# Meristems and Their Role in Primary and Secondary Organization of the Plant Body

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**Abstract**

This chapter deals with meristems and their importance in the organization of the primary and secondary plant body. The meristem concept is explained with particular reference to initials, stem cells and permanency of initials. A classification of meristems is provided, followed by the organization of SAM, RAM and vascular cambium. The genetic basis of the organization and behaviour of these three meristems is dealt with in detail along with their hormonal control. An account on intercalary meristem, metamers and modules, origin of nodes and internodes, axillary buds, apical dominance, primary and secondary thickening meristems and phellogen is also provided.

**Keywords**

Axillary bud • Genetic control of meristems • Intercalary meristem • Lateral roots • Metamer • Phellogen • Quiescent centre • Root apical meristem (RAM) • Shoot apical meristem (SAM) • Vascular cambium

## 4.1 Introduction

An adult vascular plant is a complex chimera with contiguous cells and tissues of varied structure, symplastic domains, functions and ploidy organized into distinct axial (roots and stems) and appendicular organs (leaves and flowers). Some kind of complementary physiological mechanisms must operate, whereby the mutualistic relationships of the cells, tissues and organs during development are regulated by an overriding holism and by a pervasive tendency towards

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homeostasis (Wardlaw 1968). Hence, the adult plant is often considered as a complex of several reaction systems operating at different levels (cell, tissue and organ levels) and at different topographic locations in order to maintain the integrity of the whole plant, both structurally and functionally. The basic defining morphological feature of the adult plant is its architecture which is defined as the three-dimensional organization of the plant body (Reinhardt and Kuhlemeir 2002). Although architecture is easily noticed and striking in the shoot system, a similar, although in a less-striking, form may occur in the root system. In tropical tress 23 basic architectural patterns have been recognized (Hallé et al. 1978); it is likely that an equal or more number of architectural patterns might occur in temperate trees and in herbaceous taxa of all regions (Krishnamurthy 2015). These various architectural patterns are due to differences in the branching patterns which in turn are brought about by the differential behaviour of the apical meristems of the main stem and branches as well as by the number and differential developmental behaviour of the axillary meristems. A variation in root architecture in a similar way is the result of differential behaviour of the main root apical meristems and the lateral root meristems.

## 4.2 Concept of Meristem

During early embryogenesis, cell divisions occur almost throughout the embryo, but as the embryo matures, the addition of new cells to the embryo body is gradually restricted to certain of its regions only. This restriction of cell divisions to specific regions of the plant body continues as the embryo, via a seedling, becomes the adult plant. The, thus formed, *growth centres* remain embryonic and are maintained as such almost throughout the life of the plant. Hence, the adult plant body becomes a composite mixture of embryonic and matured tissues. These growth centres continue to add new cells to the adult plant body and are called *meristems*. This fact made Bower (1947) to say as follows: 'the life of the higher plants may be described as an *indefinitely continued embryology*, the increase in the number of parts being in a geometric ratio'. This restriction of cell divisions to specific meristems in the adult plant body is an evolutionarily advanced feature, since in many lower plants almost all cells are involved in cell division. Thus, in higher plants there is a clear-cut division of labour between the different parts of the plant body. The localization of growth centres (meristems) is an important feature of distinction between plants and animals. The term 'meristem' is derived from the Greek word 'meristos', which means 'division'. The tissue that organizes the meristem is called *meristematic tissue*. There are cells in each meristem that maintain it as a perennial source of new cells. These cells are called *initials*. Their derivatives give rise to mature cells of the plant body with or without intervening cell divisions in them.

The concept of initials needs to be discussed at this juncture. A given initial in any meristem produces two daughter cells after division, out of which one continues to act as an initial while the other invariably becomes the precursor of an adult cell. The concept of initials and derivatives often goes with a qualification, i.e. the two are not inherently different from one another, but that the two do have some differences, when we speak of them with specific reference to some categories of meristems such as vascular cambium (see for more details, a later page in this chapter). Unlike the derivative cell, the initial must recreate complex patterns of transcriptional activity with each mitotic division and self-renewing. But, how this is moulded and maintained is not very clear. Initials also are characterized by very infrequent divisions (low mitotic index) and prolonged division cycle when compared to their derivatives. It is commonly believed that many cells of the meristem serve as initials, mainly because of their specific location in the meristem and not because of their inherent properties. In animals, a fixed category and numbers of initial cells are formed during embryonic development itself, but in the adult animal, the tissues and organs are maintained throughout the animal's life by populations of such cells that reside within these tissues and organs. These cells are termed *stem cells* (Weizman 2000). Animal stem cells

are often compared to plant's initials and several biologists, especially in the last 15 years, have adopted the term 'stem cells' for initials (Veit 2006) or to their most recent derivatives. There is often a confused usage of both these terms together, while describing the shoot apical meristem. For instance, Fletcher (2004) states as follows: 'The stem cells are not permanent initials'. Laufs et al. (1998) stated as follows: 'it is now widely assumed that central cells function as stem cells and serve as initials or source of cells for the two other zones of the shoot apical meristem'. Bowman and Eshed (2000) mention, 'the central zone acts as a reservoir of stem cells ….. It should be noted that these cells do not act as permanent initials….'. Vernoux et al. (2000) said as follows: 'It is now generally accepted that the central zone acts as a population of stem cells…. and generating the initials for the other zones, while maintaining itself'. Meyerowitz (1997), while characterizing the shoot apical meristem as a 'group of stem cells', designated its central zone as the 'zone of initials'. It is not clear from the above whether stem cells are equivalent to initials or stem cells give rise to initials or stem cells are permanent while initials are not and vice versa. The authors of this chapter advocate the use of the more appropriate and conserved term 'initials' (Evert 2006; Krishnamurthy 2015), although, while quoting, other researchers use 'stem cells' employed by them. It should, however, be stated here that more and more people are using the stem-cell terminology and the word 'initial' is fast disappearing from meristem literature. In fact, meristems are now called stem-cell niches (Scheres 2007; Bitonti and Chiappetta 2011).

Another important aspect that needs to be discussed here relates to the *permanency of the initials*: whether the initials that make up a meristem at any one particular time continue to remain as initials till the entire life span of the meristem? Some experiments carried out by Ball and his associates (Soma and Ball 1963) and Newman (1965) on shoot apical meristems (SAMs) have indicated that it is not so. Ball and his co-workers did two types of experiments to come to this conclusion. In one, they put India ink dot on the surface at the tip of the SAM and traced its fate through time-lapse photography. They found the dot got split into several dots, got radially displaced and finally formed parts of the internode or leaf primordia. The carbon dot eventually got totally lost from the tip of the SAM. In another experiments, they slightly injured the surface and subsurface cells of SAM through a microneedle and traced the fate of these injured cells. As expected, they also got displaced from SAM and formed part of internode or leaf after some time. These results indicated that the cells of SAM (i.e. initials) are not permanently located in the meristem but are replaced by newer initials. Newman (1965) also concluded that no cell in SAM is a permanent cell of the meristem. The meristem can be compared to an office manned by a number of initials. Once the people of an office get retired or transferred, their place is taken over by other people. In a similar way, the office of the meristem is manned by different sets of initials at different times. Newman, in fact, compared the SAM to a fountain of water, in which water coming out of the single nozzle (pore) (compared to the central mother cell zone) splits to radiating pattern (comparable to the derivative cells of the meristem), thereby implying that neither the water coming out of the nozzle (i.e. initials of meristem) nor the water that gets split in a radiating manner (i.e. derivative cells) is a permanent feature of the nozzle (i.e. meristem) but gets replaced every time by fresh water (i.e. fresh initials). The concept of nonpermanency of initials can be extended to other types of meristems as well, although no experiment has been so far carried out on them (Krishnamurthy 2015).

After a thorough review of literature on meristems as well as by doing investigations on meristems, Swamy and Krishnamurthy (1978) have shown that meristems, whether apical or lateral, are always constituted of a relatively quiescent group of cells and that the actively dividing cells are located around this group in apical meristems or located in files of cells between actively dividing files of cells in lateral meristems. Two physiological features characterize this quiescent zone: the extended mitotic cycle and the low mitotic index of its constituent cells. The quiescent

zone/file of cells not only forms the ultimate source of all dividing cells of the meristem but also acts as a reservoir of cells to aid in the reconstruction of the meristem if the dividing cells are damaged for some reason (Krishnamurthy 2015).

## 4.3 Classification of Meristems

Four principal categories of meristems are recognized in higher vascular plants: *apical* (in the shoot and root apices), *lateral* (vascular cambium and cork cambium or *phellogen*—both running parallel to the long axis of the stem and root of gymnosperms and dicots), *intercalary meristem* [a group of meristematic cells intercalated between mature tissues and derived either from shoot apical meristem (*residual* type) or from dedifferentiated mature cells (*resumptive* type)] and *diffuse* meristems (which are highly localized, temporary groups of cells that divide in all planes). Apical and intercalary meristems promote the longitudinal growth (growth in length) of the plant organ, while lateral meristems promote growth in girth or latitudinal growth. Apical meristems are small and lateral meristems are thin, but together they produce the entire plant, with several thousand times more mass/volume than them.

Many developmental botanists distinguish the meristematic initials and their most recent derivatives from their partially differentiated but still dividing cells under the name *promeristem* (Evans and Barton 1997; Barton 1998), *protomeristem* or *primary meristem*. Promeristem is classified further into the following categories based on the tissue region produced from it: *protoderm* (that gives rise to the epidermis), *procambium* or *provascular tissue* (that gives rise to primary vascular tissues) and *ground meristem* (that gives rise to ground tissue). Promeristems often exhibit different but characteristic patterns of cell division and growth depending on the plant organ in which they are present. Consequently, they often get special names such as the *mass meristems* (undergo cell divisions in all planes), the *rib meristem* (which through regular transverse divisions produce parallel, longitudinal files of cells) and the *plate meristem* (in which cell divisions are primarily anticlinal to result in flat structures like leaf, petal, sepal etc.)

## 4.4 Shoot Apical Meristem (SAM)

The SAM is the most distal region (above the youngest leaf primordium) of the shoot apex of the main shoot as well as of the branches. The SAM ranges in width from about 40 μm as in *Luzula* and *Syringa* to about 2,300 μm as in *Cycas revoluta*, with most species having a width range of 120–300 μm. The width may also differ depending on the age of the plant, with embryonic and seedling apices having less width than those of adult plants. The height of the SAM (as measured from the base of the youngest leaf primordium to the summit of SAM) also varies greatly. SAM of water plants like *Elodea* and *Hydrilla* has the greater height. In all plants, the height varies with the *plastochronic changes* (the changes that happen in SAM during the period between the formations of two successive leaf primordia) with the least height during early leaf primordial initiation and the maximum height sometime after leaf initiation.

### 4.4.1 Organization of SAM

Vascular plants have two basic categories of SAM, one with a single large and morphologically and cytologically distinct *apical cell* and the other with a multicellular SAM. Single apical cells characterize many pteridophytes like *Equisetum* and many ferns. The apical cell is superficially located and more commonly inverted pyramidal in shape (Fig. 4.1). The base of the pyramid is towards the surface of the apex, while the other three facets are 'embedded' in the body of the shoot apex. Its derivatives are formed in an ordered pattern from three of its inner facets. In some water ferns, the apical cell is three sided, and new cells are cut off from two of its facets only to result in bilaterally symmetrical shoots. The term *merophyte* is often used to refer to the immediate derivatives of the apical cell.

Although the apical cell was considered by early plant morphologists to be the ultimate source of all cells of the shoot, some others concluded that the apical cell is active only in young plants.

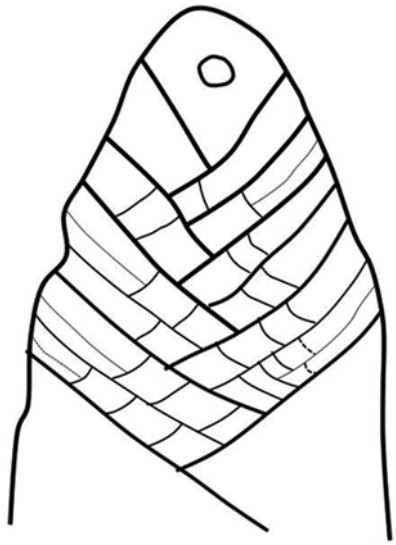

**Fig. 4.1** Diagrammatic representation of L.S. of shoot apex of *Equisetum* showing the apical cell and planes of its division and formation of derivatives (merophyte) (Krishnamurthy 2015)

However, radiolabelled DNA precursor supply to SAM with single apical cells showed that the apical cell divides mitotically. However, it is relatively quiescent when compared to its immediate derivatives.

In taxa like *Selaginella*, the SAM appears to have more than one apical cell arranged in a single anticlinal row. While some consider it as a transitional SAM between SAM with single apical cell and SAM which is multicellular, others consider it only as SAM with a single apical cell. Multicellular SAM occurs in *Lycopodium*, *Isoetes*, a number of ferns, gymnosperms and angiosperms. Two major theories have been proposed to explain the organization of the multicellular SAMs. One is the *tunica-corpus* theory proposed by Schmidt (1924), according to which the SAM has a superficial mantle of cells constituting the *tunica,* which encodes an inner *core* (Fig. 4.2). The number of tunica layers varies from one to three (L1, L2 and L3) depending on the species. Generally, all cells of tunica divide only anticlinally, and because of this feature, only the layered structure of the tunica is maintained. The cells of the corpus divide in all planes, thereby adding to the bulk of the SAM. It has

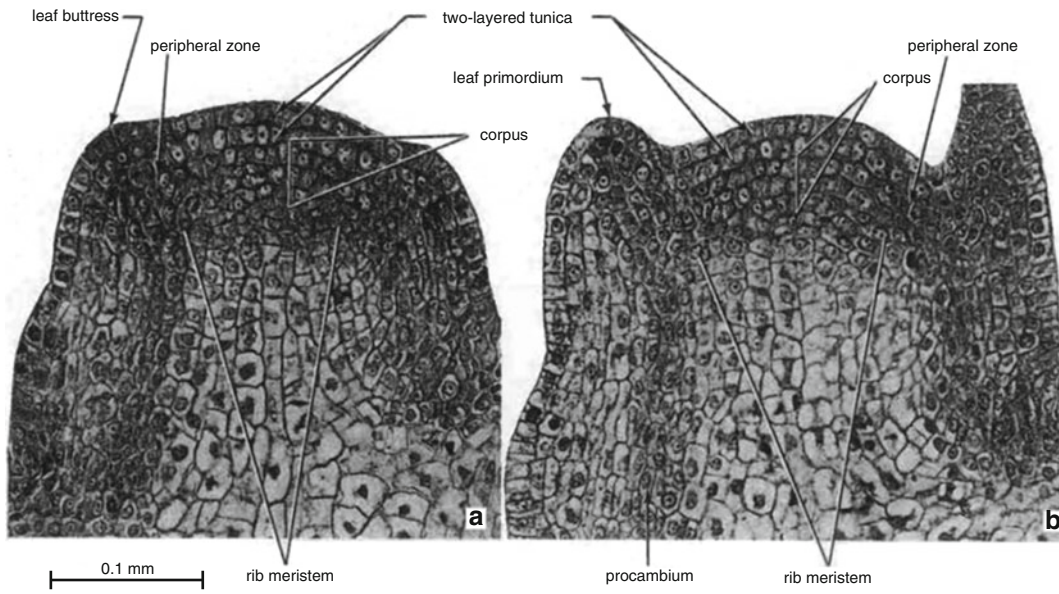


**Fig. 4.2** L.S. of shoot apices of *Solanum tuberosum* exhibiting tunica-corpus organization (After Sussex 1955)

been suggested that each layer of tunica arises from a small group of separate initials, and the corpus has its own initials beneath those of the tunica. Clonal analyses made on naturally occurring and experimentally induced *chimeras*, as well as on polyploidy induced by colchicine on SAM cell layers, are cited as providing a strong evidence for the one to three initials in each tunica layer. The main merit of the tunica-corpus concept is that it is very simple and explains the organization of SAM only without implying any relation to histogenesis in the derivatives of SAM, although the epidermis of subjacent shoot is derived from L1 layer of tunica. The tunica-corpus organization could not be found in the SAM of gymnosperms other than that of in *Gnetum* and *Ephedra*.

