The Plant Cell, Vol. 4, 1029–1039, September 1992 © 1992 American Society of Plant Physiologists

REVIEW ARTICLE

Vegetative Apical Meristems

June I. Medford

Department of Biology, 506 Wartik Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802

INTRODUCTION

A fascinating aspect of plant biology is that a small cell group is the origin of shoot systems as varied as redwood trees or Arabidopsis. The first recognition that shoot systems originate from small groups of cells was by Kaspar Wolff in 1759 (cited in Cutter, 1965). These small groups of cells are known as shoot apical meristems. Because the shoot apical meristem is the origin of the shoot, it can be described to "make" the shoot. The apical meristem "makes" the shoot through four functions: initiating new organs, initiating new tissues, communicating signals to the rest of the plant, and maintaining itself as a formative region. How these functions are integrated within the apical meristem to produce the shoot has been the question of many studies and provides continual intrigue.

Many recent studies have focused on processes in floral meristems (e.g., Coen and Meyerowitz, 1991). Yet, before the floral (and inflorescence) meristem forms, the vegetative apical meristem is the shoot's source of cells. This earlier state of the apical meristem describes a set of tissues and organs with distinct characteristics. Furthermore, processes in vegetative meristems may influence processes in later types of meristems. This review will discuss the remarkable properties of shoot apical meristems, with a specific focus on vegetative apical meristems. The discussion has three goals: to clarify terminology, to review molecular and genetic data, and to suggest areas where studies may further our understanding of how the meristem makes the plant.

BACKGROUND AND TERMINOLOGY

Terms and Their Usages

Apical meristems have been the focus of many studies in plant development. These studies often described diverse plant species with apparently distinct types of shoot apical meristems. Because the apical meristems were (and are) often seen as diverse, a wide variety of terms have come to be associated with them. For example, the terms shoot apex, shoot apical meristem, and promeristem are used at times synonymously and at times distinctly by different authors. As molecular and genetic approaches are applied to studies of apical meristems, common definitions of terms will enhance our progress. In fact, molecular and genetic studies on apical meristems may even lead us to appreciate that, despite differences in the details, the organization and functioning of all shoot apical meristems are essentially alike (Wardlaw, 1957).

The overall similarity in the organization of shoot apices allows terms such as shoot apical meristem and shoot apex to be defined in a simple, functionally significant manner. The shoot apical meristem is the distal-most portion of the shoot and comprises two groups of cells: the initial or source cells and the cells that are the progenitors for tissues and lateral organs (Wardlaw, 1957; Cutter, 1965). By contrast, the shoot apex comprises several cell and tissue types: the apical meristem itself, a region just proximal to the meristem where lateral organ primordia are formed, a subapical region where the shoot widens and primordia enlarge, and the region of maturation, where differentiation becomes apparent (Wardlaw, 1957; Cutter, 1965). With these definitions, reference to the shoot apex is restricted to a small portion of the shoot, typically including only three to six leaf primordia. The shoot tip can then be defined as that portion of the shoot comprising all tissues and organs distal to the still-differentiating leaves. The number of leaves in a shoot tip would vary depending on the rate of differentiation. The above definitions are based on decades of studies and are used widely today (Steeves and Sussex, 1989).

Two examples will illustrate how careful attention to the difference between the apical meristem and the shoot apex has enhanced or could enhance our understanding of the complex processes occurring in meristems. First, transcripts of genes such as proliferating cell nuclear antigen (PCNA) have been localized to the apical meristem and young leaf primordia (Kosugi et al., 1991). Recent studies have shown that PCNA plays a role in a DNA excision repair process (Shivji et al., 1992). Because the apical meristem is the source of cells for the shoot, DNA repair processes might be limited to the apical meristem. However, the PCNA localization in both the apical meristem and developing leaf primordia suggests that DNA repair activity is apparently not limited to the apical meristem. By contrast, some studies leave gaps or misconceptions because they do not distinguish between the shoot apical meristem and the shoot apex. For example, a developmental analysis of the

expression of elongation factor 1α was done using a promoter–GUS fusion (Ursin et al., 1991). GUS expression was reported in the apical meristem and the apical dome. However, the published figures (Figures 4A and 4B of Ursin et al., 1991) show GUS expression in regions of the shoot apex and vascular tissue at the side of the apical meristem but not in the apical meristem itself. Excluding an artifact, the results are open to various interpretations. For example, it is possible that elongation factor 1α is not transcribed in the apical meristem, suggesting that translation is not prominent in meristems. However, translation is a process found in all active cells. Another possible interpretation of the data is that the elongation factor gene could be among those repressed in the meristem (see below).

