arabidopsis roots contain an average of 17 epidermal and 17 cortical cells per cell file. At 4 days after germination, there was no increase in the number of cells in the rml1 mutants and a very limited increase in rml2 (approximately onefold compared to 10-fold in the wild type) indicating limited functionality of the meristems in these mutants. Both mutants also showed highly vacuolated cells and differentiation of the root tip as evidenced by the apical formation of vascular tissue and root hair emergence. Only rml1 was able to form lateral roots, and the laterals that it formed terminated after the same number of divisions as the primary root, indicating a strong genetic component to the number of root cells. When the RML1 gene was cloned, it was shown to encode γ-glutamylcysteine synthetase (Vernoux et al. 2000), the first enzyme in glutathione biosynthesis. Glutathione is a reducing agent that acts as a redox buffer. Either genetic or pharmacological inhibition of glutathione biosynthesis causes cell cycle arrest and defects in auxin signaling that affect QC maintenance, suggesting that the QC may be important for maintaining cell divisions in the root.

X. Importance of the QC in Maintaining Root Growth

The QC, as defined by Clowes (1956a,b, 1958a), is a region of low mitotic activity that lies at the apical pole of the stele at the point of convergence between the endodermal and cortical cell layers. The QC can be distinguished from other cells in the root on the basis of their weak (or slow) incorporation of radiolabeled nucleotides or nucleic acid precursors, indicating low mitotic activity (Clowes 1956). In some roots with large QCs like those of Zea mays, the number of QC cells can range from 200 to nearly 1000. In contrast, the number of QC cells in Trifolium repens L. is usually small, 2 cells on average (Clowes 1984), and in Arabidopsis 4–7 cells comprise the QC (Dolan et al. 1993). The relative degree of quiescence varies between different species of roots, between different roots on the same plant, and even between cells in the same QC, with cells at the center of the QC showing the highest degree of quiescence. In general the QC cells of roots with open meristems divide more frequently (normalized to the root cap initials) than those of closed meristems. Likewise as a trend, there is higher mitotic activity in small QCs than in large. The size of the QC may change throughout the life of the root (Clowes 1984). In some cases, changes in the size of the QC correlate with changes in the patterning of the root, particularly the vasculature (Feldman and Torrey 1976).
It has been suggested that all roots have, at least at some point in their development, a QC (Jiang and Feldman 2005). In *Arabidopsis* the QC forms during the late globular stage of embryo development (Figure 3.6; Scheres et al. 1994). After fertilization, the *Arabidopsis* embryo divides asymmetrically to produce a basal cell, which forms the extra-embryonic suspensor and an apical cell, which will give rise to all cells of the embryo proper with the exception of the QC and the CIs. The QC and CIs form from the hypophysis, which is recruited from the suspensor. The recruitment of the hypophysis correlates with the establishment of an auxin maximum in the basal domain of the embryo. Factors that disrupt auxin signaling interfere with specification of the hypophysis (Christensen et al. 2000; Benjamins et al. 2001; Friml et al. 2004; Michniewicz et al. 2007). The auxin maximum at the base of the embryo results in the degradation of BODENLOS (BDL). Degradation of BDL frees the MONOPTEROS (MP) transcription factor from repression allowing the expression of the TARGET OF MONOPTERUS 7 (TMO7) protein in the basal region of the embryo proper. Upon expression, TMO7 moves from the basal region of the embryo into the apical cell of the suspensor to specify this cell as the hypophysis. The hypophysis then divides to produce an apical lens-shaped cell and a basal cell (Schlereth et al. 2010). During this division, high auxin levels seem to segregate to the basal cell, whereas cytokinin predominates in the apical cells. Auxin in the basal cell promotes expression of ARR7 and ARR15, which inhibit cytokinin signaling (Muller and Sheen 2008). This partitioning of auxin and cytokinin signaling is required for the proper specification of the basal cell as columella and the apical cell as QC. *Arabidopsis* mutants (e.g., dominant alleles of BDL or loss-of-function alleles of MP) that fail to form a hypophysis fail to make a root.

Not all species specify the QC through recruitment of a hypophyseal cell. Instead, in some plants, the meristem and QC form from a small group of (apparently) disorganized cells at the root pole (von Guttenberg 1968; Jiang and Feldman 2005). In other species, all cells in the root meristem are mitotically active at the time of radicle emergence, and the QC forms post-embryonically (Clowes 1958a,b; Alfieri and Evert 1968). Roots that show constitutively or nonconstitutively determinate root growth either lack a QC entirely or they progressively lose their QC as the root grows (Shishkova et al. 2008). For example, in *S. gummosus* (Rodriguez-Rodriguez et al. 2003) and *A. filiculoides*, a QC is never formed (Clowes 1956, 1958b; Nitayangkura et al. 1980), and root growth is constitutively determinate. When Sanchez-Calderon et al. (2005) grew *Arabidopsis* seedlings on low-phosphate medium, the roots initially had recognizable QC cells and maintained some degree of root growth. The loss of QC markers by day 8 correlated with the inability to rescue root growth when returning the root to normal-phosphate medium. Likewise markers of the QC are either absent or misexpressed in both the *shr* and *scr* mutants (Di Laurenzio et al. 1996; Helariutta et al. 2000; Nakajima et al. 2001) and in other *Arabidopsis* single and double mutants that show constitutively determinate root growth. These results suggest that the presence of a QC is a prerequisite for indeterminate growth.

Two mechanisms have been proposed for the role of the QC in supporting indeterminate growth. The first suggests that the QC serves as a reservoir for cell replacement. During the growth of the root, initial cells are lost either due to wounding or due to the normal aging process. In order for the root to continue to grow, these cells must be replaced. The QC is a potential source of replacement cells. Experiments by Clowes (1967) in which root growth was perturbed through drought, wounding, or cold treatment found that after a state of imposed quiescence (referred to by Clowes as dormancy), growth could resume when the plants were transferred back to normal growing conditions. The basis of this recovery was traced back to cell divisions within the QC. Likewise similar results were found when roots were

FIGURE 3.6 Specification of the hypophysis in the *Arabidopsis* embryo. An increase in auxin in the basal region of the embryo relieves repression of MP by BDL. This allows expression of TMO7, which moves into the top cell of the suspensor. This along with high auxin specifies this cell as the hypophysis (HYP). Later in growth of the embryo, when the hypophysis divides, high auxin is segregated to the lower daughter. High auxin promotes expression of ARR7 and ARR15 inhibiting cytokinin (CK) signaling. Cytokinin is required in the upper lens-shaped cell to specify the QC. (Images modified from Scheres, B. et al., Development, 120, 2475, 1994.)
exposed to ionizing radiation (Clowes 1963, 1965) or in the roots of incense cedar during overwintering (Wilcox 1962). The thought is that stressors like cold treatment, drought, or ionizing radiation preferentially damage cells that are actively dividing. This means that the initial cells and their daughters would suffer the most damage. Since the QC has low mitotic activity, these cells are in a sense protected from loss. When the stressor is released, the QC can then divide to replace any injured cells and reform a functional meristem.