Foster (1938) proposed the *cytohistological zonation concept* to explain the organization of SAM of gymnosperms (Fig. 4.3). This concept is based not only on the planes of division in cells of different zones of SAM but also on the degree of cytochemical differentiation and the extent (mitotic index) and duration (mitotic cycle) of cell divisions of the constituent cells in different zones. Thus, this concept recognizes different cytohistological zones in the SAM. Subsequently, similar zones have been recognized in angiosperm SAM. Cytohistological zones were then superimposed on tunica-corpus organization.

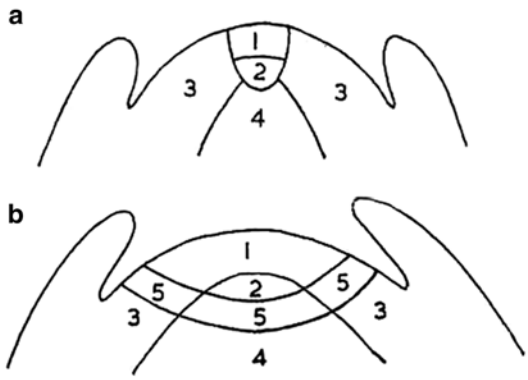


**Fig. 4.3** (**a**, **b**) Diagrammatic representation of zonation patterns in the shoot apical meristems. *1* Apical initials, *2* central mother cell zone, *3* peripheral zone, *4* rib meristem, *5* cambium-like transition zone (Krishnamurthy 2015)

The zones initially recognized and subsequently confirmed in angiosperms are *apical initials*, *central mother cell zone*, *peripheral zone* and *rib meristem* (Fig. 4.3). Rib meristem lies below the central mother cell zone or is separated from it, in some cases by a very narrow *transition zone*. The apical initials are believed to contribute cells to the surface layer by anticlinal divisions and to the central mother cell zone by periclinal division. The central mother cell zone gives rise to the rib meristem (and transition zone) as well as to the peripheral zone (also called by some *flank meristem* or *flank zone*). The peripheral zone gives rise to the leaf, axially buds and the peripheral parts of the stem, while the rib meristem gives rise to the pith. The zonation concept underwent changes and reinterpretations by the French botanists, who recognized the following zones: the *waiting meristem* (*méristéme d'attente*) or *apical zone* (zone *apicale*), the *initiating ring* (*anneau initial*) and *medullary meristem* (*méristéme medullaire*). The first zone is equivalent to Foster's apical initials and central mother cell zone, the second to flank zone and the third to rib meristem. However, renewed cytological, cytochemical, ultrastructual and autoradiographic studies as well as surgical experiments and in vitro culture studies have helped to characterize the features of the cells of each zone. Also, investigators outside France reaffirmed the zones recognized by Foster (1938).

The central mother cell zone is the most important zone of SAM. It is the ultimate progenitor of all other zones of SAM. It has the following cytological characteristics: largest cells in SAM, vacuolated, fairly thick-walled, low RNA content, least RNA synthesis, filamentous mitochondria, large nucleoli, undifferentiated plastids, the least mitotic index (i.e. very few cells were in division at any time of observation) and the greatest cell doubling time (i.e. prolonged cell cycle) which ranged, depending on plants, from >40 to 250 h. Its cells are least affected, when SAM is damaged by environmental or other factors even when other cells are damaged and hence are able to regenerate the SAM. Some investigators even go to the extent of saying that the stem cells (initials of SAM) are located in the

upper region of central mother cell zone (Pautler et al. 2013) (but in fact, all its cells are the true initials). In contrast, the flank zone has the following contrasting cytological characteristics: smallest cells in SAM, free of vacuoles, thin walled, high RNA content, greatest RNA synthesis, normal mitochondria, small nucleoli, the greatest mitotic index (i.e. almost all its cells divide) and the least cell doubling time which ranges, depending on the plant, from 11 to 137 h, i.e. approximately two times quicker than in central mother cell zone. The other two zones are intermediate between these two zones with reference to their cytological characteristics (Krishnamurthy 2015).

### 4.4.2 Elaboration of SAM

A number of studies have been done on the origin of SAM in the developing embryo and its subsequent elaboration during its growth into the adult plant. For instance, the primary SAM in *Arabidopsis* is reported to become apparent relatively late in embryogenesis, after the cotyledons are initiated (Barton and Poethig 1993). However, a critical analysis of embryogenesis in plants revealed that the SAM is initiated in the midglobular embryo stage itself in the form of *epiphysis* (Swamy and Krishnamurthy 1977; Krishnamurthy 1994, 2015; Raghavan 2000), which is the embryonic equivalent of the central mother cell zone of the adult SAM. Very cleat structural, cytological and cytochemical differences are seen between the epiphysis and the cells around them. These differences are noticed even before the heart-shaped stage, which represents the stage of initiation of the two cotyledons. In other words, the cotyledons are the first organs formed by the epiphysis and its surrounding cells that together constitute the embryonic SAM. The viability of the embryonic SAM is associated with a change from globular to cordate shape of the embryo. Further differentiation in SAM is temporarily halted when it becomes dome shaped just before its dormancy as the seed containing it also becomes dormant (Krishnamurthy 2015). However, in many taxa such as legumes, this embryonic SAM is active and produces a number of leaf primordia before becoming dormant. It becomes again active with seed germination, gets elaborated and starts exhibiting the different cytohistological zones.

### 4.4.3 Control of SAM Activity

#### 4.4.3.1 Genetic Control

The establishment of SAM requires the activity of *SHOOT MERISTEMLESS* (*STM*) gene in *Arabidopsis*, which is reported to be first expressed in one or two cells of the late globular embryo in the epiphyseal region. The *stm* mutations result in loss of function, i.e. these mutants produce seedlings with normal root and hypocotyl but lack SAM. *STM* mRNA is detected in the central mother cell zone and peripheral zone of the adult SAM but not in the developing leaf primordia. *STM* gene is, thus, required for the activity of SAM and the formation of leaf primordia. The expression of *STM* gene throughout the SAM but not in the leaf founder cells (see more information on founder cells in Chap. 6) prevents the apical meristem dome from premature differentiation by repressing the leaf primordium-specific regulator *ASYMMETRIC LEAVES1* (*AS1*) (Fig. 4.4) (see also Byrne et al. 2000). A well-defined SAM is also absent in the 'buds' regenerated on cultured explants of the *stm* mutants indicating that this gene controls both the embryonic and adventitious SAMs. The STM gene of *Arabidopsis* has been cloned and its predicted amino acid sequence shows a strong homology to the homeodomain (coded by the homeobox gene) of the *KNOTTED1* (*KN1*) gene of maize, the initial expression of the transcripts of which is seen in the early differentiating SAM of maize embryos and continues to be seen during postembryonic stages as well in the adult plant SAM. In rice, a gene similar to the *KN1* called *Osh 1* (for '*Oryza sativa* homeobox') is found; the mutation in this gene causes clump formation by SAM, and probably this gene maintains cells of SAM in the undifferentiated state. The *no-apical-meristem* (*nam*) mutation in *Petunia* species causes the non-differentiation of

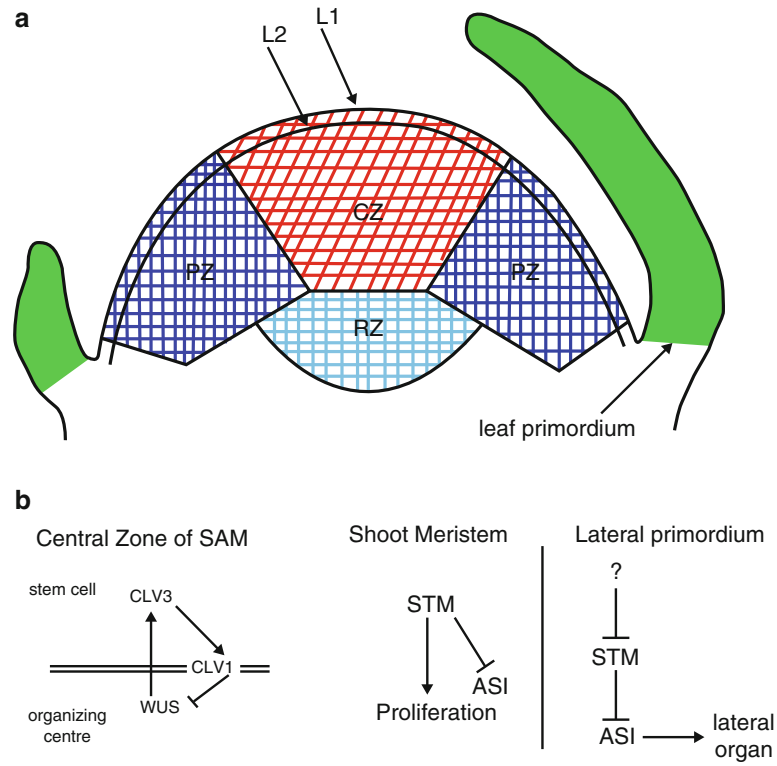


**Fig. 4.4** (**a**) Schematic diagram of the *Arabidopsis* shoot apical meristem (*SAM*) with developing lateral organs. The infrequently dividing central zone (*CZ*) contains organizing centre with overlying stem cells. Frequently dividing cells in the peripheral zone (*PZ*) give rise to lateral organs, whereas divisions below the rib zone (*RZ*) contribute to growth of the shoot axis. (**b**) Regulatory pathways active in the shoot meristem. Shoot stem cells are maintained by the *WUS-CLV* feedback loop. WUS expression in organizing centre confers stem-cell identity. The *CLV3* ligand secreted by stem cells is thought to bind to the receptor *CLV1* which in turn represses *WUS* expression (denoted by T-bar). *STM* maintains proliferation in shoot meristem by repressing expression of *AS1*. *STM* is repressed (denoted by T-bar) in lateral primordia permitting activation of *AS1* expression that is required for lateral organ development (Vijayaraghavan et al. 2005)

SAM in embryos. It is believed that the novel protein encoded by *NAM* gene functions in intercellular signaling, the absence of which probably inhibits cell division around the epiphysis during transition from cordate to torpedo embryos. A putative *STM* orthologue has been expressed in the SAM of poplar and it is named *ARBORKNOX1* (*ARK1*) (Groover et al. 2006). *KNOX* group of genes such as the above are reported to bind directly and either activate or repress GA biosynthesis genes, thereby modifying the levels of GA in SAM and boundary regions. They are also reported to regulate cytokinin biosynthesis, as discussed in the next section (Pautler et al. 2013). Another mutant, similar to *stm,* is *zwille* (*zll*). This also does not express the differentiated characteristics of a SAM in that the initiation of the meristem is blocked at the torpedo-shaped stage of the mutant embryo. The *zll,* like *stm*, is likely to control both embryonic and adventitious SAM (in cultures). A closely similar mutation, referred to as *pinhead* (*pnh*) affects the SAM during embryogenesis, as well as the axillary bud meristem, but not SAM formed in cultured explants. In addition to *STM* gene, *WUSCHEL* (*WUS*) gene is required for the maintenance of initials' function in SAM. In *wus* mutants, the initials undergo differentiation into derivative mature cells and no longer remain as initials. *WUS* expression begins at 16-celled stage of the embryo in advance of

*STM* expression. In the fully developed SAM, *WUS* expression is restricted to a small group of central cells between the L3 layer (the initial layer of the corpus) and the rib meristem, and this expression persists throughout shoot development. *WUS* is not expressed within the initials but the signaling must occur between L3 and central cells. Further studies indicate that a short 57 bp *cis*-acting element in WUS promoter mediates the effects of diverse regulatory pathways controlling *WUS* expression (Baurle and Laux 2003). Thus, *WUS* is a positive regulator of SAM maintenance. It is likely that *WOX4* of rice may be similar to *WUS* as a positive regulator of SAM maintenance.

Mention must also be made of *MGOUN* (*MGO*) gene of *Arabidopsis*, which, like *WUS*, contributes to the precise definition of SAM. Cell divisions in SAM of *mgo* mutant embryos are distorted to some extent as in the SAM of *stm* mutant embryos leading to the formation of a larger-than-normal SAM. In these embryos, the corpus (central mother cells) region undergoes cell proliferation to form a fasciated structure. Hence, it may be stated that *WUS* and *MGO* genes are particularly required for maintaining the structural and functional integrity of SAM, specifically its central mother cell zone. However, we do not yet know whether the central mother cell zone and peripheral zone of SAM are differentially regulated. In addition to *STM* and *WUS* genes which regulate differentiation in SAM, there are two other *Arabidopsis* genes that regulate SAM size by repressing the overactivity of initials. These are *CLAVATA* (*CLV*) genes (Fig. 4.4b) (*CLV1*, *CLV2* and *CLV3*) and *EXTRACOTYLEDON* (*XTC*) genes. Mutation in these genes increases the production of more cells in the central mother cell zone and thereby its size (Fletcher 2002). This accumulation of cells is due to a failure of differentiation of flank meristem cells. *CLV3* expression is mainly restricted to L1 and L2 layers and to a few L3 cells in the central zone of SAM and probably marks the initials in these layers. *CLV1* expression underlines the L1 and L2 layers. As already mentioned, *WUS* gene expression is in the cells of central mother cell zone (the 'organizing cells' of the entire SAM) which confers initial cell identity to the overlying neighbouring cells, and the signals from *CLV1*/*CLV3* regions act negatively to dampen such activity. *CLV3* protein secreted by the initials in the apex moves through the apoplast, binds to a *CLV1*/*CLV2* receptor complex and causes the downregulation of *WUS*, maintaining the appropriate amount of activity of initials throughout development. In other words, *CLV* genes are negative regulators of SAM maintenance. Thus, a negative feedback loop exists between *CLV-WUS* which maintains a balance between the proliferation of promeristem cells and their loss through differentiation and the initiation of leaves in the flank meristem (Fletcher 2002, 2004; Schoof et al. 2000; Ha et al. 2010; Aichinger et al. 2012). The genetic relationship and molecular function of *FON1* and *FON2* in rice plant are very similar to those of *CLV1* and *CLV3* (Pautler et al. 2013).

Many more genes are needed for the correct organization of SAM in the embryo as well as its elaboration in the adult plant of *Arabidopsis*. The *EMBRYONIC FLOWER* (*EMF*) regulates the typical tunica-corpus organization right from the cordate embryo stage onwards; its effect is seen in adventitious SAM of callus. The meristem directly forms the reproductive meristem. The other genes of *Arabidopsis* involved in regulating SAM are *ULTRAPETALA 1 and 2* (*ULT 1 and 2*), *HANABA TARANAU* (*HAU*), *HAIRY MERISTEM* (*HAM*), *PHAVOLUTA* (*PHAV*), *PHABULOSA* (*PHAB*), *REVOLUTA* (*REV*), *CORONA* (*CAN*), *POUNDFOOLISH, STIP, FASCIATA1 (FAS1)* etc. The *FLATTENED SHOOT MERISTEM* (*FSM*) of rice controls size and shape of SAM; in *fsm* mutant, the SAM is flatter and smaller.

Some of the above genes are included under class III homeodomain-leucine zipper (*HD-ZIP III*) genes. These are believed to be targets of miRNAs 165 and 166. Overexpression of miRNA 166 by activation tagging results in downregulation of the *ATHB9*/*PHV*, *ATHB-14*/*PHB* and *ATHB-15* genes and concomitantly causes an enlargement of SAM (Zhou et al. 2007). It was further shown that overexpression of miRNA 165 causes a drastic reduction in the transcript levels of all five *HD-ZIP III* genes in *Arabidopsis*

(*IFL1*/*REV*, *ATHB-9*/*PHV*, *ATHB-14*/*PHB*, *ATHB-15*, *ATHB-15*/*CNA*/*ICU4*). The miRNA 165 overexpressors display prominent phenotypes reminiscent of loss-of-function mutants of *revphbphv* and *rev*/*ifl1*, including loss of SAM (Zhou et al. 2007).