Attention to terms is particularly important in the growing body of molecular studies that describe genes expressed in shoot apices. For example, barley shoot tip cDNA clones with homologies to histone and ribosomal protein genes were described as meristem specific (Kohler et al., 1992). Numerous studies have shown that histones play a role in chromatin structure and, hence, development. With this in mind, it is possible that meristem-specific histones are important for functions of the apical meristem. However, because histone and ribosomal protein genes are usually highly expressed in all dividing tissues and because expression of individual gene family members was not defined in this study, descriptions of these genes as "meristem specific" is not substantiated. Precise statements about the specificity of molecular probes will be important for understanding the complex processes and regulation thought to be present in the shoot apical meristem.

Common usage of terms is as important as careful attention to differences among terms, and it will improve our understanding of development. In many such studies, the authors have used their terms carefully and have been consistent within their own work. However, discrepancies arise when various authors use different definitions for the same terms. For example, Kelly et al. (1990) used "shoot apex" as defined here (i.e., consisting of the apical meristem, young leaf primordia, etc.), whereas both Fahn (1982) and Sattler (1988) used "shoot apex" to describe what is defined here and by Cutter (1965) as the shoot apical meristem. Although such variable usage was common in the past, molecular and genetic studies provide an opportunity (and requirement) for uniform usage of the terms such as shoot apical meristem and shoot apex.

Shoot Apical Meristems in Vivo

One reason for the difficulty in distinguishing the shoot apex from the apical meristem is that the shoot apical meristem is surrounded by more mature tissues. Figure 1 shows the dissection of a Brassica shoot necessary to reveal the apical meristem. Figure 1B shows the plant following the removal of all fully expanded leaves. This illustrates the shoot tip as described above. Each subsequent figure shows the removal of two more leaves until the shoot apex is revealed in Figure 1E. After all the mature and maturing leaves are removed, the shoot apical meristem is finally revealed as a small group of cells located between the youngest leaf primordia (Figure 1G). In Brassica, in addition to being surrounded by the developing leaves and leaf primordia, the apical meristem is also in proximity to differentiating cortical and pith tissues. The large size difference of shoot tip and shoot apex relative to the apical meristem implies that studies analyzing potential regulatory molecules in the shoot tip or shoot apex will reflect regulation in developing leaves and stems rather than the apical meristem.

Diagrammatic View of Shoot Apical Meristems

Figure 2A shows a diagram of a shoot apical meristem and shoot apex as defined by Cutter (1965) and Wardlaw (1957). The shoot apical meristem is typically a small (approximately 100 μm in diameter), dome-shaped group of 800 to 1200 cells. Both the size and shape of shoot apical meristems vary tremendously at different points in development and among various species. For example, whereas the apical meristem in tobacco is about 100 μm in diameter (Poethig and Sussex, 1985), the young apical meristem in Arabidopsis is about 35 × 55 μm and contains approximately 50 to 70 cells (Medford et al., 1992). Beyond the small dome, all other tissues and organs at the distal end of a shoot constitute the shoot apex (Figure 2A, stippled areas).

Figure 2B diagrams the regions and functions that numerous studies have defined within the apical meristem. Two different concepts have been used to define regions of the apical meristem, both originating with the histogen theory of Hanstein (1868). One concept is that of layers. This idea arises from the fact that chimeric shoots can be constructed that have genetically different components in separate cell layers in the apical meristem (Satina et al., 1940). The contribution of each cell layer to mature parts of the shoot can then be determined by examining the chimeric identity of the tissues and organs. Because three distinct layers in most angiosperms could account for all cells in the mature shoot, the apical meristem was defined as having three cell layers (L1, L2, and L3) (Satina et al., 1940). The L1 is the outermost layer, and cell divisions in this layer are restricted to the anticlinal plane (perpendicular to the surface; see Figure 2B). The L1 forms the epidermis in differentiated parts of the shoot. Cells in the second layer, or L2, divide predominantly in the anticlinal plane but also divide in the periclinal plane (parallel to the surface) when organs are forming. Cells in the third layer from the surface, the L3, divide in both anticlinal and periclinal planes and provide cells for the interior portion of organs and stems. The concept that the apical meristem has a depth of three cell layers has been important for understanding shoot development. As studies of apical meristems accumulate, it may be of interest to reexamine those cases such as *Pelargonium zonale* (geranium), where cells in chimeric shoots behave differently from those in nonchimeric shoots (Thielke, 1948; Clowes, 1961).