The second mechanism by which the QC may promote or maintain indeterminate growth is based largely upon the observation that in all Arabidopsis mutants, which lack a functional QC, the surrounding initial cells cease divisions and terminally differentiate. These results suggest that the QC acts as an organizing center that maintains the initial cell population in an undifferentiated state. The assumption is that the QC produces a short-range signal that affects cell behavior in a concentration-dependent manner such that the cells that are in direct contact with the QC receive the highest concentration of this signal and are therefore specified as initials. When the initial cells divide, their daughter cells are displaced from the QC and hence undergo some degree of differentiation. Cells in the larger meristem (those which undergo transit-amplifying cell divisions) receive even less of the QC-derived signal and finally cease division once they are significantly removed from the influence of the QC and begin the process of cell expansion and differentiation. Therefore, distance from the QC should affect both cell fate and competence. If the QC was lost, one would predict that the meristem is also lost.

To test this hypothesis, the four cells that comprise the QC were ablated in the Arabidopsis root. Following ablation, the QC was rapidly replaced by cells in the stele, in a region that correlated with the new auxin maximum (van den Berg and Willemsen 1997; Xu et al. 2006). Similar results were found when the QC cells were surgically excised in maize roots (Feldman 1976). However, if only one of the four QC cells was specifically ablated in the Arabidopsis root, replacement was delayed. In the time frame before replacement, van den Berg and Willemsen (1997) found that the columella initials that were in contact with the ablated QC failed to divide and instead differentiated as columella, whereas those in contact with the intact QC cells behaved normally. CEI cells in contact with an ablated QC cell continued to divide but did so periclinally in a manner similar to the CEDs, suggesting that the presence of the contacting QC cells prevents the CEI cells from adopting the CED cell fate. These roots now had essentially dedicated initials (as opposed to a common cortical endodermal initial) that gave rise separately to cortex and endodermis. As this is something that is observed in older roots, perhaps as the root ages either the QC signal wanes in intensity or the ability of the surrounding cells to respond to the signal weakens. From these ablation studies, it was concluded that a QC-derived signal maintains the initial cell population.

The concept of the QC as an “organizer” has also been tested genetically. In scr mutants both the primary and lateral roots show constitutively determinate root growth. These roots lack an endodermal cell layer and fail to maintain a QC. Upon the switch to determinate root growth, QC markers are lost, and both the former QC cells and the surrounding initials divide abnormally and differentiation eventually proceeds to the root tip (Di Laurenzio et al. 1996). In wild-type roots, the SCR protein is expressed in the endodermis, the QC, the CEIs, and their daughter cells (Malamy and Benfey 1997; Gallagher et al. 2004). To test which of these cell types is responsible for maintaining root growth, Sabatini et al. (2003) replaced wild-type SCR activity in either the QC or the CEI and their descendants. Restoration of SCR activity in the QC, but not in the CEI and the ground tissue, enabled reestablishment of indeterminate growth, suggesting that a wild-type QC is required to maintain root growth. In the roots with a rescued QC, the rate of root growth and the overall size of the meristem were decreased relative to a completely wild-type root; however, differentiation of the initial cells and QC was inhibited. These results suggest that the loss of the QC is the primary defect that results in precocious determinate growth in scr plants.

More recent results suggest that the requirement for SCR in root maintenance can be partially bypassed by the downregulation of retinoblastoma-related (RBR) activity (Wildwater et al. 2005). RBR both promotes cellular differentiation and inhibits E2F transcription factors, which promote mitosis. Although RBR is uniformly expressed in the root meristem, RNAi against RBR can partially rescue the stem cell maintenance phenotype of the scr-4 mutant. These results indicate that one of the roles of SCR in the QC is to reduce RBR activity (through a yet unidentified mechanism) and therefore inhibit differentiation. In the maize root, low levels of ZmRBR2;1 are found in the QC as compared to the root cap or the proximal stem cells, indicating that RBR activity in maize may also correlate with QC identity and that in maize RBR activity may be regulated at the level of transcription (Rymen et al. 2007; Jiang et al. 2010).

A prediction of the QC as “organizer” model is that initial cells achieve their progenitor cell status in the meristem based upon position relative to the QC and not based upon some inherent quality of the initial cell. This hypothesis was also tested using cell ablations. If CEI cells were ablated, cells from the adjacent pericycle layer could divide periclinally and invade the position formerly occupied by that ablated CEI. In this position, the pericycle daughter cells adopted the behavior of a CEI. They divided anticlinally to produce a CED; the CED in turn divided periclinally to produce functional endodermis and cortex cell layers. Likewise cortex cells were able to divide and functionally replace E/LRCI cells that were ablated, indicating that position relative to the QC maintains the initial cell status (van den Berg et al. 1995; van den Berg and Willemsen 1997). It is noteworthy that in these experiments, there was no indication of division by the QC to replace the lost initial cells, as has been suggested in other systems. This may be due to the limited damage caused by the ablation experiments. It may be that the QC only divides if a significant population of initial cells is lost. For example, the initial cells may provide a mobile signal that inhibits division of the QC, and loss of one to two cells does not significantly reduce this signal.
XI. Reformation and Maintenance of a QC

The finding that the QC is replaced when all four cells are ablated (van den Berg and Willemse 1997; Xu et al. 2006) suggests that position rather than lineage also determines which cells become part of the QC. One of the key signals for QC formation and maintenance in the mature root is auxin, as it is during embryonic formation of the QC. An auxin maximum, which correlates with the position of the QC is maintained at the root tip by the cell-to-cell movement of auxin (via auxin efflux—PIN and influx—AUX carriers) from the shoot (Blilou et al. 2005; Grieneisen et al. 2007) and localized production of auxin in the root tip (Peterson et al. 2009). Genetic or physiological disruption of this auxin signal results in either loss of the QC or respecification of the QC at the position of the new auxin maximum, generally proximal to the original QC (Xu et al. 2006). This indicates that a continuous auxin signal is required to maintain the root meristem. The primacy of this auxin signal then sets up the potential for a self-organizing system in which auxin specifies the QC and the QC in turn recruits the initial cells (Grieneisen et al. 2007). Indeed, self-organization of the root meristem has been demonstrated in experiments in which the tip (QC and initials) is excised from the maize root (Feldman 1976). In these experiments, cell divisions are initiated in the stump (predominantly in the pericycle cells), and a new meristem reforms. However, this meristem is only active following the establishment of a new QC. Similar results are seen in pea (Rost and Jones 1988). Interestingly the ability of auxin to respectify the meristem has its limits. If in addition to the root cap, the QC, and the initials, the transit-amplifying cells are also removed, a new meristem is not specified. Instead, lateral roots form at right angles to the root stump (Feldman 1976; Rost and Jones 1988). These results indicate that not all cells are able to respond to the auxin maximum with reformation of a QC.