#### 4.4.3.2 Hormonal Control

The SAM, besides being controlled by genes, is controlled by hormones (Veit 2009). The SAM shows a characteristic placement along the proximo-distal polar axis. This polarized placement that is evident from the embryo onwards depends on the complex patterns of hormonal synthesis, transport and accumulation, particularly of auxin. Relatively low levels of auxin are a prerequisite for the normal organization and activity of SAM, probably through the effect of auxin on the expression of *CUC* genes and the activation of *STM* gene. Localized elevation in auxin levels, particularly in the flank meristem, triggers leaf initiation, probably accompanied by the downregulation of *KNOX* genes in leaf founder cells (see Chap. 5 in this volume). How auxin regulates *KNOX* gene expression in SAM is not yet clear. Low auxin levels in SAM may be related to the relatively nonpolarized cell growth in the corpus region. There is also suppression in GA activity in SAM and this suppression may be regulated by *KNOX* genes. Cytokinins are also important in regulating the SAM. Reduction in cytokinin level suppresses SAM activity, while an elevated level promotes SAM activity. This is evident from an analysis of *ABPHY1* gene in maize, which codes for an A-type cytokinin response regulator. Loss of *ABPHY1* function leads to great enlargement of SAM through a cytokinin-mediated cell proliferation (Giulini et al. 2004). The relationship between cytokinins and SAM activity is also revealed by an analysis of *KNOX* phenotypes. This gene expression results in a rapid increase in cytokinin levels through an isopentenyl transferase enzyme that mediates cytokinin synthesis. Available data suggest that *KNOX* genes and cytokinin mutually reinforce SAM identity. A genome-wide binding profile for *KN1* was recently identified (Bolduc et al. 2012). This study revealed that *KN1* targets auxin, cytokinin, GA and brassinosteroid hormone pathways. In *Arabidopsis*, *WUS* promotes cytokinin signaling by repressing the A-type genes *ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) and *ARR15*, whereas cytokinin positively regulates the expression of *WUS* (Gordon et al. 2009). The importance of *LONELY GUY* (*LOG*) gene function in SAM organization of *Arabidopsis* is known very recently. The biologically active form of cytokinin, which is probably catalysed by *LOG4* expression in the SAM epidermis, acts as a positional cue for patterning the *WUS* expression domain (Chickarmane et al. 2012).

### 4.4.4 Autonomy of SAM

An important question relevant to SAM is whether it can function independently of its subjacent regions of the stem. To answer this question, two different experimental approaches had been followed: surgical experiments and in vitro culture of isolated SAMs. It is evident from such experimental studies that the SAM, to a very large extent, is autonomous and that it does not need signals from other parts of the plant to direct its functional activity (Smith and Murashige 1970; Soma and Ball 1963). A critical analysis of the results of surgical experiments indicated that the central mother cell zone is the most important region of the SAM and that any surgically removed portion of SAM without at least a few cells of central mother cell zone will not regenerate the SAM (Swamy and Krishnamurthy 1978; Krishnamurthy 2015). The cultured SAM explants first establishes a bipolar axis through the initiation of one or more root primordial before again producing stem tissues.

### 4.4.5 Evolution of SAM

The structure of SAM differs among plant groups, thus suggesting its probable independent evolution in lycophytes, ferns, gymnosperms and angiosperms. Several parameters such as structure, gene expression patterns, leaf primordia initiation patterns etc. have been used as the basis for such an evolutionary analysis.

One such parameter employed is the distribution and pattern of plasmadesmal (PD) network in the SAM (Imaichi and Hiratsuka 2007). An analysis of this parameter was made in the SAMs of 17 families and 24 species of angiosperms, gymnosperms and pteridophytes. The SAMs of angiosperms and gymnosperms have low PD density per unit area, with no difference between SAMs showing tunica-corpus organization and those showing cytohistological zonation patterns. On the contrary, SAMs of ferns (including *Psilotum* and *Equisetum*) have average PD densities (more than three times higher). Interestingly, the lycopods have both fern (in *Selaginella*) and seed-plant (Lycopodiaceae and Isoetaceae)-type PD densities. In other words, SAMs with single and plural initial cells have, respectively, the fern- and seed-plant-type PD distribution, indicating their probable independent evolution. Further, the fern- and seed-plant-type PD patterns are equivalent, respectively, to the lineage-specific network (LPD) (i.e. PDs formed in expanding cell plates during cytokinesis) and interface-specific PD network (IPD) (i.e. PDs having primary and secondary origins with the latter inserted into already existing walls) proposed earlier by Cook et al. (1996). The coordinated growth of the plant requires cell-to-cell signaling and transport of regulatory proteins and/or mRNAs, and hence PD distribution densities and patterns in apical meristems with single and plural initial cells assume great importance.

## 4.5 Root Apical Meristem (RAM)

In contrast to SAM, the RAM is subterminal, does not produce appendicular organ like leaves, does not undergo periodic changes in size due to plastochronic changes, produces endogenous root branches beyond the region of active growth (unlike exogenous origin of branches in stem very near the apical meristem) and does not produce nodes and internodes. Generally, the root may or may not have a specific protoderm (or *dermatogen*), the promeristem of epidermis (the outermost layer of root is often called *rhizodermis* or *piliferous* layer). Friedman et al. (2004) raised three basic questions regarding the roots: (1) Are roots of different land plant lineages homologous? (2) Are the developmental gene programmes that give rise to roots and shoots the same? (3) Do the shared features of histogenesis in roots and shoots arise from common developmental programmes first expressed in land plant sporophytes prior to the origin of roots? All these three queries also reflect on RAM as it gives rise to roots. The first question is still unresolved. Notwithstanding the fact that the RAM and SAM differ, as stated above, cladistic analyses (Kendrick and Crane 1997; Veit 2006) support the homology of the two meristems. Both have a centrally located 'quiescent centre zone', as emphatically shown even as early as 1978 by Swamy and Krishnamurthy; also, recent molecular and genetic analysis have shown the existence of similar mechanisms of operation in the two meristems. Some believed that the shoot apex was transformed into a root apex early during the evolutionary history of vascular plants, as supported by investigations of mutations affecting both RAM and SAM and by the similarities in the expression of *SCR* and *WOX* genes in both RAM and SAM (Jiang and Feldman 2005). There are others who believe in the de novo origin of root.

### 4.5.1 Organization of RAM

Like SAM, RAM is also of two basic categories in vascular plants: with a single apical cell (Fig. 4.5) and a multicellular RAM. RAM with single apical cell is seen in many ferns, while the second type of RAM is seen in lycophytes (other than *Selaginella*), a few ferns, gymnosperms and angiosperms. In most cases of RAM with an apical cell, the cell is tetrahedral and cuts off segments on three lateral (proximal) facets to produce the body of the root; the rootcap is produced from either the fourth facet of the apical cell distally or from a separate meristem formed very early in root ontogeny.

In the multicellular RAM of a number of plants, the body of the root arises from a massive meristem comprising of three of four precursor tissue regions or *histogens* (Hanstein 1868, 1870). Each of these

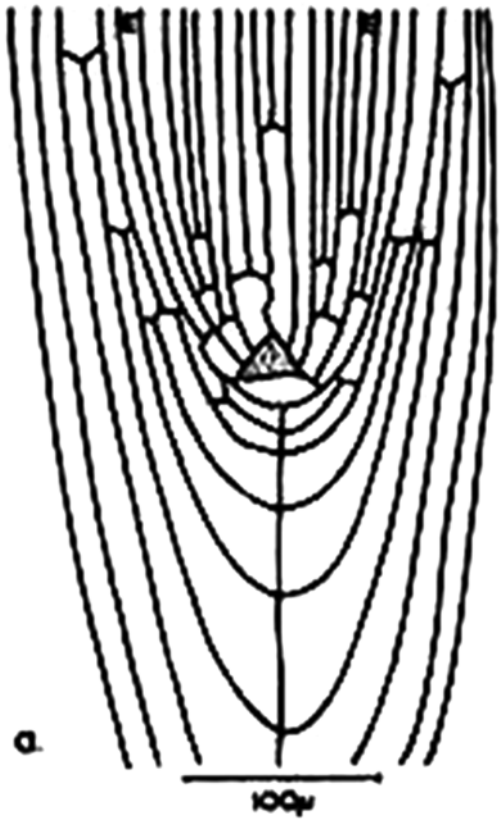


**Fig. 4.5** L.S. of root apex of *Marsilea* showing apical cell and body-cap organization and T-division patterns in its derivative cells (Clowes 1961)

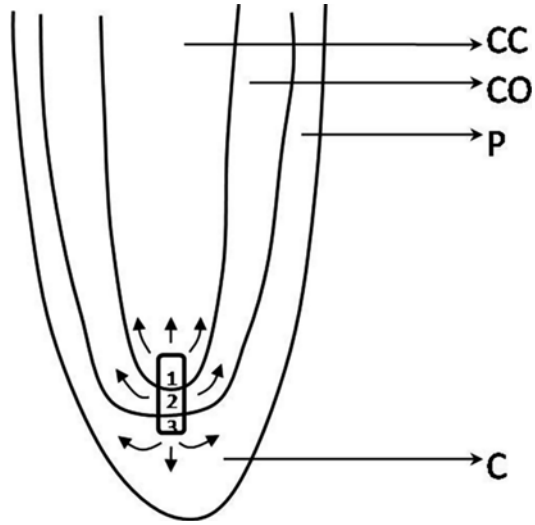


**Fig. 4.6** Diagrammatic L.S. of the root apex interpreted as per histogen theory. *1* Plerome, *2* periblem, *3* dermatogen, *C* rootcap, *CC* central cylinder, *P* piliferous layer (Krishnamurthy 2015)

histogens begins with one to many initials at the apex arranged in superposed tiers (Fig. 4.6). The histogens are called dermatogen (precursor of the root dermal layer), *periblem* (precursor of cortex), *plerome* (precursor of stele and pith, if present) and *calyptrogen* (precursor of rootcap). In RAM of some plants, the dermatogen is absent and the surface layer is formed from periblem. In some gymnosperms and angiosperms, all tissue regions of the adult root or all except the central cylinder appear to arise from a common meristematic zone; such a RAM was called *open type* (Guttenberg 1960) and the ones with separate initials belong to the *closed type* (see Heimsch and Seago 2008). The distinction between open and closed types of RAM is not always clear, sometimes even in the same plant at different developmental stages; sometimes a transition type of RAM is seen in some taxa such as *Allium cepa*. Clowes (1981) and Deysson (1980) distinguished open and closed types of RAM in a slightly different way. According to them, a closed RAM has a distinct initial layer for the rootcap, while an open RAM has interchange of cells between cortex and cap.

Whatever may be the category of RAM, whether with single apical cell or multicellular or whether of open, closed or transitional type, the RAM shows the body (körper) or cap (kappe) type of cell divisions (Schüepp 1917). The planes of those cell divisions (= *formative divisions*) that are responsible for the increase in the number of vertical files in the actively dividing region of the RAM are very important to indicate the body or cap category. Many of the files divide into two, and where they do so, a cell divides transversely; then, one of the two new cells divide longitudinally and each daughter cell of this division becomes the source of a new file. This combination of the transverse and longitudinal divisions results in an approximately inverted T- or Y-shaped wall pattern, and hence, such divisions are called *T-divisions* (Fig. 4.5). The directions of the top stroke (horizontal bar) of the T vary in different root regions. Often, in the rootcap, particularly in the columella region, it is directed towards the base of the root (cap-type division),

while in the body of the root, it is towards the apex (body-type division). In some RAM, there is a clear boundary between the 'body' and 'cap'; in others such a boundary is not clear.

### 4.5.2 Quiescent Centre Concept

This is the most important concept relating to the organization of RAM. Clowes (1961) first discovered a *quiescent centre* (*QC*) in RAM in 1954, and this discovery completely revolutionalized our understanding of the behaviour of RAM. According to Clowes, there is a *minimal constructional centre* in the RAM, which largely ceases to be mitotically active, and this centre was labelled by him as QC. The regular presence of this in RAM was proved by conventional and cytochemical staining methods, surgical experiments, irradiation and on root apices fed with radiolabelled chemicals involved in DNA synthesis followed by autoradiography (Fig. 4.7).

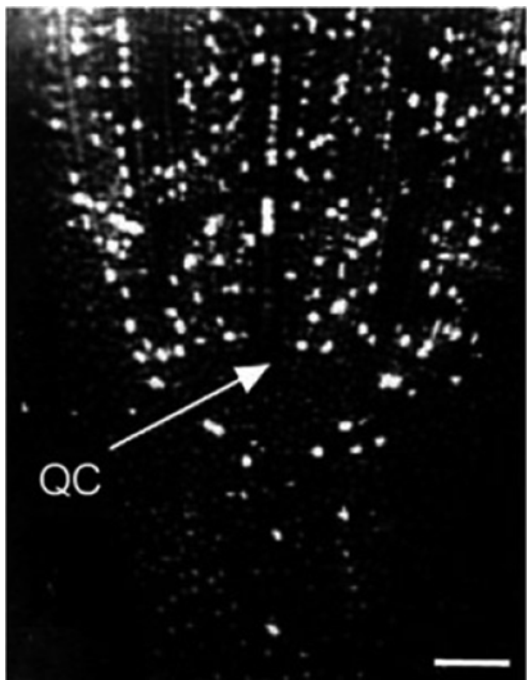


**Fig. 4.7** Autoradiograph of longitudinal sections through the *Allium cepa* RAM of seedlings exposed to [Me-3H] thymidine for 24 h: root grown in water. Figure shows the lack of DNA synthesis in Quiescent centre (QC) (Liso et al. 1988)

Mitotic activity was noticed only in cells located on the margins of QC. The QC in the RAM of a mature root is hemispherical (*Zea mays*) or discoid in shape and contains as few as four cells as in *Arabidopsis* to more than a thousand as in some very broad roots. In *Zea mays*, it has almost 1,500 cells and in *Helianthus* about 800 cells. The report of its absence in the very thin roots of rice is erroneous as QC is an integral part of each and every RAM, however small it may be. Similarly, the report of origin of QC twice in primary roots, once in embryo and then during germination with an intervening absence in the mature embryo (see Evert 2006) is also erroneous since QC occurs since its inception as hypophysis in the globular embryo throughout the life of the root (Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015).

The relatively inactive state (in terms of cell division) of the QC does not mean that its cells have become permanently inactive mitotically. QC cells do divide, although infrequently. It serves to renew the more actively dividing regions around the QC with its supply of new cells, since the dividing regions have an unstable population of cells that are displaced from time to time and need to be replenished with newer cells. By labelling nuclei with radiolabelled DNA precursors and by blocking cell cycle at metaphase with inhibitor chemicals, one can obtain quantitative data on the duration of *mitotic cycle* (*MC*) as well as number of cells actually undergoing cell division (i.e. *mitotic index*, MI) in the QC and other regions of RAM. These data indicate that the cells of QC divide approximately ten times slower than the adjacent dividing cells (Krishnamurthy 2015). Pulse labelling with tritiated thymidine has shown that the differences in the duration of mitotic cycles are largely caused by differences in the duration of *G1* phase of cell cycle. In other words, the MC takes a very long time to get completed in QC cells. The MI is also very low in the QC, i.e. only a few cells of QC divide and even these cells take a very long time to complete their division. The MC duration of QC cells of investigated taxa ranges from around 170 h to about 520 h in contrast to cells of other regions of RAM whose MC ranges from about 12 h to about 45 h.

The paucity of the mitotic activity of the cells of QC led Clowes (1961) to suggest that the actual initials of RAM are located just outside the QC, all around it. And he designated these initials as *promeristem* of RAM. However, Swamy and Krishnamurthy (1975), Barlow (1978) and Steeves and Sussex (1989) have argued that it would be more appropriate to consider the slowly dividing and sparsely dividing cells of the QC themselves as the ultimate source of cells to the entire root apex; they are the initials which are strikingly similar to those of central mother cell zone of the SAM. Hence, QC itself is to be considered as the promeristem. Some botanists like Kuras (1978) consider the root promeristem as comprising the QC as well as its immediate, actively dividing derivatives. Even today there is no uniformity in the use of terms and in the meanings attributed to these terms by different investigators, although the majority agrees that the QC is the promeristem.