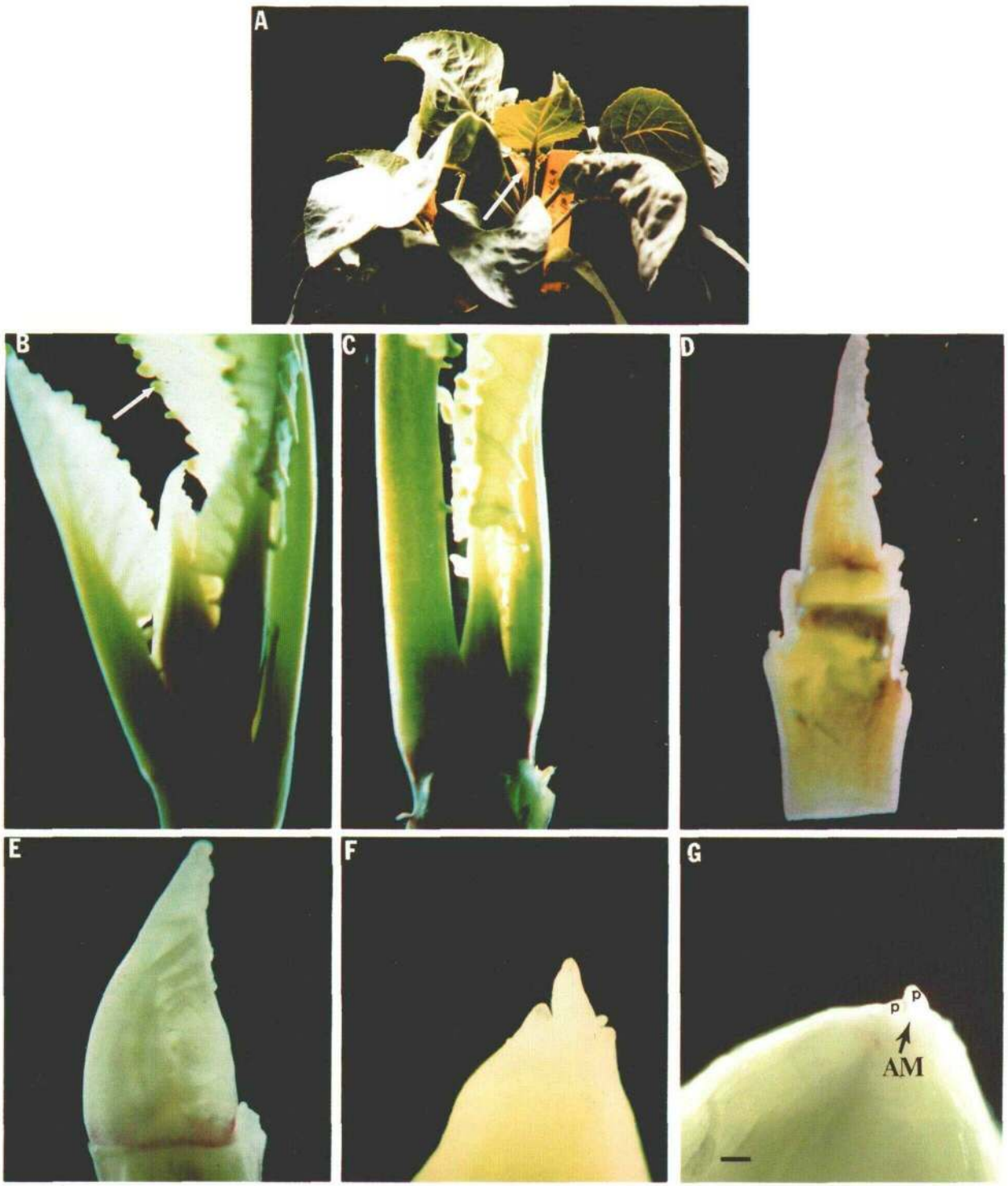

Figure 1. Dissection of *Brassica oleracea* to Reveal the Vegetative Shoot Apical Meristem.