Downstream of auxin in the formation and maintenance of the QC are the SHR and SCR genes (discussed previously) (Di Laurenzio et al. 1996; Helariutta et al. 2000; Sabatini et al. 2003), the WUSCHEL RELATED HOMEBOX 5 (WOX5) (Haecker et al. 2004), and PLETHORA (PLT) (Aida et al. 2004; Galinha et al. 2007) pathways (Xu et al. 2006). WOX5 is transcription factor in the WUSCHEL (WUS) gene family. The founding member of this family, WUS, is required for maintenance of both the shoot and floral meristems. WUS is expressed in a defined region in the center of the SAM referred to as the organizing center (OC). The function of the OC is similar to the QC in that signals from the OC maintain the surrounding stem cells in a mitotically active and undifferentiated state. WOX5 is the functional equivalent of WUS. Correct expression of WUS in the root can rescue wox5 mutants and expression of WOX5 in the QC can rescue wus mutants (Sarkar et al. 2007). WOX5 expression is initiated during embryogenesis in the upper lens-shaped cell that will form the QC immediately after the asymmetric division of the hypophysis. In the mature root, WOX5 is expressed in the QC (Haecker et al. 2004). Loss of WOX5 function results in an abnormal QC and differentiation of the distal stem cells as columella, suggesting a non-cell autonomous role for WOX5 in maintaining the fate of the columella initial cells in an undifferentiated state (Sarkar et al. 2007). There are mild effects of wox5 on the structure of the proximal stem cells; however, root growth is not precociously determinate, suggesting that other gene may redundantly function in maintenance of the proximal initials. As WOX7 is the most closely related homolog of WOX5 in Arabidopsis, this is a likely candidate (Nardmann and Werr 2007; Nardmann et al. 2007).

There are homologs of WOX5/WOX7 in rice, maize, brachypodium, sorghum, and poplar (Nardmann and Werr 2007; Nardmann et al. 2007). Analysis of the expression of the rice WOX5/WOX7 homolog, QHB in rice, as well as ectopic expression studies suggests that the role of QHB in rice root development is similar to that of WOX5 in Arabidopsis. In Arabidopsis WOX5 expression is reduced or lost in shr-1 and scr-4 mutants, indicating that WOX5 is downstream of both SHR and SCR (Haecker et al. 2004). This regulation is likely to be intact in rice, as QHB and SCR expression largely overlap, particularly in four cells at the tip of the root (Kamiya et al. 2003a; b; Haecker et al. 2004). However, the overlapping region of SCR and QHB may not define the entire QC region in rice. Analysis by Gl ewes (1956a, 1971, 1984) of QC structure in grasses suggests a large QC. Likewise examination of markers of cell divisions (CDKs) by Umeda et al. (1999a, b) in the rice roots showed broad expression in the meristem with a region (containing more than four cells) at the tip that is largely devoid of expression. This region has been interpreted as the QC. The region in which SCR and QHB overlap (Kamiya et al. 2003a) corresponds to approximately the center of the region defined by Umeda et al. (1999a, b). This may indicate a region of increased quiescence in this central region of the QC with reduced quiescence at the periphery.

The PLETHORA (PLT) genes are also downstream of auxin in maintenance of the QC but are largely independent of the WOX5 and SHR/SCR pathways (Aida et al. 2004). PLT1, PLT2, PLT3, and PLT4 are AP2 class transcription factors that are expressed in partially overlapping domains that converge upon the QC and the surrounding initial cells in the root meristem (Aida et al. 2004; Galinha et al. 2007). PLT1 and PLT2 are the most similar of the four genes. Loss of both PLT1 and PLT2 expression results in the loss of QC markers, differentiation of the initial cells, and formation of root hairs at the root tip, indicating that PLT functions in both the maintenance and the activity of the QC and initials (Aida et al. 2004). Triple mutants between plt1, plt2, and plt3 are similar to the rml1/2 mutants with no post-embryonic root growth. Quadruple mutants between plt1, plt2, plt3, and plt4 (babyboom) resemble mp (nulls) or BDL (gain of function) mutants, entirely lacking a root. Analysis of PLT1 and PLT2 expression in the presence of exogenously applied auxin or in the mp, non-phototropic hypocotyl 4 (NPH4) double mutants shows that PLT1 and PLT2 act downstream of auxin. In turn the PLTs also affect auxin activity through upregulation of the
PIN1, PIN3, and PIN4 proteins suggesting feedback between PLT and auxin levels. However, neither PLT1 nor PLT2 functions directly downstream of auxin as there is a significant delay in PLT expression after auxin application. One of the effects of ectopic expression of PLT1 is the induction of ectopic meristems that show both expression of the QC-25 marker and the formation of columnella cells in the absence of auxin accumulation. Collectively these results suggest that the PLT genes mediate QC specification downstream of auxin and that ectopic expression of PLT can bypass the need for an auxin maximum in formation of the meristem (Galinha et al. 2007). Examination of PLT genes in rice (Li and Xue 2011) and maize (Jiang and Feldman 2010) suggest similar role for the PLTs in maintaining the QC in these species.

The function of the PLTs is not restricted to the QC; they also function in the initial and transit-amplifying cells. Interestingly the function of the PLTs depends upon concentration. PLT1, PLT2, PLT3, and PLT4 are all expressed in the RAM with the highest levels of expression in the QC and the initials. In the QC and the surrounding initials, high concentrations of PLT2 promote stem cell fate. In the transit-amplifying cells, moderate levels of PLT promote mitotic activity, and even lower levels of PLT are required for differentiation. Based on the effect of PLT levels on cell behavior in Arabidopsis, an obvious candidate for regulation by PLT is RBR; however, downregulation of RBR is not sufficient to rescue plt1 mutants (Galinha et al. 2007). PLT has been shown to upregulate expression of HIGH PLOYDITY2 (HPY2), a SUMO E3 ligase whose expression inhibits endoreduplication and cell differentiation. HPY2 is expressed downstream of both PLT1 and PLT2. Loss of HPY2 expression leads to a reduction in both B-type cyclins and cyclin-dependent kinases suggesting a mechanism by which PLTs function in the root meristem (Ishida et al. 2009). It is not known precisely what creates the gradient of PLT expression, but expression of PLT1 and PLT2 is regulated in part by the expression of both HISTONE ACETYLTRANSFERASE (HAG1 a.k.a. GCN5) and ALTERATION/DEFICIENCY IN ACTIVATION 2B (ADA2b) as loss-of-function alleles reduce the expression levels of PLT (1 and 2) and abolish the PLT gradient producing root meristem defects (Kornet and Scheres 2009). In maize the ratio of zmPLT, expression in the QC relative to the initials is approximately 4:1. Based upon the high levels of zmPLT in the QC relative to the proximal initials in both Arabidopsis and maize, it is tempting to speculate that the highest PLT levels promote quiescence. However, upon decapping of the maize root, the levels of zmPLT expression actually increase in the QC concomitant with activation of mitosis in these cells, indicating that high levels of PLT, at least in the maize root, do not promote quiescence (Jiang et al. 2010).