Since QC has cells with low MI and since even its dividing cells have a prolonged MC, the QC may also be considered as a reservoir of cells to renew the RAM, if RAM becomes dormant due to unfavourable environmental conditions or becomes damaged due to various reasons. In fact, QC cells have been shown to enter into active division activity during recovery from a period of dormancy through cell cycle changes. The root apical meristem is occasionally damaged as the root forces its way through the resisting soil or damaged due to soil biota. When this damage occurs, cells of QC divide and once again organize the RAM with all its component zones including a QC. QC cells are not easily damaged by dormancy and other physical factors affect only dividing cells when they are in *S* and *G2* phases. Some of the cyclin-dependent kinases (CDKs) share a conserved PSTAIAR motif (stand for proline, serine, threonine, alanine, isoleucine and arginine sequence). A high level of this protein (CDK) has been identified by immunofluorescence studies in the QC of maize. This finding has raised the possibility that the PSTAIAR proteins might enable QC cells in readiness to enter the cell cycle after an extended interphase (Krishnamurthy 2015).

Based on an analysis of growth patterns in root apices, Barlow (1973) and Clowes (1984) have suggested that quiescence at a particular location of RAM results from antagonistic directions of cell growth in various parts of the meristem, the rootcap or calyptrogens being the most important in suppressing growth. Clowes (1978a, b) argued that the origin of QC during embryogenesis coincides with the appearance of the rootcap. If the cap is surgically removed or otherwise damaged, the QC gets activated to give rise to a new cap meristem and hence a new cap), following which quiescence again resumes in QC. This behaviour prompted Barlow and Adam (1989) to suggest that the activation of QC, after damage or removal of cap, results from an interruption or modification of signaling between the rootcap or its meristem and the QC. This signaling, according to these authors, is more likely to be a hormone, an auxin. It was further suggested that the origin and maintenance of QC in maize root apices are due to polar auxin supply and that the rootcap or its initial plays an important role in regulating the polar auxin transport towards the root tip (Kerk and Feldman 1994). High levels of auxin bring about elevated levels of ascorbic acid oxidase (AAO) resulting in the depletion of AA within QC, i.e. QC has a more oxidizing environment. Since AA is required for *G1* to S phase transition in the cell cycle in RAM (Liso et al. 1984, 1988), Kerk and Feldman (1995) proposed that depletion of AA in root apices may be responsible for the formation and maintenance of the QC. Subsequently, Kerk et al. (2000) reported that in maize AAO also oxidatively decarboxylates auxin; this is another mechanism for the regulation of auxin levels in QC and other RAM regions. An intact rootcap is vital for this physiological process to occur. There are some problems in the above attractive explanation: cells in the QC do divide, although in a prolonged manner. How do these dividing cells selectively escape when dividing cells (particularly at the margins of QC) are affected by the depletion of AA? There are roots without rootcap but not without QC. Moreover QC has its origin in hypophysis of the globular embryo stage when a rootcap or its initial is non-existent. Hence, it is

more likely that QC controls the rootcap (and other RAM tissues) and not vice versa. Moreover, it is stated in the earlier paragraph and emphasized long back by Swamy and Krishnamurthy (1975, 1978) that the QC is not only an integral part of RAM but also the ultimate source of all cells of RAM and that its prolonged MC and low MI is a built-in safeguard to regenerate the RAM if its meristematic cells are damaged by irradiation, mechanical damage, carbohydrate starvation, exposure to low temperature or exposure to ascorbic acid. But unlike the other factors mentioned above, those roots treated with ascorbic acid retained a minimal QC size or minimal constructional centre. The importance of QC is also brought to light by laser ablation studies on *Arabidopsis*. When all four QC cells are ablated, they were replaced by the initials of the root central cylinder (Scheres and Wolkenfelt 1998). Ablation of one QC cell causes cessation of cell division and the progression of differentiation in the columella and cortical initials with which it is in contact with. The major role of QC is thus to keep the initials in the dividing state. High meristematic activity around QC of *Arabidopsis* has been shown to be triggered by glutathione, a diffusible redox agent; this functions along with AA to maintain the cell's redox equilibrium. Dividing cells show an increased level of glutathione in contrast to a low level in QC. Thus, pool sizes of AA and glutathione and control of mitotic activity are linked in RAM. Some researchers believe that QC itself may be the site of hormone synthesis and this itself might prevent the QC cells from active mitosis. This is proved by the fact that exposure of maize RAM to TIBA, an inhibitor of auxin transport, causes a burst in mitotic activity in QC.

How does the QC originate and get elaborated as the plant develops from the zygote? As early as 1975 itself, Swamy and Krishnamurthy (1975) have analysed in detail the origin of QC in tap (primary), lateral and adventitious roots. Unfortunately this work was overlooked by most western botanists. In primary root (radicular root), QC originates as hypophysis, a cell first demonstrated and named by Hanstein (1870). It gets differentiated at the micropylar pole of the globular embryo with the simultaneous appearance of epiphysis on the exactly opposite pole of the embryo. Although there are slight variations in the origin of hypophysis (see details in Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015) as traced by different embryologists, it was categorically demonstrated that the hypophyseal cell and its immediate derivatives remain histologically conspicuous by relatively poor stainability and a relatively 'dormant' and quiescent state. During seed germination, this histological distinction remains unchanged, although the size of hypophysis and its derivative cells slightly increase through slow addition of cells. Thus, the hypophysis and QC are a continuum, and two separate terms are not necessary and QC or QC initials should replace hypophysis (Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015). The so-called hypophysis has the reduced capacity to incorporate radiolabelled DNA precursor and low poly(A)-RNA as revealed by in situ hybridization using [$^3$H] poly(U) as a probe (see Raghavan 2000). The same cells of hypophysis that fail to bind [3h] poly(U) do not incorporate [$^3$H] thymidine in the QC of seedling in *Capsella bursa-pastoris*. The hypophyseal origin of QC is also demonstrated by the *hobbit* (*hbt*) mutant of *Arabidopsis*. This mutant has an aberrant hypophysis specification during embryogenesis and its seedling lacks a recognizable QC (Willemsen et al. 1998). A precise delineation of cell determinants associated with the nondividing cells of QC, the dividing cells of dermatogen and the cells of the growing root must be taking place during the initial divisions of the hypophyseal cell (Krishnamurthy 2015). Hence Aida et al. (2004) must be wrong in believing that the four-celled *Arabidopsis* QC is not composed of stem cells; in fact, all these four cells are primordial stem cells (see earlier discussion on the concept of initials in this chapter). This conclusion of Aida et al. (2004) has also been questioned by Jiang and Feldman (2005), who regard these four cells as structural initials (stem cells) as opposed to the surrounding, more actively dividing functional initials cells.

The origin of QC in lateral and adventitious roots is shown to be de novo (Clowes 1961;

Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015). In lateral roots, Clowes (1961) demonstrated that the origin of QC coincided with the arrest of DNA synthesis in the concerned cells after the lateral root initiation proceeds for a time. The de novo origin of QC in the adventitious roots has been traced through development studies made on *Commelina benghalensis* (Swamy and Krishnamurthy 1975; Krishnamurthy 2015).

As already discussed, the presence of QC is very vital for the effective functioning of the root, particularly in deciding the indeterminacy of root growth. If QC is lost, the RAM becomes determinate. This has been demonstrated in root primordia that are transformed into thorns as in some palms such as *Cryosophila* and some bamboos and in the cactus *Pachycereus* (Rodriguez-Rodriguez et al. 2003). In this cactus, immediately after germination, the primary root becomes determinate but instead several lateral roots emerge. This primary root loses its QC in a molecular mechanism similar to that noticed in *Arabidopsis HOBBIT* or *PINOID* mutants. The unpublished observations of one of the authors of this article, Krishnamurthy, indicate that the RAM becomes arrested in growth and stubby in mycorrhizae-infected roots of *Lycopodium* and *Pinus* essentially due to the disorganization of QC. However, little is known of the mechanisms leading to QC disappearance and determinate root growth.

### 4.5.3 Genetic and Hormonal Control of RAM Activity

The formation of hypophysis/QC is influenced by mutants in *MONOPTEROS* (*MP*) (Berleth and Jürgens 1993), *BODENLOS* (*BDL*) (Hamann et al. 1999), *SHORT-ROOT*, *SCARECROW*, *PLETHORA* (*PLT*) and *HOBBIT* genes. Some molecular genetic analysis made on RAM revealed what may be analogous to *WUS* activity in the QC that is noticed in SAM (Kamiya et al. 2003; Haecker et al. 2004). Artificial activation of this *WUS*-like gene promotes RAM, while overexpression of CLV-like peptides leads to depletion of RAM (Hobe et al. 2003; Fiers et al. 2005).

Many root-specific genes that are transcribed in the cortical derivatives have been known and identified. The one fairly well-studied gene is *Axis-abundant 92* (*AX-92*) of water-imbibed seeds of *Brassica napus*; this has been isolated from a cDNA library made to poly(A)-RNA. The transcripts of this gene are detected in the ground meristem and cortex of seedling root but are not present in significant amounts either in rootcap or the vascular tissues. Hence, it defines initial cortical parenchyma differentiation events and is first expressed in the cortical region of torpedo embryo radicle of transgenic plants (Deitrich et al. 1992). In contrast to this, transcripts of one of maize *ZRP3* (for '*Zea* root preferential') cDNA clones are found in several inner cortical layers, while transcripts of another clone of *ZRP4* are expressed in endodermis and three to four exodermal layers of outer cortex. The maximum expression of *ZRP3* gene is confined to a height of 2 cm while that of *ZRP4* to a height of 8 cm from the root tip. A *ZRP3* homologue is known in bean root, and this is expressed in cortical meristem with a gradually decreasing gradient from root tip towards root body (Choi et al. 1996).

In addition to genetic control, RAM activity is also controlled by plant growth regulators. In *Arabidopsis*, specification of RAM in embryo occurs as a consequence of *PIN1*- and *PIN4-dependent* accumulation of auxin. The auxin in preglobular embryos originates either in the suspensor or in the embryo sac (Friml et al. 2003), but by globular stage, the expression pattern of *PIN*-dependent transporters changes and auxin production begins. With further development of embryos, auxin production continues and increases at their apical ends. Genes mediating auxin response include *BDL* (which affects auxin sensitivity), *MP* (which affects polar auxin transport) and *AUXIN RESISTANT1* (which affects auxin response), the mutants of all of which do not form an embryonic root. *HOBBIT* mutants have incorrect hypophysis (QC) development and a reduction in auxin reporter gene expression and accumulate the *AXR3/IAA7* repressor of auxin responses. Some information on the importance of auxin on QC/RAM of adult roots was already provided. The apico-basal polarity of

RAM depends on the complex pattern of synthesis, transport and accumulation of growth regulators, mainly auxins, right from the embryo stage onwards. High auxin levels in the central basal domain of the *Arabidopsis* embryo trigger the expression of *PLT1* and *PLT2* genes, which are *AP2* class transcription factor genes. These genes along with *SCR* and *SHR* genes that control radial patterning help in the establishment of an organized RAM with the correct positioning of QC (Jiang and Feldman 2005). These are expressed as early as eight-celled embryos. *PLT* and *SCR*/*SHR* operate via parallel pathways that appear to converge to a subset of target QC-specific promoters (Fig. 4.8). High *PLT* expression promotes stem-cell fate (QC), while the lower and lowest expression promotes derivative cell proliferation and differentiation, respectively (Galinha et al. 2007). Through a feedback mechanism, PLT proteins promote *PIN* expression and maintain stem-cell niche. A histone acetyltransferase activity, general control nonrepressed protein5 (GCN5), is also necessary to promote *PLT2* expression (Fig. 4.8) (Kornet and Scheres 2009). CDKB1 and CDKB2 and CYCB1;1 (cell cycle kinases) are likely to be putative targets of *PLT2*. *PLT1* and *PLT2* induce the expression and/or accumulation of *HIGH PLOIDY2* (*HYP2*), a nuclear-located ligase, which is expressed in the dividing cells of RAM, and a negative regulator of endoreduplication. Thus, auxin maintains root meristem homeostasis through *PLT1/2* expression and high *HYP2* expression, which prevents endoreduplication and promotes cell proliferation.

The GRAS family transcription factors *SCR* and *SHR* are required for correct function of RAM; *SHR* is expressed in provascular cells adjacent to QC and endodermal initial layer (Fig. 4.8), where it interacts with *SCR*. This interaction results in the activation of target genes required for QC identity, stem-cell niche homeostasis as well as for cell divisions in RAM (Bitonti and Chiappetta 2011). Recently, a number of putative SCR-interacting proteins have been identified (Fig. 4.8), which specifically interact with their N-terminal domain. Their c-terminal domain interacts only with SHR. The former

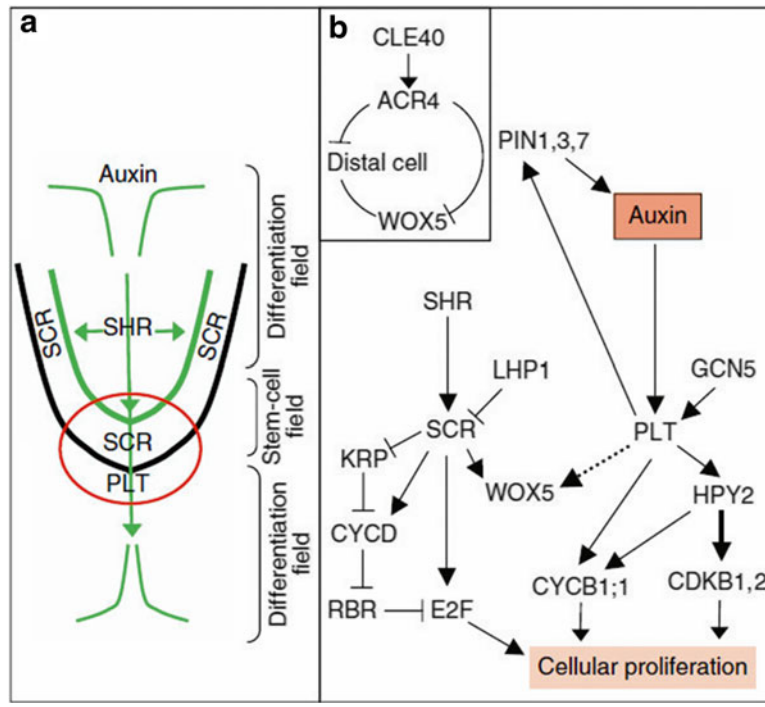


**Fig. 4.8** Scheme depicting (**a**) auxin polar flux (Adapted from Vernoux and Benfey 2005) and (**b**) its interaction with a transcriptional network in root patterning (see text for further details). *Arrows* indicate positive regulation; *barred lines* indicate negative regulation (After Bitonti and Chiappetta 2011)

interaction is found to be versatile and is necessary to repress further cell divisions, while the latter is necessary to activate asymmetric cell division. Among the SCR-interacting proteins, LIKE HETEROCHROMATIN PROTEIN1 (LHP1) is highly expressed in the root elongation zone. LHP1 plays a role in cortex formation by acting together with *SCR* in preventing further asymmetric cell divisions. Thus, it can be concluded that the stem-cell niche can be identified as the domain where the highest expression levels of *PLT1*, *SHR* and *SCR* overlap. Perhaps all these (*PLT*, *SHR*, *SCR*) do not control the same target genes with the possible exception of *WOX5*. *WOX* expression is confined to QC by maintaining the stem-cell state and most likely involved in the control of QC-specific gene expression.