(A) Intact plant.
(B) Removal of all mature leaves to reveal the shoot tip. For orientation purposes, the same leaf is indicated by an arrow in **(A)** and **(B)**.
(C) to **(G)** Subsequent removal of two leaves. The shoot apex can be seen in **(E)** and the shoot apical meristem in **(G)**. AM, apical meristem; p, primordium. Bar = 100 μm.

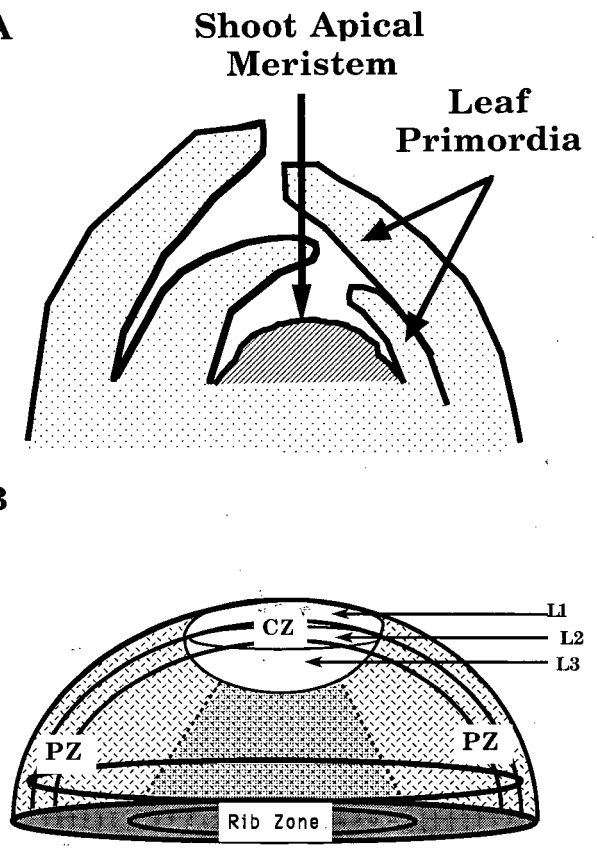

Figure 2. Diagram of the Shoot Apical Meristem.

(A) Comparison of the shoot apex and the shoot apical meristem. The entire figure represents the shoot apex, whereas the shoot apical meristem is represented by the dome-shaped, shaded area.
(B) Zones and layers within the shoot apical meristem. The central zone (CZ) is a small, oval-shaped, distally located group of cells that serves as the source of cells for other parts of the meristem. The peripheral zone (PZ) is located to the side and beneath the central zone. Organ initiation takes place in the peripheral zone. The rib zone forms the boundary between the meristem and the rest of the plant and is the source of the interior tissues of the stem. The zones are drawn with distinct lines, but in vivo the zonal boundaries are less distinct. L1, L2, and L3 correspond to the genetically defined three cell layers from the exterior of the meristem to the interior, respectively.

A second idea used to define regions of the shoot apical meristem is that of zones. Evidence for meristem zones has been suggested in numerous studies examining cytological features and cell division patterns (Esau, 1977; Steeves and Sussex, 1989; Lyndon, 1990). Zonation patterns are remarkably uniform among angiosperm apical meristems (Wardlaw, 1957) but are apparent only when the meristem is active (Clowes, 1961). Figure 2B shows a diagram of the three meristem zones: the central zone, the peripheral zone, and the rib zone. The central zone, which is a group of cells located at the distal end of the apical meristem, includes cells from all three layers. Cells in the central zone divide less frequently and have more prominent nuclei than cells at the sides or peripheral zone (Rembur and Nougarede, 1977; Nougarede and Rembur, 1978; Steeves and Sussex, 1989; Lyndon, 1990). In addition, central zone cells are thought to function as stem cells, but, unlike mammalian stem cells, these cells are not permanent (Ruth et al., 1985). In spite of their impermanent nature, central zone cells act as initials (or source cells) for other regions of the apical meristem and hence the shoot.

Peripheral zone cells arise from cells of the central zone. The peripheral zone extends around the meristem in a doughnut or inner-tube shape (Buvat, 1952). The peripheral zone's main function is the formation of lateral organs (mainly leaf primordia) that are positioned at mathematically precise points. Active regions of the peripheral zone (i.e., in relation to the pattern of leaf initiation) are thought to oscillate about the central zone in both the radial and vertical dimensions (Catesson, 1953).