XII. Stem Cell in the Root Meristem

Based upon the different activities of the QC, some classify the QC cells as stem cells, whereas others associate it with the niche (reviewed by Jiang and Feldman 2005). The difference between these two groups lies in their definition of stem and niche cells (see Section XIII for more details). In a review article on stem cells, Morrison et al. (1997) came to the conclusion that “since different people define stem cells in different ways (for examples see Hall and Watt 1989; Potten and Loeffler 1997), formulating a generally acceptable definition can lead to a conclusion similar to that of U.S. Supreme Court Justice Stewart in regard to pornography: “It’s hard to define, but I know it when I see it.” However, there are two features that universally appear in definitions of stem cells. They are the ability to divide and to self-maintain so that with each division the stem cell is able to “pass on” stem cell identity to at least one of its daughter cells. Other characteristics that are often ascribed to stem cells are the ability to regenerate an organ or tissue after wounding, a low rate of cell division compared to its daughter cells and a relatively undifferentiated state with respect to other cells within the tissue or organ (Potten and Loeffler 1997; Morrison and Spradling 2008; Li and Clevers 2010). By this definition, both the QC cells and the initial cells in the root meristem could be classified as stem cells. However, it is clear that they are not identical populations of cells and there are probably differences in their “stemness.”

In the context of the QC versus initial cells, it is useful to look at how Potten and Loeffler (1997) regard stem cells. In assigning the label stem cell, they point out that as stem cells are defined based upon their potential (i.e., how they will act in some uncertain future state), it is useful to distinguish between two different types of stem cells: actual stem cells and potential stem cells. Actual stem cells are ones that at the time of examination fulfill the key characteristic of a stem cell—they are undifferentiated, proliferative, and actively maintaining the stem cell population. In contrast, potential stem cells have the ability to behave as stem cells, but at the time examined, they are largely quiescent. There are multiple examples of these in animals, and often these cells exist within or near a population of actual stem cells. For example, within an intestinal crypt, actively cycling crypt-based columnar (CBC) cells exist at the base of the crypt among Paneth cells. CBC cells are multipotent stem cells that can reform a mini-gut in culture. Above the CBC cells are the label-retaining cells (LRGs) so named due to their retention of a pulse of bromodeoxyuridine (BrDU). As cell divisions would dilute the BrDU staining, these cells are relatively quiescent. It is thought that the LRC cells are potential stem cells that may be activated due to loss of or damage to the CBCs (reviewed by Potten and Loeffler 1997; Crosnier et al. 2006; Li and Clevers 2010).

Experiments in which the cells of the intestinal crypt are irradiated have shown that there are indeed functionally different populations of stem cells within the crypt (Ijiri and Potten 1986). With moderate to low doses of radiation, the actively cycling stem cells are killed, but at least two potential stem cell populations still exist, which become activated to repopulate the crypt. Higher doses of radiation kill the second population of cells, and still higher doses are required to deplete all mitotically competent cells. It is thought that the first cells to be lost in the
crypt are those undergoing transit amplification, followed by the CBC cells, and then the LRCs (Ijiri and Potten 1986; Potten and Loeffler 1997; Barker et al. 2008). In the framework of the root, the crypt irradiation experiments (at least on their surface) are very similar to the root irradiation experiments performed by Clowes (1963, 1965). That is, after irradiation, the initial cells are lost, and the QC activates to repopulate the root meristem. Based upon this behavior, it is tempting to adopt the animal nomenclature and classify initial cells as actual stem cells and the QC cells as potential stem cells. Indeed, in nomenclature proposed by Barlow, the actively dividing initial cells that surround the QC are classified as functional initials and the cells in the QC, structural initials as indicating that these cells are variations of the same general type (reviewed by Jiang and Feldman 2005).

One of the problems in defining stem cells in plants has been the idea that all plant cells are essentially potential stem cells—under appropriate conditions able to regenerate either the entire plant or specific organs. This is in contrast to animal cells, where regenerative potential is limited to small pockets of stem cells found within defined regions of the animal (Sugimoto et al. 2011). The notion of the totipotent plant cell is largely based upon two observations: (1) the ability to regenerate entire seedlings from callus tissue in culture and (2) the capacity of wounded organs to regenerate following injury. During the formation of callus in tissue culture (as opposed to after wounding), mature plant tissues are treated with the appropriate cocktail of hormones to produce callus. The callus tissue is then coaxed (again through the application of the appropriate concentrations of phytohormones) into producing an entirely new plant. This progression is often thought of as a process of cellular dedifferentiation (from mature leaf or root) back to an embryonic or naïve state (callus) followed by redifferentiation. However, when the actual cells participating in the generation of callus were examined in Arabidopsis, they tended to be either xylem pole pericycle cells in the root or cells associated with the xylem poles in organs that lack pericycle. Interestingly, the cells that give rise to crown roots in rice are also associated with the xylem poles, perhaps suggesting a special role for these cells in plant growth (Atta et al. 2009; Reboullat et al. 2009). When the expression profiles of the callus tissues were examined, they were not undifferentiated cells; instead, they resembled, independent of the source of the callus tissue (root, cotyledon, or petal), cells in the root meristem, with subependimal expression of WOX5, SCR, SHR, and PLT1. Interestingly cellus formation was blocked in plants harboring a mutation in the ABERRANT LATERAL ROOT FORMATION (ALF4) gene, which is required for the initiation of lateral roots. ALF4 is a nuclear-localized protein that maintains the pericycle cells in a “mitotically competent” state, which again suggests that the pericycle plays a key role in the formation of callus (Sugimoto et al. 2010). The pericycle therefore may represent a large population of potential stem cells that are able to generate de novo meristems not only during the process of lateral root formation but also in response to stress or damage. (For more details about the pericycle role in lateral roots development, see Chapter 6.) These results suggest that plant cells may not be all that different than animal cells in their developmental potential and that only specific cells within the plant have regenerative potential.