It was already mentioned that a feedback loop exists in SAM between *WUS* and *CLV* genes. The closest homologues of *CLV* in RAM are members of *CLV3/ENDOSPERM SURROUNDING REGION* (*CLE*) family. Some of the *CLE* genes like *CLV3*, *CLE19* and *CLE40* act to reduce RAM size, while others (*CLE41*) promote cell proliferation in stele. *CLE40* controls cell division in the distal root meristem, and the CLE40 protein is believed to be secreted from columella cells into QC and represses *WOX5* expression therein (Fig. 4.8). Probably this effect is brought through CLE40's putative receptor, ARABIDOPSIS CRINKLY4 (ACR4), which is mainly expressed in the distal meristem and locally restricts cell division activity interfering with columella stem-cell maintenance (Bitonti and Chippetta 2011) (Fig. 4.8). The *CLE40*/*WOX5* pathway of RAM parallels *CLV3*/*WUS* pathway in SAM.

Cytokinins are also implicated in RAM activity. A decreased cytokinin signaling leads to enhanced root meristem growth, while elevated cytokinin levels suppress RAM activity. This is evident from an analyses of *PASTICCINO* (*PAS*) 1, 2, 3 phenotypes that show suppression of cytokinin responses. Probably PAS gene controls the functional balance between cytokinin and auxin (Viet 2006). More recent work has indicated an antagonistic, and at times transient, interaction between auxin in determining positional information specifying cell fate or histogenesis in developing RAM (Bitonti and Chiappetta 2011). Cytokinins promote cell differentiation at the boundaries between the division and elongation zones by suppressing auxin signaling and transport, while auxin promotes cell division by suppressing cytokinin signaling (Ruzicka et al. 2009). Increased cytokinin levels reduce root meristem size and inhibit root growth, by modulating *PIN* expression and hence auxin distribution. This interplay depends on the convergence of both cytokinins and auxin on the same target gene, *SHORT HYPOCOTYL* (*SHY2*), which encodes an IAA class repressor protein of the auxin signaling pathway (Fig. 4.9). *SHY2* prevents the activation of auxin-responsive genes by negatively regulating *PIN1,3,7*. *SHY2* controls auxin and cytokinins in opposite ways. Auxin drives SHY2 protein degradation through SCF $^{TIRI}$, while cytokinins promote SHY2 expression through AHK3/ARR1 (a signal pathway). Ethylene and auxin and brassinosteroids also interplay. Both these interplays

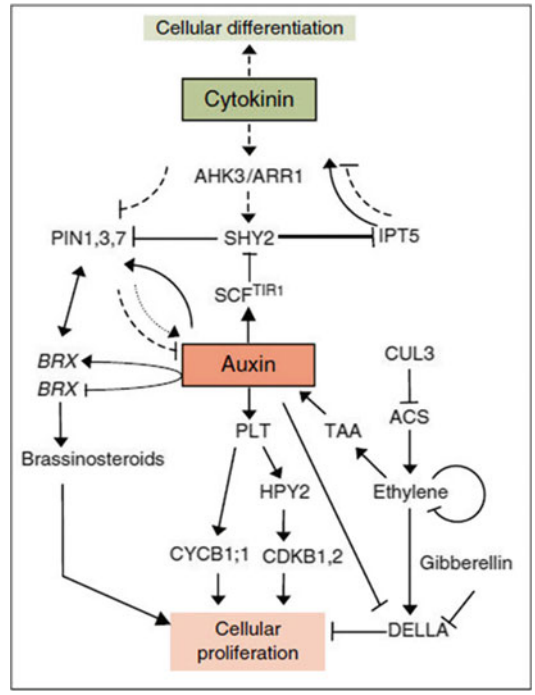


**Fig. 4.9** Scheme depicting hormone interaction in transcriptional network underlying root patterning (see text for further details). *Arrows* indicate positive regulation; *barred lines* indicate negative regulation (Bitonti and Chiappetta 2011)

affect cell proliferation in RAM (Fig. 4.9) (Binonti and Chiappetta 2011).

### 4.5.4 Autonomy of RAM

The autonomy of RAM has been investigated using surgical experiments as well as in vitro culture of isolated RAM. Culture studies involving progressively shorter lengths of root apex as well as surgical experiments have shown that the RAM is autonomous to a great extent and that it has an inherent capacity to organize itself. Culture of longer segments of root apex of pea plant do much better than shorter ones, although the latter's growth can be improved through suitable alteration of the nutrient medium through vitamins, sucrose and micronutrients at the appropriate ratio. Culture of QC (about 1,500 cells) of maize roots regenerated a well-organized root of normal structure on a culture medium supplemented with kinetin and IAA (Feldman and Torrey 1976). Surgical experiments have shown that even very thin longitudinally split RAM portions can regenerate a whole RAM if they contain at least a few QC cells (Swamy and Krishnamurthy 1975).

## 4.6 Intercalary Meristem

*Intercalary meristem* (*IM*) was defined already. IM is more common in monocots than in dicots or other vascular plants, and it plays an important role in the longitudinal growth of internode and floral stalk or scape (Swamy and Krishnamurthy 1979). The detached IM category is more common and is found in internodes of grasses and many other monocots, and in *Equisetum*, while the resumptive type is seen in the gynophore of peanut plant. It may be located in the internodal region just above the node (as in many grasses and *Equisetum*), a little away from the node, i.e. not at the base of the internode but a little removed from the base (*Avena* and *Triticum*) or just below the ovary in the pedicel's uppermost region in gynophore of peanut or in the upper region of the scape. Initially cell division is seen throughout the internode/floral axis but then becomes gradually restricted to specific regions of internode/floral axis. IM should not be confused with the *uninterrupted meristem* (*UM*) that Fisher and French (1978) speak of. UM, according to these authors, is a region of cell division derived from the subapical meristem of shoot apex that progressively gets confined to the upper part of the internode or floral/reproductive stalk and that it is continuous with the subapical meristem (i.e. uninterrupted) and not isolated from the apex by mature tissues. Swamy and Krishnamurthy (1979), however, have shown that UM is not continuous from subapical meristem as there are mature nodal tissues that separate it from the subapical meristem. Hence, they advocated that only two types of meristematic activity are involved in internodal ontogeny: (1) subapical meristematic activity of the rib meristem that is seen in all plants with a distinct internode to a variable extent and the wave of differentiation of the internodal tissues is acropetal with progressive restriction of the division activity to the more and more apical regions of the internode before it finally ceases and (2) the IM activity where the IM is either derived from subapical meristem, with gradual restriction of division activity to any region of the internode/floral axis/spike axis where it is very much prolonged contributing significantly to internodal tissues, or derived by dedifferentiation of mature tissues and contribute division activity for a very long time. UM, thus, is a quickly fading subapical meristematic activity, while IM is a prolonged subapical division activity restricted to a particular locus of the internode.

## 4.7 Development of Stem

The primary body of the stem is essentially contributed by SAM, IM and UM activities of shoot.

### 4.7.1 Metamers and Modules

There are three levels of morphological organization in multicellular plants: *cell*, *metamer* and *module (*Fig. 4.10) (Barlow 1994). The cell is the first level and its autoreplication results in the

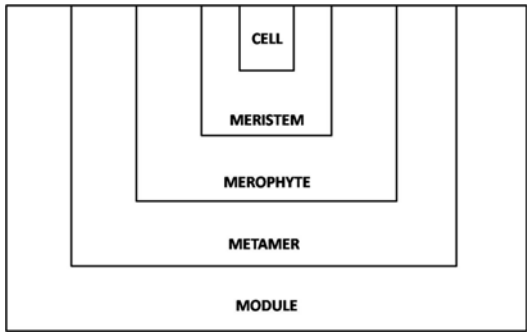


**Fig. 4.10** The hierarchical composition of a module illustrating its interrelations to the lower levels of cell, merophyte and metamer (Modified from Barlow 1994; Krishnamurthy 2015)

**Fig. 4.11** Long and short shoots of *Ginkgo biloba*; here short shoots are lateral (Robert W. Ridge 1987. Reproduced with permission from International Christian University)

replenishment of meristems which constructs the second level, the metamer. The metamers together organize the third, more complicated, level, the module. All these three levels/units are related to one another in a hierarchical fashion. Modules in combination organize the vast variety of shoot (and root) systems. The shoot metamer or *phytomer* is a unit that has one quadripartite set of leaf, node, internode and bud and represents the fundamental unit of shoot construction. The term *merophyte* (Douin 1923) is applied to each daughter cell derived from the single apical cell of lower vascular plants. Though the anatomical constitution of a metamer may vary according to the phyllotactic patterns of the plant, conceptually, the respective parts of a metamer per se, rather than their construction that is crucial for the definition of a metamer. A module is an axis formed of metameters. The growth of the module will be seen as long as the main SAM is active, while branching of the module is effected by axillary meristems. In many temperate tree taxa, the sequence of metamers is arranged into morphologically dissimilar groups because of dormancy, with some modules fully developed and others partially. These seasonally produced units of growth are called *submodules*; these have definite metameric structure but are only a part of the complete module. Fully developed modules of some temperate trees can be *long shoots* and *short* or *dwarf* shoots (sometimes also called *spur shoots*). The long shoots are vegetative while short shoots often bear reproductive organs. The short shoots may be *terminal short shoots* (derived from terminal SAM, with lateral shoots becoming prominent) as in *Terminalia* or *lateral short roots* as in *Ginkgo* (Fig. 4.11) and *Pinus*. The short shoots often have poor subapical rib meristem activity (hence internodes are telescoped) and usually do not bear lateral branches.

### 4.7.2 Origin of Nodes and Internodes

The groups of cells that ultimately form part of the node and internode are specified simultaneously with the initiation of leaf primordia on the SAM. The morphological definition of node and internode is decided, at least partly their topographical relationship with the adjacent leaf primordium. Hence, the plastochronic changes in the SAM have a greater significance in defining node and internode than merely deciding leaf production (Swamy and Krishnamurthy 1974). The histological definition, as different from spatial definition, of prospective node and internode has largely gone unnoticed (Barlow 1994). For instance, the appearance of tannin idioblastic cells in *Sambucus racemosa* is restricted only to the internode, and hence its location in the shoot apex will distinguish the internode from node (Zobel 1989a, b), where otherwise it is difficult. Hence, node and internode are not simply

passively defined by their position relative to the accompanying leaf primordium but are defined before the primordium becomes visible (Swamy and Krishnamurthy 1974; Lyndon 1987; Barlow 1994). Once it is delineated, the internodal growth is contributed not only by subapical rib meristematic activity (seen in all plants, although to a variable extent) but also by IM and, to a limited extent, by UM, as already discussed. The subapical meristem is recognized as one of the five morphogenetic zones in the shoot apex (Fig. 4.12) by Wardlaw (1957, 1968). The final form and behaviour of the internode is largely decided by this meristem (Romberger 1963; Swamy and Krishnamurthy 1979). In some taxa, this meristematic activity extends from 1.9 to 18.1 cm below the apical dome depending on the taxon and may extend to the seventh internode from top or even beyond. The plane of cell division in this meristem is almost exclusively transverse. The activity of this meristem is almost absent or extremely limited in acaulescent and rosette plants and short shoots, and GA is implicated in deciding the extent of this activity (Sachs et al. 1959, 1960; Sachs and Lang 1961). The nodal region, from the beginning, does not elongate and the constituent cells are also telescoped and very short. In monocots, it is marked by a nodal plexus, from the beginning, which is made of anastomosic provascular/vascular tissue. The internode consists of a dermal layer, a cortex and a stele (with or without pith) with a ring of collateral (xylem and phloem on the same radius) vascular bundles in dicots and gymnosperms, while in monocots, the dermal tissue is followed by a ground tissue in which vascular bundles are embedded. There are significant variations from this basic plan in respect to tissue distribution, stele types, distribution of vascular bundles and structure of vascular bundles (Evert 2006; Krishnamurthy 2015).

The node is the region from where leaf traces originate, and a gap or *lacuna* (= parenchymatous region) is seen in the stele where a leaf trace departs from the leaf. Based on the number of gaps associated with each leaf, the nodes are usually classified into *unilacunar*, *trilocular* and *multilacunar* types. Monocot nodes have a nodal plexus and leaf gaps cannot be recognized. More details on these nodal types and their phylogenetic and taxonomic significance can be seen in Krishnamurthy (2015).

### 4.7.3 Procambialization and Primary Vascularization in Stem

The most prominent feature of nodal and internodal differentiation in the shoot apex is the blocking out of the *procambium* from which the primary vascular tissues are derived. The height at which this blocking out (as different from ground tissue) happens varies, but in most taxa, it

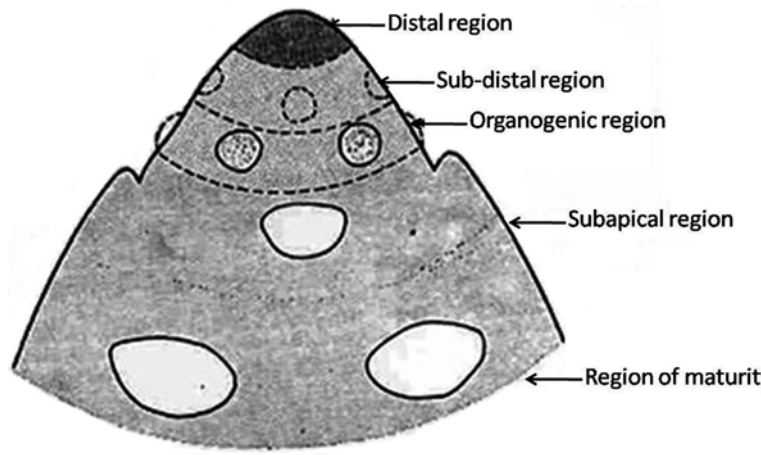


**Fig. 4.12** Diagrammatic representation of a shoot apex as a system of contiguous interrelated zones (Based on Wardlaw 1957)

is stated to happen at almost the level of the youngest leaf primordium. Some people speak of a progenitor for procambium called by names such as *meristem ring*, *prodesmogen* or *residual meristem*, but it is very difficult to distinguish this progenitor from procambium and also because the progenitor is stated to give rise to interfascicular parenchyma as well. The actual timing, location and identification of procambialization are matters of great dispute mainly because of interpretational problems and also because of the actual definition of procambial tissue (Esau 1943, 1965). Procambium in this location is usually identified by the small cross-sectional area, dense and vacuole-less cytoplasm and elongated cells, but these have been contested as not unique features of procambium by many researchers. It is very disappointing, and often frustrating, that till date we do not have a meaningful concept of procambium and unique markers for it. Most people associate procambium with the leaf primordia, mostly influenced by the researches of Professor Esau. These people go to the extent of asserting the entire procambial system and the primary vascular tissues derived from it as being made of leaf traces. Leafless taxa also have vascular bundles in their stems; also removal of leaf primordial in taxa with leaves also does not prevent vascular tissue formation in stems (Krishnamurthy 2015).

The other problem in procambialization relates to its wave of differentiation. Most people speak of a basipetal longitudinal wave extending from leaf downwards into the stem. There is no real proof of it although basipetal wave is observed in monocots for lateral traces. In the junction region between the stem and leaf, the so-called procambial strand is made of vacuolated parenchyma cells (and not procambial cells) which connect stem procambium with leaf procambium. The xylem elements that differentiate in this transition region is also morphologically very different and are unlike primary xylem tracheary elements that normally differentiate from procambium. From the transition region, procambialization into the leaf is acropetal, but is basipetal in the internode. The transverse wave of procambial differentiation is both centripetal and centrifugal so as to increase the transactional area of its occupation; surrounding non-procambial cells also contribute to the procambial mass.

The longitudinal wave of primary vascularization from the procambium follows the wave described for procambialization. The transverse wave is bidirectional in each procambial strand with the protophloem elements arising first towards periphery of the strand and then extending towards the centre of the strands while the protoxylem elements arising first in the strands towards the centre of the stem and extending towards the periphery of the strand. The metaxylem and metaphloem meet at the centre of the procambial strand, often leaving a few cells of procambium in the middle in dicots and gymnosperms but often not in monocots; however, these procambial cells become parenchymatous. The concepts of protoxylem and metaxylem (and protophloem and metaphloem) are often disputed (Easu 1965; Krishnamurthy 2015), since all possible distinctions proposed between them so far have exceptions.