At the base of the shoot apical meristem, and serving as a transition zone between the apical meristem and shoot, is another set of cells, known as the rib meristem or rib zone (Figure 2B). Cells in this region form a border between the meristem and fully differentiated cells. Because of this, the rib zone is sometimes considered to be distinct from the apical meristem. It is included in the discussion here for clarity. Cells in the rib zone are arranged in longitudinal files and contribute to tissues in the central portion of the stem. Like the cells of the peripheral zone, cells in the rib zone are thought to be derived from impermanent initials in the central zone. The rib zone has two possible functions. First, it forms the cells for the center of the stem, and, second, it may act as an organizing center for the shoot (Sachs, 1991). The second function requires that signals entering or leaving the meristem be transmitted through plasmodesmata or by apoplastic means because there are no vascular connections between the meristem and the rest of the plant (Sachs, 1991).

How do the two concepts of meristem organization—layers and zonation—relate to each other? As shown in Figure 2B, various zones contain cells from all three cell layers. However, although the evidence from chimeras suggests that the apical meristem in most angiosperms is three cell layers deep, data from zonal and microsurgery studies (see below) suggest that the apical meristem can be functionally defined to a greater depth. The ideas can be reconciled if programming of the immediate derivatives of meristem initials is a progressive process. Specifically, the epigenesis hypothesis of Sachs (1991) could also be applied to apical meristems. According to the hypothesis, programming cell fate is a slow process requiring continuous exposure to a determining substance. If cells in the apical meristem are programmed in a similar manner, then it would be possible to define the meristem as three cell layers even though molecular and cytological localization indicate a greater depth because of the slow progressive nature of programming.

EXPERIMENTAL STUDIES WITH MICROSURGERY

Even from the lucid descriptive studies, the shoot apical meristem and shoot apex can be seen as distinctive parts of the shoot. The importance of this distinction can be seen in functional analysis. How can the functions of apical meristems be studied? One way is to perturb the system with small surgical cuts and observe the results. These experiments suggest important concepts from which testing with molecular and genetic approaches can begin.

The experimental studies offer three hypotheses for testing: that the apical meristem is a self-regulating unit, that the apical meristem plays some role in specifying determinate growth, and that the apical meristem influences the positions where organs are formed. Two types of experimental evidence support the idea that, although specific zones and cell layers can be defined within the meristem, the shoot apical meristem operates as if it is a unit in itself. The first type of evidence comes from experiments in which the apical meristem is surgically isolated from the rest of the plant and placed in culture. When the meristem is cultured, it continues to function (i.e., to initiate organs and tissues) independent of any outside signals (reviewed in Steeves and Sussex, 1989). The only requirements for the culture medium besides basal salts are inositol and IAA (Smith and Murashige, 1970).

The second type of evidence for the concept of a self-regulating meristem comes from experiments surgically splitting the apical meristem. If the meristem is cut into a small piece, it will first reform the entire apical dome prior to assuming any type of function (Lopriore, 1895; Pilkington, 1929; Snow and Snow, 1951; Sussex, 1952). This is true even when the piece of the apical meristem is as small as 1/20th of the original meristem (Sussex, 1952). The above experiments point to the exceptional properties of shoot apical meristems. Organs are initiated from the meristem's peripheral zone, and tissues from the meristem's base. Yet, these functions will not occur unless the entire meristem is intact. How do cells in spatially separate regions like the peripheral zone "know" that the meristem is or is not intact? There must be some type of constant communication signal within the shoot apical meristem. It would be interesting to see whether this putative signal is altered as tissues and organs are formed.

A second concept from surgical experiments is that the apical meristem determines which cells adopt a determinative growth pattern. If the apical meristem and newly initiated leaf primordia in ferns are surgically separated from one another, the primordia (with a normally determinate growth pattern) alter their development and form another shoot apical meristem (with an indeterminate growth pattern) (Wardlaw, 1950). The same switch from determinate to indeterminate growth cannot be reproduced in angiosperms. However, when the developing leaf primordia of angiosperms are separated from the apical meristem, the primordia develop without a dorsiventral symmetry (Sussex, 1951). Hence, the differences between angiosperms and ferns need not reflect distinct developmental strategies but could be a matter of timing. Specifically, angiosperm primordia cells could become determined faster (although not to the point of becoming a dorsiventral organ) than cells in fern primordia.