Stem cells are defined not only based upon their future behavior but also relative to the behavior of their daughter or neighboring cells. Potten and Loeffler (1997) suggest that within an organ, there may exist a continuum of different cellular identities. At one end of the spectrum is the naïve stem cell that has a sustained capacity for proliferation and at the other is the mature differentiated cell that has lost the ability to divide. In between these two extremes are transit cells, which have some aspects of stem cells but lack the ability to self-maintain. These cells are thought to be on a pathway that will lead to differentiation. Along this pathway, these cells divide one to several times to amplify the number of cells in the tissue. Some of these divisions may be asymmetric resulting in daughter cells that themselves may also divide asymmetrically to adopt different cell fates as is seen in the development of the ground tissue layers and the epidermis in the rice root. As transit cells are proliferative even in the absence of stem cells, they have some tissue regenerative potential. By assigning a proliferative function to the transit cells, the number of times that the actual stem cells need to divide is reduced; this may reduce the genetic load of the stem cells. A similar benefit is seen in having a population of quiescent, potential stem cells. Because these cells are not mitotically active, they are less sensitive to cellular damage and mutation.

Recent papers looking at DNA damage (Curtis and Hays 2007; Yoshiyama et al. 2009), chiefly double-strand breaks (DSBs), showed that in the RAM the initial cells and their daughters are particularly sensitive to DSB and that they respond by activating a cell death pathway. During this process, the QC cells are spared (Fulcher and Sablowski 2009). Therefore, QC cells may represent a protected population of stem cells with less genetic load than the initial cells.

**XIII. Stem Cell Niche Concept in the Root**

The concept of a stem cell “niche” was first suggested by Schofield (1978) with respect to hematopoietic stem cells (HSCs). He proposed that within the organism, stem cells are found in association with other cells that “determine [their] behavior.” He saw these associated cells as preventing the maturation of the stem cells. By preventing maturation, the niche cells played a role in maintaining the proliferative potential of the stem cell. As cells are displaced from the niche, they lose their stem cell identity and become first-generation colony-forming cells (what Potten and Loeffler (1997) would refer to as a transit cell). However, susceptible cells, which have not undergone terminal differentiation may also enter the niche and may be induced into a stem cell fate. This appears to be the case for the transit-amplifying cells in the intestinal crypt; if they are transplanted back into the base of the crypt, they can adopt the properties of intestinal stem cell (Barker et al. 2008). Likewise during both oogenesis and spermatogenesis in Drosophila, the progeny of the germ cells.
line stem cells (GSCs) retain the ability to serve as replacement cells for at least three generations after their production (Xie and Spradling 2000; Morrison and Spradling 2008). Therefore, the role of the niche is both to maintain the stem cell population and to recruit new cells upon loss of the native stem cell population. Repopulation of the niche in animals may occur via migration of transit cells back into the niche or through division of active or quiescent stem cells (Morrison and Spradling 2008). Interestingly Wilson and Trump (2006) suggest that the niche may at the same time promote both a quiescent (potential) and active stem cell population. At the center of the niche where inhibitory signals are highest, the quiescent stem cell population would be maintained. However, the actual stem cells would reside at the edges of the niche where the signals inhibiting mitosis are weaker. In the root, there is both an auxin and the PLT gradient with maxima at the QC. These two signals maintain both the QC and initial cell populations in the root apex.

Repopulation of a stem cell niche is classically illustrated by the ability of germline stem cells (GSC) in contact with cap cells in the Drosophila germarium to change their pattern of cell division and replace ablated GSCs during oogenesis. It is thought that the cap cells comprise the niche and through direct signaling maintain the GSC population. However, signaling between the GSC is also important for stem cell maintenance, as the presence of a neighboring GSC affects the orientation of cell division and hence the fate of the daughter cells. This is similar to the results of van Den Berg et al., where the ablation of a root initial cell affected the orientation of division in the contacting initial so that new stem cells were generated (van den Berg et al. 1995; van den Berg and Willemsen 1997).

As the process of oogenesis in Drosophila is strikingly similar in its geometry to the process of root development in Arabidopsis, analogies have been drawn between the different cell types. The cap cells in the ovariole have been likened to the QC cells in Arabidopsis and the GSCs to the initials (Xie and Spradling 2000). Like the cap cells, the QC appears to have both stem cell maintenance and inductive capacity, as cells that come into contact with the QC through asymmetric division of the initials adopt a stem cell fate. This signaling function has led some to consider the QC as a niche as opposed to a stem cell population (reviewed by Jiang and Feldman 2005; Ivanov 2007). However, the presence of a signaling function does not necessitate the classification of the QC as part of the niche. Li and Clevers (2010) suggest that mutual signaling between active and quiescent stem cells may in fact reinforce differences between these two populations. In addition, there are examples of signaling between stem cells in several systems where neither cell type is considered a niche cell. Jiang and Feldman (2005) classify the QC as both stem cells (founder cells) and niche cells suggesting that the two populations are largely overlapping in the root meristem. However, it should be noted that the definition by Jiang and Feldman deviates from the stem cell niche concept as defined by Xie and Spradling (2000) who stated that “a requirement for intercellular signals does not by itself indicate the presence of a niche. A true niche should function independently of resident stem cells.” While the QC may not significantly contribute to the initial cell population in Arabidopsis, it does divide, and in other systems, the contribution to root growth may be significant. Therefore, if the terms stem cell and niche cell are applied to the root meristem in the same way that they are in animal systems, the QC should be classified as a stem cell population.

What is the niche then in the root? In animals a distinction is made between stromal and epithelial niches (Scadden 2006; Morrison and Spradling 2008). In “stromal” niches, distinct and specific cells in the niche (e.g., the cap cells in the anterior end of the ovariole) control the fate of the resident stem cells. These cells are separate from the stem cells, and they exist in the absence of an active stem cell population. In contrast, “epithelial” niches are “devoid of specialized cell types” that control stemness. Instead, a localized domain defines the niche. Scadden (2006) suggests that some niches may be “composed of extracellular matrix and other non-cellular constituents” that control stemness. For example, ovarian follicle cells (FSCs) exist in a dynamic environment in which they maintain no continuous contacts with any specific cell type (Nystul and Spradling 2007). As an auxin maximum is the primary signal for formation and maintenance of the root meristem (Grieneisen et al. 2007), it may be useful to think of the root stem cell niche as an epithelial niche that induces the activity of the QC and surrounding stem cells. Cells with the highest levels of auxin express WOX5, SCR, and high levels of PLT and therefore become quiescent stem cells (QC), whereas the surrounding initials experience lower levels of auxin and become active stem cells. Homogeneous interactions between the quiescent and active stem cells then help to orchestrate the behavior of the entire stem cell population and maintain growth. It has been suggested in animals that the lack of a requirement for heterologous specialized cell types in an epithelial niche may lead to more flexibility in the positioning of the niche and the ability of the niche to move (Scadden 2006; Morrison and Spradling 2008). This may be particularly true in plants where relative movement of cells within an intact organ is severely limited.