### 4.7.4 Axillary Buds and Branches

In some lower vascular plants, bisection occurs in the SAM itself to result in true *dichotomous branching*. When a branch occurs laterally, at or near the shoot apex, from the axillary buds, the branching is called *monopodial*, and this is the most common type of branching in seed plants. In many angiosperms, the SAM of the main stem becomes a floral meristem, and its function is taken over by an axillary meristem which again ends after some time in a floral meristem and this process continues. This type of branching is called *sympodial* branching (Reinhardt and Kuhlemeir 2002). Genes regulating sympodial branching have been recorded. In the tomato mutant *self-pruning* (*sp*), the sympodial branching units are successively reduced. The dominant *SP* gene is orthologous to *CEN* and *TFl* genes of *Antirrhinum* and *Arabidopsis,* respectively.

Details on the histological origin and structure of axillary buds can be found in Evert (2006) and Krishnamurthy (2015). Details on additional or

accessory axillary buds and adventitious buds are provided in Krishnamurthy (2015). Experimental evidence indicates that the axillary buds are determined by the 'subtending' leaves (Snow and Snow 1942). If a leaf primordium is removed surgically before its 'axillary' bud is initiated, the bud would fail to develop, but even if a small portion of the base of the leaf primordium remains after surgical removal, the axillary bud is triggered to develop (Snow and Snow 1942). This observation is supported by an analysis of the *Arabidopsis phabulosa-1d* (*phb-1d*) mutant, where the abaxial (lower surface) leaf fate is transformed into adaxial (upper) leaf fate resulting in axillary bud development on the underside angle that the leaf makes with the stem instead of the normal upper side angle of the leaf (McConnell and Barton 1998); this emphasizes that the basal region of the adaxial leaf fate plays an important role in bud development. Transcripts of members of a family of maize genes called *KNOX* and *ROUGH SHEATH* (*RS*) are expressed in the axillary bud (and internode) near the base of leaves. *KN1*, *KNOX* and *RS* genes together are reported to predict the sites of axillary bud and its associated metameric components.

Another important phenomenon associated with axillary buds and branching is *apical dominance*. It is commonly observed that the actively growing terminal bud often slows down or even inhibits the development of the axillary (= lateral) buds into branches and that if it is curtailed through pruning, axillary buds sprout into branches. This phenomenon is called apical dominance. This is intimately related to the branching habit of the plant. A very well-coordinated control of apical dominance and the activity of lateral buds is responsible for the evolution of the 23 basic architectural patterns recognized in tropical trees. Apical dominance is strictly enforced in tall and unbranched plants like coconut and papaya but is highly flexible in branched and bushy taxa. Auxin, cytokinin and a few other less characterized chemical factors have been shown to mediate apical dominance through their regulation on axillary buds. Application of auxin on excised terminal bud region prevents axillary bud development and brings back apical dominance. Similarly, application of cytokinin to axillary buds of an apically dominant plant released their inhibition to develop into branches (Cline 1991) showing the interplay of auxin and cytokinin in apical dominance. Transgenic tobacco plants incorporated with a chimeric gene that encodes for the enzyme isopentenyl monophosphatase show a vast increase in cytokinin and the extensive development of lateral branches (Li et al. 1992). Transgenic *Petunia* incorporated with an IAA biosynthesis gene promoted apical dominance by preventing branches (Sitbon et al. 1992). The presence of a third hormone that plays a central role in apical dominance by regulating axillary bud dormancy is based on studies made in *Arabidopsis*, rice and pea mutants with increased branching. The existence of this signal, an unknown carotenoid-based hormone, was proved by reciprocal grafting experiments. This hormone moved acropetally from the roots into the shoot. The levels of root-synthesized terpenoid hormones called *strigolactones* (*SLs*) were reduced in these mutants and an exogenous application of SLs rescued the shoot branching phenotypes. Hence, SLs are a novel and specific inhibitor of axillary bud outgrowth. Not much is known about the receptor of SL, but it is likely that *DWARF14* gene product may be the receptor (Pautler et al. 2013).

Some information on genetic control of apical dominance and branching is now available. The maize mutant *barren stalk 1* (*ba1*) encodes a basic helix-loop-helix (bHLH) transcription factor that is required to establish axillary meristems (AMs) in vegetative (and reproductive) stages (Gallavotti et al. 2004). Compared to teosinte, the closest wild relative of maize, and in the *teosinte branched1*(*tb1*) mutant, most nodes are branched, while in wheat, branching is limited to occasional nodes and hence apical dominance is present (Reintardt and Kuhlemeier 2002). Some parallels are there between *TB1* gene, that suppresses branching (Doebley et al. 1997) and the *CYCLOIDEA* (*CYC*) gene of *Antirrhinum majus,* that suppresses the growth of floral organs. In rice, when *lax1* mutant is combined with the *monoculm* (*moc1*) mutant (double mutant), vegetative AMs are completely abolished and highly

reduced in number in *lax1 lax2* double mutants (Tabuchi et al. 2011). The *GRASSY TILLERS1* (*GT1*) mutant has reduced AMs (and hence tillers) in rice. The *lateral suppressor* (*ls*) mutant gene of tomato also suppresses axillary bud development into branches.

## 4.8 Development of Root

Whether one accepts the QC as the promeristem, supports the view that the meristematic initials are present along the margins of QC or considers both the QC and the meristematic initials as the promeristem, there is no difference of opinion regarding the fact that the root tissues are developed from this region. Cells in this part of RAM are believed to be controlled by their position and positional information from their more mature derivatives rather than by any intrinsic characteristic when they give rise to the different regions of the root. This is proved by laser ablation experiments carried out in *Arabidopsis* RAM (Scheres and Wolkenfelt 1998; Van den Berg et al. 1997). When any cell of the different histogens or the QC is selectively ablated, an adjacent cell takes over its role; however, if derivatives of these initials are ablated, for example, cortical derivatives, the cortical initial was unable to generate the cortical cells including endodermis. This is contrary to the more traditional view that the RAM is an autonomous pattern-generating machine.

Both the root body and the rootcap are envisioned as consisting of files of cells emanating from the promeristem (Rost 1994). However, the limits of the classically recognized meristematic, elongation and maturation zones (Ivanov 1973) are not clear-cut, since all the tissue files of any of these three zones do not end at the same level in the root body. Cell division, cell expansion and cell maturation overlap not only in different tissue regions but also in the different cell files of the same tissue region and even in individual files. What regulates the differences between adjacent cell files and what regulates the spatial modulation of the transition points between cell division and expansion and between cell expansion and maturation (Ivanov 1973) are not clearly studied (Rost 1994). Transition points can move within a cell file relative to the growth rate of the root, since in fast-growing roots, differentiation events occur farther from the root tip than in slow-growing roots. Cell division activity in different tissue layers stopped at various distances from RAM, and it invariably extended for greater distance in the dermal layer than the remaining tissue regions. Attention was already drawn to the *formative divisions* or T-divisions (radial or periclinal divisions) that makes one cell file into two thus increasing the diameter of the root body. The other type of division is the *proliferative division* or transverse division which occurs within each file. Each file apparently maintains a more or less fixed number of proliferative divisions (Rost 1994). For instance, the tracheary element precursor cell in *Vicia faba* root divides five times, while parenchyma cells divide seven times. The proliferative divisions have at least two functions: (1) they provide a continuous supply of new cells to ensure the growth of the root, and (2) they coordinate cell length through the restriction of number of cell divisions in each file. Groups of cells of common ancestry, called *cell packets*, are often seen in the different files after proliferative cell division.

Usually, the cortex matures first; the prospective metaxylem cells can also be distinguished very close to the apex, especially in taxa without pith. The primary xylem and phloem differentiate at different radii, and in a mature root, they are arranged alternately as seen in T.S. The number of radiating groups of the exarch xylem and phloem may vary from two to several depending on the taxon and sometimes in the same taxon depending on the size of the root.

### 4.8.1 Lateral Roots

Lateral roots are endogenous in origin and are positionally related to pre-existing vascular tissues, especially opposite to protoxylem poles. They always arise far away from RAM, through periclinal cell divisions in pericycle, although in ferns they arise close to the RAM, through cell divisions in endodermis (not from pericycle).

The lateral root primordium is almost akin to the radicle, but the QC arises de nova in lateral roots. The lateral primordium pierces through the cortex before it emerges out. Auxin promotes lateral and adventitious root formation (Aloni et al. 2006). Exogenous application of auxins promotes lateral root initiation and development in many plants, especially in stem and root cuttings. Other than auxins, thiamine, nicotinic acid, adenine and one or a few micronutrients are needed for lateral root development. The importance of auxin is revealed by the study of the *Arabidopsis* mutants such as *superroot* (*sur*) and *aberrant lateral root formation* (*alf*). The former produces many adventitious roots from parts other than roots of seedlings due to an elevated level of auxin. The second mutant groups overproduce lateral roots (in *alf1-1*) or no lateral root at al1 (in *alf4-1*), while in *alf3-1* mutant, the growth of lateral root primordia produced by pericycle is arrested and they do not emerge out. These mutants can be rescued by auxin treatment. The other genes involved in lateral root ontogeny are genes that code for cell size regulatory proteins and cyclins such as *CDC2* and *CYC* (Martinez et al. 1992), while *CYC* gene promotes their growth out of the parent root cortex. Cytokinins inhibit lateral root formation and reverse the auxin effect. Ethylene promotes both lateral and adventitious roots.

## 4.9 Meristems Involved in Latitudinal Growth

*Latitudinal growth* is also called growth in girth or thickness and is seen in root and stem. It is normally seen in dictos and gymnosperms once after primary longitudinal growth is over, but in some monocot stems, a type of latitudinal growth is seen simultaneously with primary growth. This primary latitudinal growth is due to a meristem called *primary thickening meristem* (*PTM*). Secondary latitudinal growth takes place through a *secondary thickening meristem* (*STM*) in some monocot stems and through a *vascular cambium* (*VC*) in the stems and roots of dicots and gymnosperms.

### 4.9.1 Primary and Secondary Thickening Meristems (PTM and STM)

In many arborescent, semi-arborescent and a few herbaceous monocot taxa, PTM is found (DeMason 1994; Swamy and Krishnamurthy 1974, 1979); these plants lack a true vascular cambium. PTM is found often from the embryo stage onwards and continues till the vegetative life of the plant. PTM occurs at the shoot apex, but yet the meristem is classified as a lateral meristem; it is also a primary meristem as the name implies. The most characteristic feature of the taxa with PTM is that the shoot apex produces a very quick succession of leaf primordia even when the plants are still in the seedling stage so that the SAM appears to be sunken below the surrounding leaf primordial tissues. Also, the adult diameter of the plant is often obtained even in the seedling stage itself (Fig. 4.13). The PTM occurs at the bases of all these leaf primordia at progressively declining angles from the youngest to the oldest leaf primordia, thus making the central cylinder of the stem wide and the cortex narrow. The sequence of events in the organization of PTM can be divided into three stages that almost occur simultaneously. The first event is the initiation of PTM cell files in the crown, which happens immediately adjacent to the base of the SAM and must occur by anticlinal divisions in the youngest cell files. During the second event, there is elongation of these cell files by periclinal cell division activity in the PTM followed by cell enlargement along the length of the files. In the third event, there is a reorientation of the cell files to a horizontal plane. The cell files repeatedly cut off cells which contribute to increase the width of the stem. The period of maturation of growth in girth of stems is centrifugal in the central cylinder and centripetal in the cortex (DeMason 1994). The tissues derived from PTM include ground tissue and procambial strands.

A number of arborescent and semi-arborescent species of monocots show thickening growth by STM (Fig. 4.13). This has been wrongly called by many botanists vascular cambium. There is functional and developmental relationship

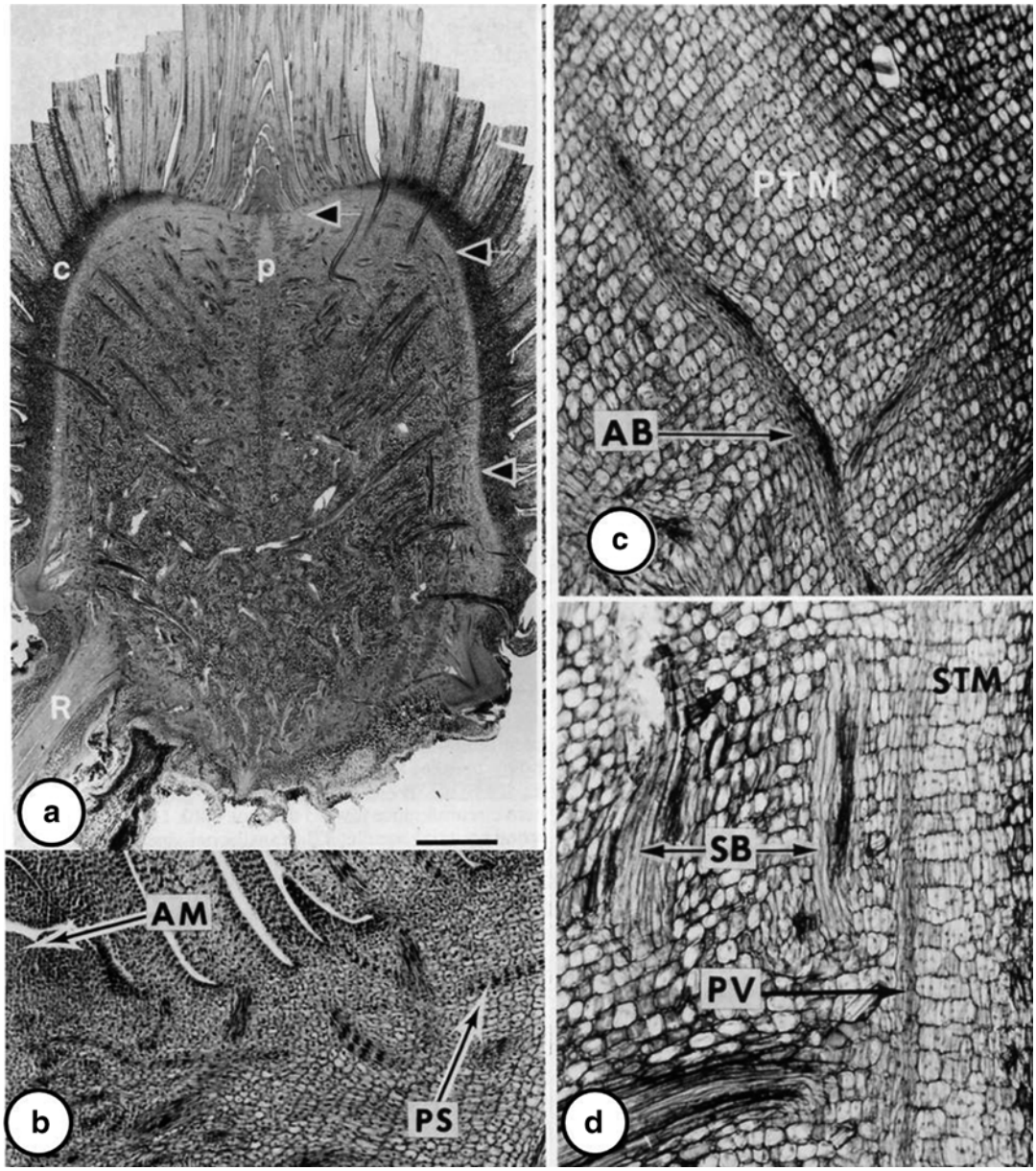


**Fig. 4.13** *Yucca whipplei*. (**a**) Median L.S. of the apex of 1-year-old stem. *Arrows* indicate primary secondary thickening meristems; (**b**) L.S. of apical meristem and the subjacent primary thickening meristem; (**c**) meristematic activity confined to a recognizable zone of tangentially flattered cells; (**d**) secondary thickening meristem region. *AB* primary axial bundle, *C* cortex, *P* pith, *PS* procambial strand, *PTM* primary thickening meristem, *PV* secondary provascular strand, *SB* secondary axial bundle, *STM* secondary thickening meristem (Diggle and DeMason 1983)

between the PTM and STM (DeMason 1994). If the two meristems are developmentally related, then the function of the STM should be related to the function of PTM, PTM and STM should be longitudinally continuous in an actively growing stem and the STM should arise for the first time in the stem in relation to the PTM. Studies on taxa with STM have shown that all three criteria proposed above are fulfilled. Thus, the PTM and STM form a continuum. The STM produces

derivative cells mainly towards inside, although in some taxa, it may also contribute parenchyma cells towards outside. The derivatives produced towards inside develop into parenchyma cells as well as into vascular bundles or strands containing xylem and phloem that get embedded in the parenchyma tissue. In some taxa, the parenchymatous derivatives may become fully or partially differentiated into sclerenchyma, particularly around the vascular bundles.