The third concept that arose from surgical experiments is that the meristem has some effect on organ positioning. Organs initiated from the vegetative meristem are positioned in the shoot apex with remarkable precision. The precision is such that spirals of contact points between leaves can be described by a mathematical equation, the Fibonacci series (each term in the series is the sum of two preceding terms, e.g., 1, 1, 2, 3, 5, 8). How molecular and genetic information in the apical meristem determines such a precise placement of lateral organs is not known.

Several studies addressed the positioning concept with surgical experiments. The first experiments involved cutting the shoot apex of an angiosperm, Lupinus, to isolate the apical meristem from leaf primordia at various stages of development (Snow and Snow, 1931, 1933). These workers suggested that the positions at which leaves arise are determined by spatial constraints in the shoot apex such that new primordia are formed where there is the first free space or minimum area between pre-existing leaf primordia. To explain the absence of primordia formation in this region, this area does not include the distal-most portion of the apical meristem. The second type of experiment, from Wardlaw (1949), suggested that the positioning of leaf primordia was not due to physical constraints. Wardlaw performed surgical experiments similar to those of Snow and Snow but used a plant (Dryopteris, a fern) in which the large size of the apical meristem prevents physical contact between leaf primordia. The data Wardlaw obtained were similar to that of Snow and Snow. However, because spatial constraints could not be operating in this system, Wardlaw suggested that an inhibitory substance that prevents the outgrowth of primordia originates from both newly formed leaf primordia and the apical meristem.

The idea of inhibitory substances was not unique to Wardlaw but originated with Schoute (1913) and was strongly supported by Richards (1948). Given that Catesson's analysis (above) indicates that the active division zones oscillate in both a vertical and radial dimension, a model predicting an inhibitor or morphogenetic gradient should also take into account the three-dimensional aspects of the meristem and developing primordia. Hence, a simple model in which developing leaf primordia emerge at positions where an inhibitor is at the lowest point seems unlikely to be the entire explanation for a three-dimensional shoot apical meristem (Figure 1B). This model could be correct if there is either a polarity to the inhibitory substance or an additional component, however. For example, the inhibitory substance could be produced by the apical meristem and developing primordia and be transported only basipetally. This would allow incipient primordia to develop in regions of the apical dome above the older primordia. A second possibility is that a change in some other component (e.g., a receptor)

in addition to an inhibitor is involved. This idea is implied in Schoute's field concept, where positioning involves the formation of a growth center around which primordia develop (Schoute, 1913; Wardlaw, 1965).

A third theory on positioning suggests that the orientation of cellulose microfibrils in cells (Green, 1985) is an important determining factor. This idea does not involve results from microsurgical experiments and will not be discussed here. Several excellent reviews discuss this idea in depth (Green, 1985, 1986).

Although experiments in which the apical meristem is cut provide numerous concepts about how the meristem functions, such experiments have caveats because the perturbation produces a wound, adding an unknown factor to all of the experimental results. This may be an especially significant problem given the suggestion that jasmonic acid, a molecule important for transduction of wound signals, has morphological effects on the apical meristem (Ravnikar and Gogala, 1990). However, the surgical experiments do provide a starting framework. Moreover, they collectively suggest that at least two of the four functions of apical meristems, initiation of organs and maintenance as a formative region, involve a complex series of intercellular communications within the shoot apex and perhaps within the meristem itself. The challenge ahead is to use the power of genetics and molecular biology to verify and/or modify these decades-old concepts about apical meristems.

GENE EXPRESSION IN APICAL MERISTEMS AND SHOOT APICES

Some studies have begun to ask about the nature of the molecules that allow meristems to have such a central role in development. Various approaches have been used in attempts to isolate meristem-specific genes. These approaches include using homology to known probes from mammalian systems, to differential hybridization of cDNA clones, to the use of plants with apical meristem amplification such as cauliflower (Kosugi et al., 1991; Medford et al., 1991a; Pri-Hadash et al., 1992; Shahar et al., 1992). Table 1 presents a summary of genes expressed in shoot apices and apical meristems. To facilitate comparison, the terminology has been standardized to that defined here. For clarity, the shoot apical meristem and shoot apex are listed separately even though there is some degree of redundancy (see above). In many cases, the expression was examined only with RNA gel blots, and the pattern was described as in the shoot apex, meaning that it is not known whether these genes are expressed in the shoot apical meristem. By and large, most of these genes are expressed in the apical meristem as well as the shoot apex. Many of these genes are typical for regions of active cell division and metabolism (e.g., histones, ribosomal proteins, and dUTPase [Koning et al., 1991; Medford et al., 1991a; Pri-Hadash et al., 1992]) and do not reveal many ideas about how meristems function. However, the studies on gene expression do support the idea that the meristem contains distinct zones. Specifically, several genes (histones H3 and H2B and cruciferin [in late embryogenesis]) are expressed in a region defined from cytological studies as the peripheral zone (Fernandez et al., 1991; Medford et al., 1991a; Kohler et al., 1992).