Considering the root meristem as an epithelial niche may also help to explain recent results by Sena et al. (2009) who surgically ablated the tips of wild-type Arabidopsis roots at different positions in the root meristem. As was seen in experiments with maize and pea (Feldman 1976; Rost and Jones 1988), regeneration of the primary root was unsuccessful if the ablation removed the entire meristem. However, if the root cap, QC, and proximal meristem were removed, the entire root tip could regenerate. During the regeneration process, cells from all layers within the stump divided and reformed the tip. In maize, regeneration of the root resulted in the establishment of the QC prior to reformation of the meristem (Feldman 1976). This was not the case in Arabidopsis. In fact, root tip regeneration was possible (although slightly reduced) in genotypes that fail to maintain a QC. The authors interpret this as evidence that root regeneration is possible in the absence of a stem cells niche (Sena et al. 2009; Sena and Birnbaum 2010). However, if one considers the QC as a potential stem cell population (as discussed earlier) as opposed to a
signaling component of a “stromal” niche, then the absence of a niche is not demonstrated by the lack of a QC. In the context of an epithelial niche concept, following ablation auxin levels rapidly increased at the tip. Once the auxin maximum is achieved, it induces stem cell identity in responsive cells, and the root tip reforms. In the time frame prior to the establishment of a new auxin maximum, the transit-amplifying cell is able to undergo limited divisions. In wild-type plants, a functional meristem and niche are regenerated. In the scrt or plt mutants, the niche reforms, but growth is determinate due to defects in stem cell responses mediated by PLT and SCR. Considering the root meristem as an epithelial niche also eliminates the need for distinct new definitions of the niche in plants as compared to animals as was suggested by Tullio et al. (2010) who stated that “… QC cells derivatives can serve as a source of new initials. This feature of the QC is a remarkable difference in comparison to animal ‘stem cell niches,’ which, as far as we know, cannot replace stem cells proper.” In the case of the epithelial niche concept, the QC cells are part of the stem cell population not the niche cells. In animals there are clear examples of stem cells generating new stem cells and the de novo generation of new niches (reviewed by Jones and Wagers 2008).

**XIV. Importance of Cell Signaling in Root Growth**

In both the shoot and the root, cell-to-cell signaling is required to maintain the normal function of the stem cells and their daughters. Perhaps the best understood example of this is the CLAVATA (CLV)–WUS signaling pathway. The primary signaling molecules in these pathways are the CLEs, secreted peptides that in their processed and functional form are approximately 13 amino acids long. In the CZ of the SAM, the CLV3 peptide is expressed in the L1, L2, and upper region of the L3 layers. The CLV3 peptide signals to cells in the L3 that express the RPK2, CLV2/CRN, and CLV1 receptors and repress the expression of WUS and therefore both the number of stem cells and the size of the QC in the SAM (for recent review, see Katsir et al. 2011).

In the Arabidopsis root, it has long been suspected that CLE peptides also play a role in maintaining the RAM, as ectopic expression of CLV3, CLE19, or CLE40 (or application of the purified peptides encoded by these genes) leads to termination of root growth. Mutations in clv2 ameliorate this phenotype (Fiers et al. 2004, 2005). Recently CLE40 has been identified as the endogenous CLV signal in the root (Stahl et al. 2009). CLE40 is expressed in the differentiated cells in the columella (Figure 3.7A). The CLE40 peptide acts through the ARABIDOPSIS CRINKLY 4 (ACR4) receptor to restrict WOX5 expression to the quiescent center cells (QC). Loss of cle40 or ACR4 leads to an expansion of the columella initials and an expansion of WOX5 expression beyond the QC, but has no effect on the proximal initials. An important difference between the CLV–WUS pathways in the shoot and the root is that in the SAM, the CLV3 and WUS domains partially overlap and WUS (which itself has recently been shown to move between cells [Yadav et al. 2011]) is required to turn on CLV3. In the root, WOX5 and CLV40 are in distinct domains. In addition in the shoot, stem cells produce CLV3, while in the root, differentiated cells produce CLV40. These results suggest that shoot and roots may have differently adapted the use of the CLV–WUS pathway. Remarkably this pathway may have been co-opted by cyst-forming parasitic nematodes as well. While CLE peptides are largely plant specific, there are non-plant species that express and release CLE peptides during the process of colonization. Expression of the CLE encoding Hg-SYV46 gene from Heterodera glycines Ichinohe is able to rescue the clv3 mutant, suggesting that the CLE peptides secreted by these plant pathogens are functional in the CLV–WUS pathway (Wang et al. 2005).

Another recently discovered class of signaling peptides in root development is the root meristem growth factors (RGFs); three functionally redundant homologous peptides that undergo posttranslational modification by tyrosylprotein sulfotransferase (TPST) (Figure 3.7B; Matsuzaki et al. 2010; Zhou et al. 2010). In situ hybridization revealed that RGF1 is confined to the QC and columella stem cells, while RGF2 and RGF3 are mainly expressed in the innermost layer of central columella.
cells. However, immunostaining showed that RGFs diffuse into the meristematic region (Matsuzaki et al. 2010). Genetic analysis revealed that the RGFs and the associated TPST1 are required for maintenance of the root stem cell niche in part through regulation of the auxin (PIN3 and PIN7) and PLT pathways. Loss of either the RGFs or the TPST results in a stunned root with a reduced meristem. This phenotype can be partially rescued by either external application of the purified sulfonated peptides or ectopic expression of PLT2.

The secretion of mobile peptides is only one of the signaling pathways that functions in root development. The other prevalent pathway is signaling through plasmodesmata (PD). In both Arabidopsis and in Azolla, a switch to determinate growth is correlated with a decrease in plasmodesmatal connectivity, suggesting that symplastic signaling may be a requirement for meristem maintenance (Gunning 1978; Zhu et al. 1998a; Baum et al. 2002). Plasmodesmata (PD) are membrane-lined intercellular channels that connect the cytoplasm of neighboring cells. Primary PD are formed during cytokinesis by the deposition of new cell wall and membrane material around endoplasmic reticulum derived tubules. All clonally related cells therefore are (at least potentially) connected by primary PD. Secondary PD form after cell division and are inserted into already existing cell walls. Thus, symplastic continuity is possible between both related and unrelated cells. The network and degree of connectivity between cells creates symplastic domains. The formation and maintenance of these domains are under developmental control. Likewise the structure and transport capacities of PD change over developmental time and are responsive to environmental conditions. For example, exposure of plants to aluminum induces the closure of PD (Sivaguru et al. 2000) and decreases both root elongation and mitotic activity in the root meristem.