### 4.9.2 Vascular Cambium

*Vascular cambium* (*VC*) is a typical lateral meristem that contributes to latitudinal growth or *secondary growth* of stem and root of gymnosperms and dicots. The reports of a VC in monocots (Rangarajan and Swamy 1980) and pteridophytes (Bhambie 1994; Cichan and Taylor 1990) and in the appendicular organs like leaf are erroneous since their vascular meristems have neither the typical structure nor the characteristic activity and behaviour of VC. The VC normally is bifacial (Larson 1994) and forms *secondary xylem* or *wood* on the inside and *secondary phloem* or *bast* on the outside (Fig. 4.14). The fusiform initials of only seed plants show anticlinal divisions, but not the so-called cambial initials of pteridophytes. The duration of production of wood and phloem by VC decides the degree of growth in girth of the plant axis, and this prodcution happens till the life of the tree to result in very massive stems as in some giant conifers and *Eucalyptus*. Secondary growth through VC involves highly plastic developmental processes, which are reflected through extensive anatomical and functional variations that are observed both within individual plants and among plants. The degree of VC activity in herbaceous taxa is very limited. In most taxa, there is only one cambial ring, but in some lianes and trees, more than one ring is formed to result in successive zones of wood and phloem.

#### 4.9.2.1 Origin of Vascular Cambium

We must speak of origin of VC in any plant from two angles: in terms of evolutionary origin and in terms of physical origin. Although Cichan and Taylor (1990) have spoken of an independent origin of VC in arborescent lycopods, sphenopsids, *Rhacophyton* (all pteridophytes) and seed plants, for reasons mentioned in the previous paragraph, only seed plants should be considered as having a true VC. The fern *Botrychium* once considered to have a VC is now proved to be without it. Krishnamurthy (2005) has discussed in detail the various aspects of evolutionary origin of VC and had concluded that it evolved with the evolution of eustely, true arborescence and branching habit in vascular plants. The plants had tried different

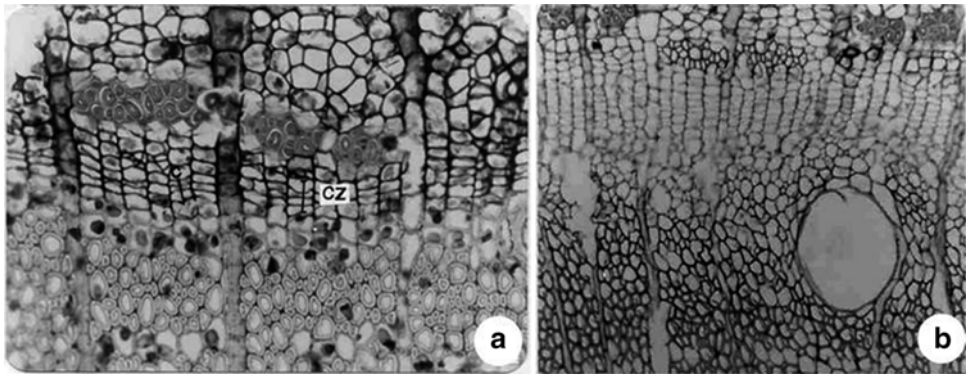


**Fig. 4.14** (**a**) T.S. of stem of *Dalbergia sissoo* showing dormant cambial zone (*CZ*) with secondary xylem below and secondary phloem (with fibres) above cambial zone; (**b**) T.S. of stem of *Albizia amara* showing actively dividing cambial zone, secondary xylem (below cambium) and secondary phloem (above the cambium) (Photographs courtesy of Dr. N. Venugopal)

methods of meeting with arborescent habit, but selection pressures prevailed in retaining VC as the best suited to taxa with arborescent branching habit, i.e. gymnosperms and dicotyledons. The bifacial and true VC of extant seed plants may share a common evolutionary origin that predates the divergence of gymnosperms and angiosperms (Spicer and Groover 2010) and may even predate the origin of seed. Within angiosperms, results from molecular phylogenetic analysis and character state reconstructions support the idea that a VC is a feature of both basal angiosperms and early-diverging eudicots, but it is not clear whether VC had a single or multiple origins.

With reference to the physical origin of VC in gymnospermous and dicotyledonous taxa, the consensus of opinion (although wrong) among many plant morphologists is that it originates from procambium. They believed that after production of primary xylem and primary phloem, the leftover procambium of the vascular bundle gives rise to the VC after little or no modification. According to these people, procambium and VC are to be looked upon as two developmental stages of the same meristem (Esau 1965) and that separate terms like procambium and cambium are needed only for convenience (Sterling 1946). This is in spite of the fact that there are very distinct differences between procambium and VC in terms of structure and organization (Fahn et al. 1972; Swamy and Krishnamurthy 1980; Krishnamurthy 2015). All vascular plants have procambium, but only dicots and gymnosperms have a VC; then why all vascular plants do not have a VC? These people have not answered this question so far. In an elaborate analysis, Swamy and Krishnamurthy (1980) not only have given evidences for non-procambial origin of VC in both stems and roots but also have shown that procambium is not at all required for the origin of VC. They have also shown that in all instances, parenchyma cells give rise to VC (Swamy and Krishnamurthy 1980; Krishnamurthy 2015). The parenchyma cells of dictos and gymnosperms are more totipotent and require fewer inputs to redifferentiate into meristematic cambial initials than those of pteridophytes and monocots. They also need only very few changes to become cambial initials as they are structurally very close to fusiform initials of VC than the latter are to procambial cells. There are four types of VC, based on topography (Krishnamurthy 2015): (1) The primary vascular tissue has the form of an almost continuous vascular cylinder in the internode so that the interfascicular regions are either absent or extremely narrow. Hence, the VC forms a continuous cylinder so also the secondary vascular tissues formed from it. (2) The primary vascular tissue forms discrete strands/bundles, but the VC (due to conversion of interfascicular parenchyma cells into VC cells) and the secondary vascular tissues formed from it form a continuous cylinder. (3) The VC forms a continuous cylinder, but it cuts off secondary vascular tissues only in the fascicular regions but not in the interfascicular regions where only parenchyma cells are cut off. So both primary and secondary vascular tissues have the appearance of a system of discrete strands. (4) The VC does not form a continuous cylinder but occurs as strips in the fascicular region only; hence, secondary vascular tissue production is restricted only to the fascicular regions.

#### 4.9.2.2 Structure of Vascular Cambium

The VC consists of two morphologically distinct types of initials: *fusiform* and *ray initials* (Fig. 4.15). These, respectively, give rise to the vertical and horizontal systems of secondary vascular tissues. The ratio of fusiform to ray initials varies in VC of different taxa (Iqbal 1994). In some taxa, ray initials are totally absent leading to secondary raylessness in the vascular tissues. The relative arrangement of the two types of initials in the VC also varies with taxon. In non-storeyed (also spelled as *non-storied*) or *non-stratified* type of VC (Fig. 4.15a), the fusiform and ray initials, as seen in TLS, have highly overlapping arrangement. In *storeyed* (also spelled *storied*) or *stratified* type of VC, both these initials or at least the fusiform initials have a more or less distinct tiered arrangement, as seen in TLS (Fig. 4.15b). While the former type is the predominant type seen in all gymnosperms and the majority of dicots, the stratified type is seen in some members of Malvaceae,

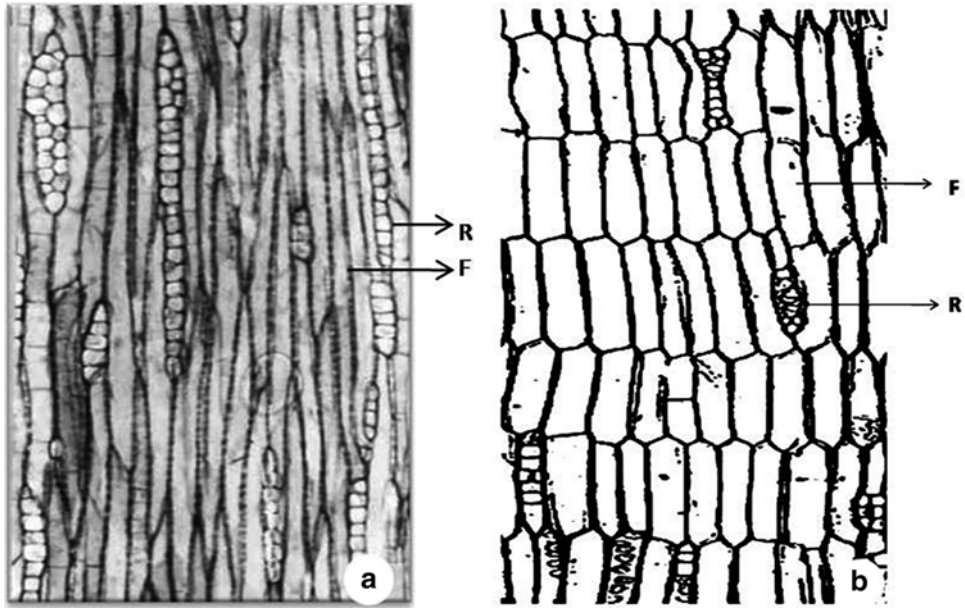


**Fig. 4.15** (**a**) T.L.S. of non-storeyed vascular cambium of *Albizia amara*, (**b**) T.L.S. of storeyed cambium of *Aeschynomene aspera. F* fusiform initial, *R* ray initial (**a** Courtesy of Venugopal; **b** based on Phillipson et al. 1971)

Leguminosae, etc. Moreover, the fusiform initials in the stratified type are shorter (around 120 μm), while they are considerably longer in the non-stratified type (around 450 μm in dicots to up to 9,000 μm in some conifers). However, the length of the fusiform initial (called genetic length) varies in the same plant with age—shorter in young trees, reaching the maximum at around 60 years and then maintaining a constant length.

The fusiform initials, which form the vertical system of VC, are tangentially flattened, pointed at both ends and possesses between 8 and 32 facets (with an average of 18–21); each initial will be in contact with at least 14 other cells. The volume of these initials is tens or hundreds of times of the initial of SAM. The ratio between length and width varies from 30:1 to 600:1 depending on the taxon. These initials are highly vacuolated in order to minimize the amount of cytoplasmic materials synthesized and to reduce energy expenditures at each cell cycle. Thus, the vacuoles economically extend the reach of the cytoplasm. Ultrastructurally, the cells are similar to vacuolated parenchyma cells. The cytoplasm contains larger mitochondria, differentiated plastids, peroxisomes, ribosomes, lipid bodies, dictyosomes, ER, microtubules, lomasomes and storage substance. The cell walls have fibrillar architecture so as to enable the regulation of direction of growth in the expanding wall. The wall also meets the conflicting demands of cohesion and extensibility of the cells and their derivatives. Radial (R) walls are usually thicker than tangential (T) walls, particularly during dormancy of cambium and it is also beaded. The chemical composition of these walls is also slightly different since T-walls are produced afresh at each periclinal division and their expansion is limited, while R-walls remain plastic and undergo a constant radial extension. The cell walls of cambial zone exhibit a far greater radial enlargement (up to 100 times) than that of tangential enlargement. Radial walls have a cellulose skeleton embedded in xyloglucans and arabinogalacturan proteins, while T-walls have xyloglucans and very little or no acidic pectins and xylans. The ray initials form the horizontal or radial systems of VC. In TLS, they are of variable height depending on the taxon, with varied number of cells and thickness. They may be *uni-*, *bi-*,or *multiseriate* (Figs. 4.15 and 4.16) and may be *homo-* or *hetero-cellular* (respectively with only


**Fig. 4.16** T.L.S of Cambial zone of *Gmelina arborea* showing multiseriate ray initial (R) (Photo by S. John Adams)

*procumbent* or *erect cells* or with both). The cells have shorter tangential than radial diameters (very clearly evident in RLS). Ray initials have well-developed cell-to-cell connections through plasmodesmata (more in tangential than in radial walls) and constitute the main symplastic route for horizontal transport of signal molecules and other chemicals.

One of the most debated aspects of VC is whether it has a single layer of initials or more than one layer, i.e. whether it is a cambial zone. The debate is mainly due to the fact that it is very difficult to identify a single initial layer even during the most dormant condition and even with more highly sophisticated instruments and techniques, when a maximum of four layers of cells are seen in the cambial zone (Fig. 4.14a). Even if a single initial layer is assumed to be present, it cannot be distinguished from its immediate derivative layer(s). This debate is made more complicated by the naming and semantic controversies, especially with reference to the application of the term 'cambium' to the component layers. For those who believe in the single initial layer concept, this term is applicable only to this initial layer, while for those who believe in the cambial zone concept, the term is applicable to the entire zone. However, the importance of VC should not be lost in the terminology conflict (Iqbal and Ghouse 1990).

#### 4.9.2.3 Activity of Vascular Cambium

The fusiform initials cut off secondary xylem on the inside and secondary phloem on the outside through tangential, i.e. periclinal cell divisions. Simultaneously the ray initials cut off xylem and phloem rays. The mechanism of periclinal division in the fusiform initial has already been described in Chap. 3 of this volume. The volume of secondary xylem cut off by the VC ring is several times more than that of the secondary phloem. Hence, the extent of outward shift of the cambial ring is determined by the volume of secondary xylem added on the inside. This should be followed by appropriate increase in the circumference of the cambial ring with concomitant and proportionate increase in the number of fusiform and ray initials, and this increase in cell number is taken care by the anticlinal cell divisions of fusiform initials. The detailed mechanism of anticlinal division is described in Chap. 3 of this volume. Once derivatives are cut off on the two sides of the cambial ring, they differentiate into secondary xylem and phloem tissue. This differentiation occurs in three phases (Krishnamurthy 2015): (1) The phloem and xylem derivatives may divide further. (2) The cells that cease dividing usually enlarge in radial direction (and in the case of large vessel elements even in tangential direction) and start differentiation events specific to each cell type of xylem and phloem. (3) Phenomena such as second wall formation and lignification in tracheary elements and in phloem and xylem fibres, sieve area development in sieve elements, development of pits and plasmodesmata etc. take place subsequently.

Within limits of genomic control, VC operates under the influence of internal physiological processes and external environmental factors. The VC invariably becomes dormant during times of extreme cold (in winter) and hot temperatures (in

summer), respectively, in temperate and tropical taxa. The activation of VC, a deterministic process, begins when stress due to extreme temperatures is removed with the approach of favourable season. Thus, cambial activity is periodic rather than continuous. Even in those tropical environments where there are no extremes of temperature, the evergreen trees growing there do show some degree of unequal cambial activity, at least during 1 or 2 months in a year. In taxa with rhythmic cambial activity, the number of times the VC becomes dormant/active is one each in a year, but in certain taxa, more than one dormant/active period may be seen within a year, depending on prevailing environment. During the dormant period, the cambial zone is reduced to three or more commonly four layers, and one of these layers is likely to be an initial layer and the one or two layers inside should be xylem derivative(s) and the one or two layers outside should be phloem derivative(s). The cells of this dormant cambial zone store a number of chemical substances and have no or fewer vacuoles. The cytoplasmic organelles become less abundant or poor; the ER invariably becomes smooth and the plasmalemma is thrown into folds. The water content is also greatly reduced and growth inhibitors accumulate.