The designation of certain genes brings confusion as to their expression patterns. For example, a gene isolated from shoot apices was designated as "meristem specific" (histone; Table 1). This designation does not distinguish whether these genes are specific for the shoot apical meristem or whether they are expressed in all dividing cells. Plant biologists frequently refer to regions with active cell division as "meristems." For example, regions of active cell division in stems are often called the ground meristem (Esau, 1977), and such regions in leaves are known as the basal meristem plate (Pyke et al., 1991). Undoubtedly, many of the genes expressed in general meristematic regions will also be found in apical meristems. However, because apical meristems have unique functions, genes that are specific for apical meristems may also exist. Furthermore, there must also be genetic differences and, perhaps, distinct genes expressed in shoot and root apical meristems because mutants of rice (Nagato et al., 1989), corn (Clark and Sheridan, 1991), and Arabidopsis (Mayer et al., 1991; Medford et al., 1992) disrupt the shoot meristem without perturbing the root meristem.

These predicted differences in meristem gene expression will result in distinct and overlapping patterns. Figure 3 shows a diagram of how meristematic gene expression can be described, assuming that there are not only distinct genes expressed in the shoot and root apical meristem, but that there are genes specific for each type of apical meristem. Meristematic genes are those expressed in areas of active cell division and the cambium (i.e., meristemtic cells in vascular tissues), as well as in shoot and root apical meristems. Meristematic genes would not be unique to apical meristems but would be common to all areas where there is active cell division and/or metabolism. Examples of meristematic genes are histone genes or genes involved in nucleic acid biosynthesis, which are needed in any dividing cell. Shoot and root apical meristems should also have unique types of gene expression (see above). However, no gene specific for shoot or root apical meristems, or simply apical meristems, has been reported to date. In the shoot meristem, several cytologically distinct types of apical meristems can be described: vegetative meristems, inflorescence meristems, and floral meristems. The cytological distinctions will presumably be reflected in both distinct and common patterns of apical meristem gene expression. Hence, Figure 3 divides gene expression for shoot apical meristems into three overlapping categories.

Although the identification of genes expressed in apical meristems has so far revealed only limited information, the exclusion of expression of certain genes suggests an interesting hypothesis as to how the apical meristem functions. In a detailed study of Brassica embryogenesis, Fernandez et al. (1991) found that napin mRNAs were excluded from the apical meristem throughout embryogenesis. Cruciferin mRNAs were

Table 1. Genes Expressed in the Vegetative Shoot Apex and Apical Meristem

Gene or Gene Product	Expression		Reference
	In Situ/GUS	Gel Blots	
Polyphenol oxidase	Protoderm (L1) and low level throughout	Throughout the plant, high in apical meristems and flowers	Shahar et al. (1992)
Cyclophilin	ND	Shoot apex and all tissues	Gasser et al. (1990)
Lectin-like	All parts of the shoot apex except the apical meristem	Shoot apex and young organs	Dobres and Thompson (1989)
Nuclear protein	Apical meristem and rapidly proliferating carrot cultured cells	Shoot apex	Smith et al. (1988)
meri-5*	Apical meristem, cell points beneath branches, floral vasculature	Apical meristem, some clones in other tissues	Medford et al. (1991a)
Histones (H2A, H2B, H3, H4)	Peripheral zones of apical meristem (H2A in specific cells of apical meristem)	Actively dividing tissues throughout plant	Koning et al. (1991); Medford et al. (1991a); Kohler et al. (1992)
PCNA	Apical meristems, immature leaves, vascular tissues	ND	Kosugi et al. (1991)
Group I cDNAs*	Apical meristem peripheral zone, procambium in leaf vascular*	Apical meristem, shoot apex, not in mature leaves	Melzer et al. (1990)
dUTPase	Apical meristem		Pri-Hadash et al. (1992)
Elongation factor 1α	Vascular tissue in young leaves and regions beneath the apical meristem	Expanding leaves	Ursin et al. (1991)
Elongation factor eIF-4A	ND	Shoot apex	Owttrim et al. (1991)
EP2 (lipid transfer protein)	Protoderm in embryos, in seedling localization in the subepidermal layers of the apical meristem's peripheral zone, around the surface of young floral organs	Shoot apex	Sterk et al. (1991)
A3	ND	Shoot apex (vegetative, transition and floral), petals, stamens, pistils	Kelly et al. (1990)
C3	ND	Shoot apex, shoot tips, pods	Williams et al. (1990)
cdc2 (p34)	ND	Shoot tip	Colasanti et al. (1991)
PAL promoter	Shoot apex, root apex, prexylem elements	ND	Liang et al. (1989)
FBPase promoter	Shoot and root apex	ND	Lloyd et al. (1991)