In Arabidopsis Zhu et al. (1998a,b) examined PD densities (PDD) between the walls of all cells in the RAM as the root aged. They found that clonally related cells within the stele and within the ground tissue had the highest density of PD. In all tissues, the density of PD increased significantly from the time of germination up to 1 week. This increase in PDD preceded a rapid increase in the rate of root growth that was seen between week 1 and week 2 post germination. Likewise a significant decrease in PDD preceded the decline in root growth that was observed between weeks 2 and 4, with the lowest PDD detected at week 4. These results suggest that a decrease in symplastic connectivity may be one of the factors contributing to a switch to determinate growth in Arabidopsis roots. This is likely the case for roots of A. filiculoides as well. Gunning (1978) showed that the apical cell of Azolla divided 55 times (on average) before growth ceased. After the 35th division, he noted that the apical cells failed to maintain the same density of PD as earlier divisions. The same observations were made of the merophytes, indicating that in Arabidopsis, decreased PDD precedes determinate root growth.

Symplastic signaling may also affect meristem organization. The presence of a single apical cell in the root meristem of ferns and lower vascular plants is often mirrored in the organization of the SAM. For example, in Equisetum both the SAM and the RAM contain prominent mitotically active apical cells that give rise to merophytes. Likewise the SAM of A. filiculoides contains a single tetrahedral-shaped apical cell that divides along its faces to produce all cells of the shoot. This similarity extends to angiosperms where plants have multiple initials in the RAMs and the SAMs (Imaichi and Hiratsuka 2007). The structural similarities between the SAM and the RAM may suggest a common origin for both of these stem cell populations (Bennett and Scheres 2010). However, it may also reflect an inherent limitation in these groups that restricts the size and number of initial cells in the meristem. For example, Imaichi and Hiratsuka (2007) provide a compelling explanation that is based upon the need for cells to communicate during development. In examining SAM structure in angiosperms, gymnosperms, pteridophytes, and lycopsods, a strong correlation was found between the organization of the SAM and the plasmodesmatal network. Angiosperms can make both primary PD (between clonally related cells) and secondary PD (between both clonally related and nonrelated cells) and they have multi-initial SAMs. In contrast, all of the pteridophytes examined by Imaichi and Hiratsuka (2007) made only primary PD and had a single apical cell in their SAM. The lycopsods were divided in their ability to make secondary PD. Interestingly, lycopsods that made both primary and secondary PD had angiosperm-like SAMs with multiple initial cells, while those that were only able to make primary PD had “fern-like” meristems with a single apical cell. The authors suggest that the inability to make secondary PD and therefore to directly communicate between non-sister cells limits the meristem to a single apical cell that is highly connected to all of its sisters by primary PD.

There are a range of different molecules that are able to pass through PD, from small metabolites to mRNAs, microRNA, and proteins. In an effort to determine what fraction of root-expressed transcription factors move between cells, Lee et al. (2006) examined the domain of transcription and domain of protein localization for 61 tissue-specific transcription factors. They found evidence for cell-to-cell movement for approximately 16% of these proteins. Movement occurred between multiple different tissue types. The capacity for movement was also widespread with many different types of transcription factors including DoF, NAC, bZIP, MADS box, and MYB domain (Lee et al. 2006) proteins moving between cells. These proteins therefore represent a large number of potential signaling molecules in root development and homeostasis.

Perhaps one of the best-characterized symplastic signals in Arabidopsis root growth is the SHR transcription factor (Nakajima et al. 2001; Gallagher et al. 2004; Cui et al. 2007; Welch et al. 2007; Gallagher and Benfey 2009; Koizumi et al. 2011). As mentioned earlier, the roots of shr-2 mutants show precocious determinate growth and defects in cellular patterning. The shr-2 mutants lack an endodermal cell layer, their QC is disrupted, and metaxylem cells replace protoxylem cells (Helariutta et al. 2000; Carlsbecker et al. 2010). The SHR transcript is expressed exclusively in the stele; however, the protein is present not only in the stele but also in the endodermis and the QC, indicating
that SHR moves from the stele into these neighboring cell layers (Figures 3.4A and 3.8). In the neighboring cell layer, SHR upregulates expression of the SCR transcription factor, which in turn maintains its own expression in the QC (Nakajima et al. 2001). In the CEI and CED cells, both SHR and SCR activate the expression of a D-type cyclin, CYCD6;1. Expression of CYCD6;1 in the CED triggers the asymmetric periclinal cell division that results in the formation of a separate cortex and endodermis. Later in root development, CYCD6;1 expression is upregulated during middle cortex formation (Sozzani et al. 2010).

SHR homologs are found in multiple species of plants. The expression patterns of OsSHR1 and OsSCR in rice along with interaction data suggest that this pathway is intact in the radial patterning of rice where the cortex and the epidermis are clonally related (Kamiya et al. 2003; Bolle 2004). In Pinus radiata D. Don, PrSHR is expressed in a pattern similar to Arabidopsis (Sole et al. 2008). In Pinus sylvestris L. and Pismum sativum L., the homologs of SCR (PsySCR and PsSCR, respectively) are both expressed in an arc between the stele tissue and the lateral root cap, which includes the endodermis. These results suggest that the SHR/SCR signaling pathway functions in monocots and in roots with an open meristem configuration (Sassa et al. 2001; Laajanen et al. 2007). The SHR signaling pathway is also not specific to the root, as experiments in leaf tissue also show movement of SHR and SHR-dependent regulation of the cell cycle (Gardiner et al. 2011).

Movement of SHR is required for its function. Nonmobile forms of SHR rescue neither the radial nor the vascular patterning defects seen in the shr-2 mutants (Gallagher et al. 2004; Gallagher and Benfey 2009; Carlsbecker et al. 2010). This is because an endodermal dependent signal is required for patterning of the xylem. In the endodermis, SHR initiates expression of miRNA165/6, which in turn moves into the stele tissue where it inhibits its target mRNA, PHABULOSA. Dominant alleles of PHB with mutations in the miRNA binding site phenocopy the metaxytem defects in shr-2. SHR therefore is at the center of a reciprocal signaling pathway in which movement of SHR from the stele activates a microRNA which itself is a mobile signal that functions in the patterning of the vascular tissue (Carlsbecker et al. 2010; Vaten et al. 2011). miRNA 165/6 isoforms are found widely in plants (PMRD database, http://bioinformatics.cau.edu.cn/PMRD/) perhaps suggesting conserved patterning pathways. It is unclear what the adaptive value of this reciprocal signal is as opposed to SHR directly activating miRNA 165/6 in the stele. However, results from Carlsbecker et al. (2010) suggest that miRNA165/6 acts in a dosage-dependent manner. Graded movement is one way to create a gradient of active miRNA 165/6. One could speculate that such a system also provides the root with a feedback loop that speeds the formation of mature, thickened vascular tissue in the absence of an endodermal layer, which itself is both a suberized and lignified tissue that may provide some structural support to the root.