There is a close relationship between bud break and cambial reactivation in temperate trees and between flushing of new leaves and cambial reactivation in tropical trees. Hence, the number of times of cambial reactivation depends on the number of times of bud break/new leaf flushing noticed in a year. This means that initiation of longitudinal growth precedes the initiation of latitudinal growth. Most researchers believe that auxins produced during bud break and in the newly flushing leaves stimulate cambial reactivation through their effect on initiating cell divisions. In temperate trees, cambial reactivation is initiated just a week to 15 days before visible bud break, but in tropical trees, it happens just after leaf flushing. During cambial reactivation, the level of growth hormones increases in its cells. The initiation of periclinal divisions is usually preceded by cell enlargement with increase water content. The cell expansion is predominantly in the radial direction. Soon a broad cambial zone of several layers is formed (Fig. 4.14b). The cytology of active fusiform initials is already described. The storage products are exhausted in and around cambial zone and used up to form new cell materials during cambial reactivation; metabolic rate increases.

Cambial reactivation in tropical trees happens mostly only once in a year and, depending on the taxon, it happens between January and early July. The same species may show cambial reactivation at different periods within these months depending on its location. For example, in teak reactivation of cambium happens in early March or between early June and early July and in *Ricinodendron heudelotii* in February to March or in December. More than one cambial reactivation/dormancy period is seen in *Holarrhena floribunda* (February to March and September) and in *Psidium guajava* (March and July). There is also variation in the production period of secondary xylem and phloem. Their production need not be simultaneous as is generally believed by many.

One major result of the nonuniform activity of VC through a year or part thereof is the production o*f growth rings*. The differences noticed in the quality and/or quantity of xylem elements produced by the VC during its active period and during its approach to dormancy cause the visibility of growth rings. Usually evergreen trees with continuous cambial activity do not show growth rings and are characterized by *diffuse porous woods* in which the diameter of vessel elements (= pores), as seen in T.S., is of uniform size. On the contrary, in trees with seasonal cambial activity vessels, elements produced during active period are with greater diameter and the diameter gradually reduces towards the dormancy period to result in *ring porous woods*. This, along with other features such as lack of vessel production, production fibres with smaller transactional diameter, thick-walled fibres or only parenchyma, dense wood etc., may mark a growth ring; this part of the wood is often called *late wood* in contrast to *early wood* formed during very active period of cambium. The so-called *annual rings* are growth rings but not all growth rings are annual. False ‘growth’ rings may result

due to the effects of drought, frost, flooding, defoliation, air pollution etc. Hence, a critical study of growth rings of a tree (called *dendrochronology*) would throw a lot of light on past climates in any area.

A study of cambial variants (i.e. deviations from the normal presence of a single cambial ring and its normal activity of producing xylem inside and phloem outside in continuous cylinders) and of the structure of the secondary xylem and phloem produced by the VC is beyond the scope of this article, and a useful review of the same can be found in Evert (2006), Spicer and Groover (2010) and Krishnamurthy (2015). However, some discussion must be focused on *reaction wood* (*RW*). The formation of RW by VC is a very important mechanism that is vital for a free species to effectively function. As the main stem bends or as a plagiotropic branch grows, it might be expected to bend downwards because of its increased weight due to production of more and more twigs and leaves. Such a phenomenon is often resisted by the formation of RW (Krishnamurthy 2007). Trees with RW have an increased wood production on either the upper or lower side of the bent main trunk or leaning branches through increased cambial cell divisions. In conifers, RW is formed on the lower side of the leaning main stems and branches, and this wood by expansion (compressive strain) pushes such stems and branches more upright and maintains a more constant branch angle. This type of RW is called *compression wood* (CW). The tracheids of this RW are rounded in T.S. and with intercellular spaces (Fig. 4.17). The tracheid walls are abnormally thick and contain more lignin than normal wood and less cellulose than usual. These tracheids by unequal expansion bring about the upward push needed to make the leaning part erect. In dicots, RW is formed on the upper side of the leaning branches and bent stems and *contracts* (tensile strain) to *pull* the bent main stem erect and the branch towards the trunk by tension to enable the branches to maintain a constant angle. This type of RW is called *tension wood* (*TW*). TW becomes more pronounced at greater leans (10–20°) both through increase in *gelatinous fibres* (*G-fibres*) (Fig. 4.18), a special category of xylem fibres, and through more highly developed G-fibres in terms of the

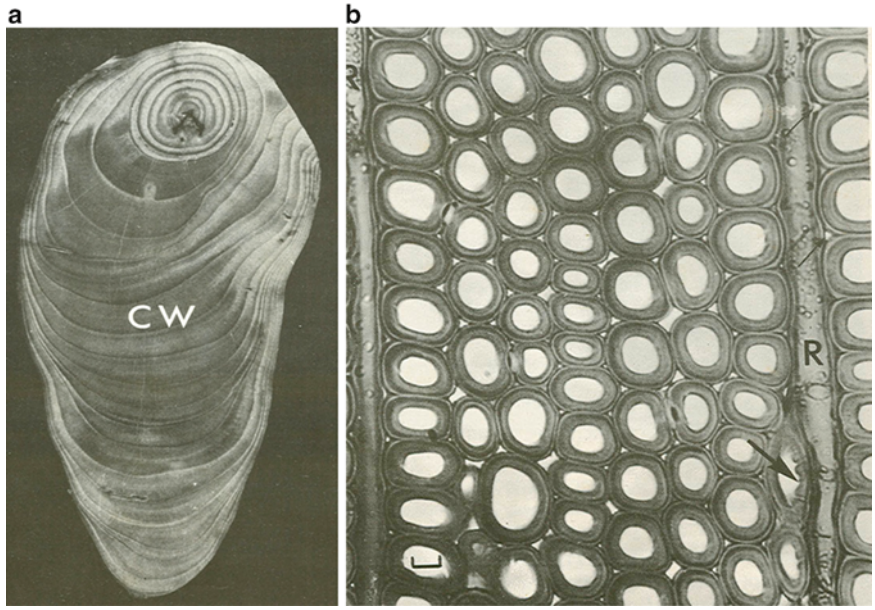


**Fig. 4.17** Compression wood (*CW*) of *Picea abies*. (**a**) T.S. of whole wood; (**b**) a portion of the compression wood enlarged to show circular tracheids with intercellular spaces. R ray (Timell 1973)

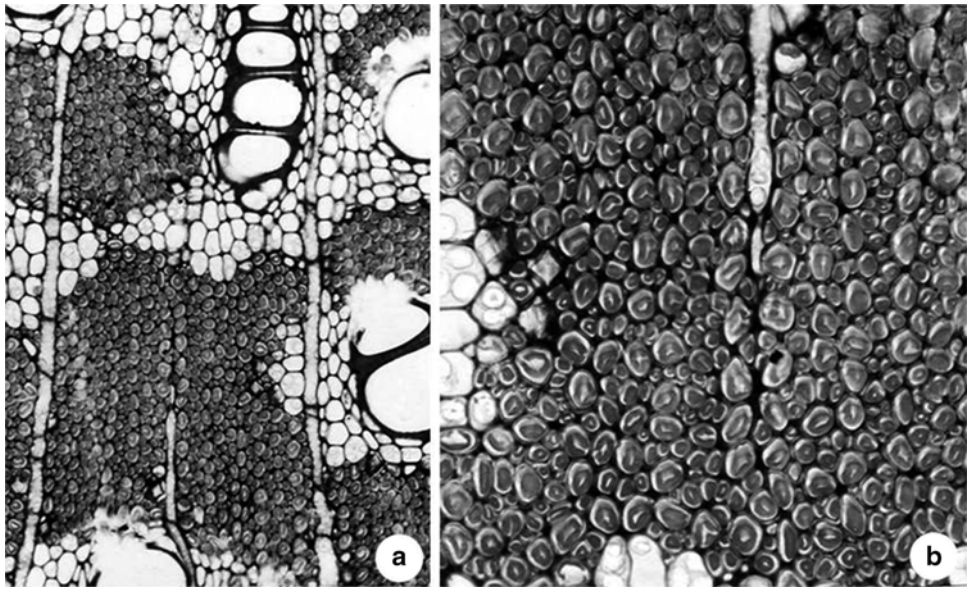


**Fig. 4.18** T.S. of tension wood of *Cassia* sp. showing gelatinous fibres (**a**); portion of the same enlarged (**b**) (Krishnamurthy 2015)

thickness of the gelatinous layer (*sg-layer*). These fibres through their selective shrinkage bring about the correction needed in the leaning stem and bending branches. These fibres have secondary walls that are thicker than those in normal wood (NW) because they have an additionally, often convoluted, cellulose-rich sg-layer that additionally has carboxylated acidic polysaccharides. TW, unlike CW, is poor in lignin. Ten fasciclin-like arabinogalactan-rich proteins are specifically expressed in TW, but not in cambial zone. Additionally extensins (hydroxyproline-rich proteins) are localized immunocytochemically close to the inner part of the sg-layer of G-fibres (Hariharan and Krishnamurthy 1995; Lafarguette et al. 2004; Jothi et al. 2010). These structural stress proteins are likely to be linked to specific mechanical properties of TW. RW could be a gravitropic response and a mechanism to maintain the plagiotropic growth of branches at a constant angle or a response to tension and pressures resulting from bending or both, but gravity appears to be more important (Timell 1986; Krishnamurthy 2007), and this may be the cause for the strains that are developed and the bending movements that these strains generate. The functional importance of RW may be summarized as follows: Each and every tree has a specific architecture, particular branching pattern and specific branch angles which are all needed to have that particular crown geometry to optimize light capture and gas exchange. Any deviation in branch angle through added weight in the form of more twigs and leaves and bending would jeopardize this nice balance in crown geometry. Hence, RW is developed to correct this bending through extension of tracheids in the CW of conifers on the underside of such bending branches or through shrinking of G-fibres in the TW of dicots on the upper side. Correction is made every time the branch angle changes to added weight (Krishnamurthy 2007).

#### 4.9.2.4 Genetics of Vascular Cambium

There is clear evidence for overlap in the genetic mechanisms controlling the SAM and vascular cambium (Groover 2005; Groover et al. 2006). This is evident from recent microarray analyses of gene expression that happens during cambial activity, which have revealed that key genes regulating SAM are also expressed in the cambial region or have paralogues that are expressed

there (Ko and Han 2004; Schrader et al. 2004). For instance, the class III homeodomain-leucine zipper (*PHA*/*PHV* and *ATHB-15*; *CORONA*) and *KANADI1* transcription factors responsible for abaxial-adaxial patterning in leaf primordia (operating in SAM), *SHORT-ROOT* (*SHR*) and potential orthologues of *AINTEGUMENTA* (*ANT*) and *PINHEAD* (*PNH*) are also expressed in the cambial region of poplar (Schrader et al. 2004; Spicer and Groover 2010). Similarly, putative *STM* orthologue called *ARBORKNOX1* (*ARK1*) and *BREVIPEDICELLUS* orthologue (*ARK2*) are known to be expressed in poplar (Groover et al. 2006; Spicer and Groover 2010). *ARK1* overexpression does not preclude secondary growth but serves to inhibit the onset and differentiation of secondary vascular tissues at the morphological level. Microarray analysis had helped to identify genes that got misregulated in response to *ARK1* overexpression. This analysis showed that 41 % of genes are up-regulated and that their proteins are involved in extracellular matrix (cell wall) synthesis or modifications, including proteins involved in cell identity and signaling, cell adhesion or cell differentiation. These gene expression differences are reflected in alterations of cell wall biochemistry and lignin composition in *ARK1* overexpression plants (Groover et al. 2006). Hence, *ARK1* is likely to act regulating cell fates of cambial derivatives through modification of the extracellular matrix. *ARK2* shows a broad expression pattern that includes not only the cambial zone but also developing secondary xylem and phloem (Du et al. 2009). *ARK2* expression levels are positively correlated with the width of the cambial zone and negatively correlated with the differentiation of lignified cell types in both secondary xylem and phloem fibres. The *Populus* orthologues of *Arabidopsis KANADI* genes *KAN1* and *KAN2* have the greater expression in secondary phloem and perhaps are required for phloem differentiation.

The patterning and polarity of cambial activity and secondary thickening are regulated by hormones and genetic mechanisms (Spicer and Groover 2010), although the details of this interplay are still poorly understood. While auxins were once proposed as simple morphogens that show a gradient across the cambial zone and its derivatives, a study of Nilsson et al. (2008) suggests that this attractive hypothesis may be an oversimplification or incorrect. Auxin-responsive genes were identified in *Populus* cambium using microarrays, but the expression levels of auxin-responsive genes across the cambial zone and its derivatives were poorly correlated with the auxin gradient. In addition, transgenic *Populus* that expresses a dominant mutant form of *PttIAA3* has altered auxin responses and has fewer cell divisions in the cambial zone and smaller lignified cell types in the wood. Hence, Nilsson et al. (2008) proposed that rather than acting as simple morphogens, auxins may regulate the expression of a few downstream regulators to affect key aspects of wood formation, particularly cell division in VC. Auxins, particularly their longitudinal gradients, also control the orientation of cambial initials and their derivatives as well as the relative rotation of cambial initials (Spicer and Groover 2010).

The class III HD-ZIP *Arabidopsis* orthologues in *Populus* such as *PHV*/*PHB*, *CAN* and *ATHB8* show the highest expression levels found in adaxial xylem tissue (Schrader et al. 2004). *Populus* plants expressing a dominant miRNA-resistant *Populus REV* transgene show patterning and polarity defects in wood and secondary phloem that include formation of ectopic cambia in the stem cortex which produce wood on the outside but not on inside. This class of genes is also involved in cambial initiation from parenchyma cells.

### 4.9.3 Phellogen

Phellogen or *cork cambium* is a lateral meristem closely related to VC. Phellogen and VC function in coordination with one another. Phellogen is located on the periphery of the axis of gymnosperms and dicots. Once the epidermal tissue are crushed by the VC derivatives, the phellogen has its origin in primary phloem, primary cortex, subhypodermis or even in the epidermis (before it is crushed). It generates through periclinal division

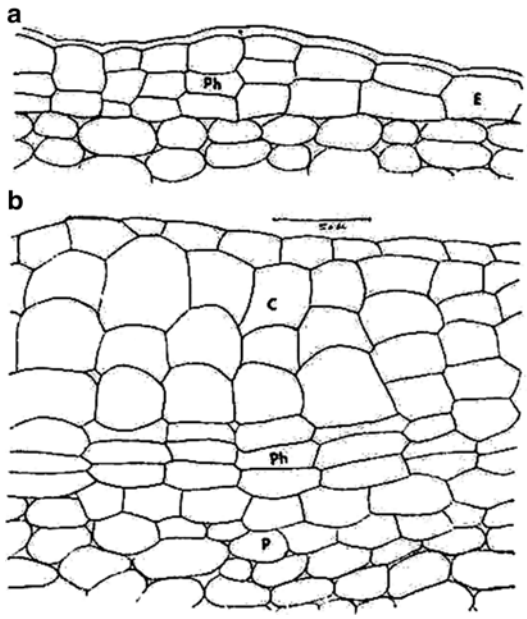


**Fig. 4.19** (**a**) T.S. of the peripheral part of the stem of *Ipomoea* sp. showing the initiation of phellogen (*ph*) from epidermal tissue (*E*). (**b**) T.S. of the peripheral part of the stem of *Nyctanthes* sp. showing derivatives of phellogen (*ph*)-cork cells (*C*) towards outside and phelloderm (*P*) towards inside (Krishnamurthy 2015)

on its outside the suberized cork tissue or *phellem* and on the inside secondary cortex or phelloderm (Fig. 4.19). In many taxa, some of the outer derivatives of phellogen develop into *lenticels*, which are structures that facilitate gas exchange in place of stomata that are lost along with the epidermis. Phellogen may function perennially or is periodically replaced by successively developed phellogens derived from internal tissues. Like VC, there is controversy regarding its unilayered or zonate nature, again because of the difficulty in identifying the initial layer.

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