Research into the mechanisms by which SHR moves has revealed interesting sets of SHR-dependent positive and negative feedback loops that control SHR movement (Figure 3.8; Sena et al. 2004; Cui et al. 2007; Welch et al. 2007; Gallagher and Benfey 2009; Koizumi et al. 2011). In stele cells, the SHR protein is present in both the nucleus and the cytoplasm and moves into the endodermis. In the endodermis, SHR is exclusively nuclear localized and moves neither back into the stele nor into the endodermis (Gallagher et al. 2004). In the endodermis two SHR-dependent transcription factors, SCR and MAGPIE (MGP), and one SHR-independent transcription factor JACKDAW (JKD) inhibit movement of SHR either back into the stele or into the cortex, presumably through trapping SHR in the nuclei of endodermal cells. Interestingly, the stele expression of a SHR-interacting protein called SIEL (for SHR INTERACTING JACKDAW (JKD) inhibit movement of SHR either back into the stele or into the cortex, presumably through trapping SHR in the nuclei of endodermal cells. Interestingly, the stele expression of a SHR-interacting protein called SIEL (for SHR INTERACTING EMBRYONIC LETHAL) is downstream of both SCR and MGP. SIEL is an endosome-associated protein that interacts with SHR in the cytoplasm of stele cells and promotes movement of SHR into the endodermis (Koizumi et al. 2011). These results indicate that in the stele, SHR promotes its own movement, whereas in the endodermis, it inhibits it. This then may provide a mechanism for concentrating SHR in the nuclei of endodermal cells. Mutations that reduce movement of SHR or SHR activity (e.g., siel-4 homozygotes or shr-2 heterozygotes) result in ectopic divisions in the ground tissue (Koizumi et al. 2011). Interestingly conditions that increase SHR movement (e.g., homozygosity for jkd-4 or SCR RNAi) (Cui et al. 2007; Welch et al. 2007) also increase the number of cell layers in the root. Regulation of SHR movement therefore may be a mechanism by which roots control the numbers of ground tissue layers that are formed.

One of the latest mobile transcription factors shown to play a role in root patterning is a novel bHLH (subfamily 14) transcription factor named UPBEAT1 (UPBI) (Tsukagoshi et al. 2010; Wells et al. 2010). UPBI regulates root growth and meristem size through the regulation of the levels of reactive oxygen species (ROS). In the root, there are two major ROS, hydrogen...
peroxides (H₂O₂) and radical oxygens (O₂⁻). O₂⁻ levels are highest in the meristem, whereas H₂O₂ is highest in the root elongation zone (Figure 3.5C). The overlap of O₂⁻ and H₂O₂ constitutes the transition zone (Dunand and Penel 2007; Dunand et al. 2007). UPB1 mRNA is present in the vascular tissue as well as in the lateral root cap (LRC) of Arabidopsis roots, while the UPB1 protein is detected primarily in the nuclei of cells in the transition and elongation zones, suggesting that UPB moves between cells. Loss-of-function mutations in UPB1 affect ROS gradients in the root resulting in an expansion of the root meristem and a concomitant decrease in the elongation zone (Tsukagoshi et al. 2010). Examination of UPB1 targets revealed that UPB1 repressed the expression of several peroxidases. Tsukagoshi et al. (2010) propose a model in which high levels of O₂⁻ promote cellular proliferation, whereas lower levels of O₂⁻ and high levels of H₂O₂ promote differentiation. As O₂⁻ and H₂O₂ form inverse but partially overlapping gradients, cell behavior can be controlled by subtle changes in the relative levels of the two ROS. Once the ratio of H₂O₂ to O₂⁻ reaches its maximal level, cell proliferation stops entirely, and cells become differentiated (Tsukagoshi et al. 2010).

UPB1 is not alone in regulating the levels of ROS in the root. One of the ways that auxin is thought to signal in the root is also through the generation of ROS. In the elongation zone, auxin induces cell-wall loosening through the formation of hydroxyl radicals that can readily react to form H₂O₂. Auxin can also interact with O₂⁻ to form O₂⁻. Therefore, depending upon the circumstances auxin could promote either cell division or cell elongation (Mori et al. 2009). It is interesting that mobile signals, like UPB1 and auxin, would function through the regulation of ROS as the generation of ROS is known to induce callose formation and block PD (Benitez-Alfonso and Jackson 2009; Benitez-Alfonso et al. 2011). Auxins move between cells via plasma membrane localized influx and efflux carriers. It is not known if they can also move via PD. Nonetheless, it is possible that one of the effects of auxin and UPB1 on ROS may be changes in PD permeability. This is probably not the only role for ROS in development however, as roles for ROS in the regulation of growth in animals have also been shown. For example, hematopoietic stem cells (HSC) are kept in a relatively quiescent state in the bone marrow by limiting the concentration of O₂⁻. Quiescence can be reversed by altering the levels of ROS (reviewed by Eliasson and Jonsson 2010) indicating a role for ROS in regulating animal as well as plant stem cells.

**XV. Conclusions**

In plants, much of the patterning of the organism occurs postembryonically through the action of meristems. The organization of the root meristem differs between different species of plants. However, in all meristems, there are populations of mitotically active cells termed initials. The initial cells in roots with a closed meristem divide in stereotypical ways so that the organization of the root is maintained relatively constant and cellular identities are stable and predictable. Initial cells have key properties that are associated with stem cells in animals including self-maintenance and a prolonged mitotic capacity. Like stem cells in animals, initial cells in the root create clones of cells that themselves may divide asymmetrically to create new cell layers or divide symmetrically (often in a series of transit-amplifying cell divisions) to increase the number of cells in the organ. Stemness is maintained through the action of both endogenous and exogenous cues, as is the size of the root meristem, elongation, and differentiation zone. The ability of the root to respond to extrinsic signals allows the root to conform its growth to suit the environment and in some cases entirely terminate growth of the primary meristem to allow the activity of lateral meristems. Genetic analysis, largely in Arabidopsis, has begun to uncover the genes and regulatory mechanisms involved in the cellular patterning of the meristems and root growth. Several of these pathways function not only in the root but also in the shoot meristem, suggesting similar points of control and regulation.

**References**


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