Expression patterns have been described using the definitions of shoot apical meristem and shoot apex in the text. Expression patterns were examined with in situ hybridization of RNA or protein probes or by localization of GUS activity. Tissue specificity was examined on RNA or protein gel blots. Asterisks indicate that multiple cDNAs have been described, and in these cases the localization is described for the key gene in the cited reference. ND, not determined.

excluded from the apical meristem until late embryogenesis, at which time they accumulated in the peripheral zone but not the central zone. Similarly, Maiti et al. (1991) found that a lectin-like gene was expressed in all parts of the pea shoot apex except for the apical meristem. These studies support the idea that the apical meristem is functionally distinct from developing leaf primordia and other tissues in the shoot apex and further support the idea that the apical meristem is a unit in itself. Moreover, they further indicate that the expression of certain genes is repressed (or these genes are never activated) in the apical meristem. It will be interesting to see whether repression of gene expression is a general mechanism used by the apical meristem.

A surprising result using expression of *Rhizobium* genes in transgenic plants may give some insight into how meristems function. The *Rhizobium nodAB* genes are more commonly referenced in discussions of roots and root nodules. These two genes are required for the production of a small, heat-stable, partially hydrophobic factor that stimulates mitosis in cultured plant cells (Schmidt et al., 1988). This factor may be

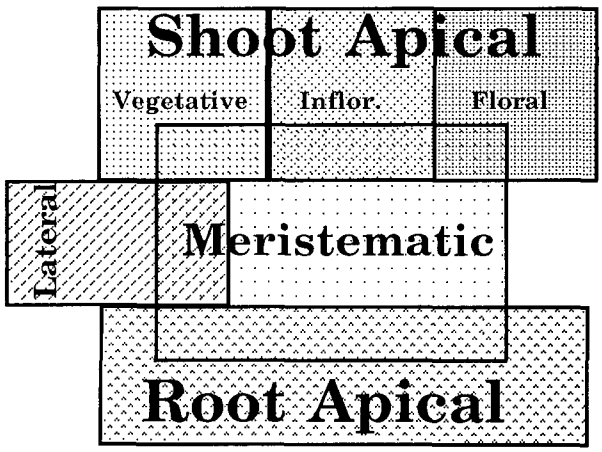

Figure 3. Diagram of Meristem Gene Expression.

Genes expressed in actively growing regions are represented in the meristematic category. A number of meristematic genes would be expressed in apical meristems (both shoot and root). In addition, the apical meristems (and perhaps lateral meristems) would differentially express a series of unique genes. Because cytological distinctions can be found among vegetative, inflorescence, and floral apical meristems, these meristems should have gene products that are distinct from one another. Although gene expression in the apical meristems is diagrammed separately for shoots and roots, some genes may be expressed in both types of apical meristems.

a precursor of the acetylated glucosamine produced by the *nodABCJ* genes (Lerouge et al., 1990), which determines host specificity. When *nodAB* genes were placed under the control of a dual promoter (1′, 2′ TR promoter) and transferred to tobacco plants, the resulting transgenic plants had bilobed or bifurcated leaves (Schmidt et al., 1991). Similar bilobed leaves reported in experimental and genetic studies correlated the result with the formation of leaf primordia containing an abnormally large number of cells. For example, when an incipient leaf primordium is surgically cut so as to include too many cells, bilobed leaves result (Sussex, 1964). Furthermore, the Arabidopsis Forever young (Fey) mutant results in the sequestering of an abnormally large number of cells into developing primordia, as well as in the production of bilobed leaves (Medford et al., 1991b, 1992). Assuming that the bilobed leaves in the transgenic tobacco plants originated from altered apical meristem signals, the results may tell us about processes in normal apical meristems. For example, could normal leaf primordia formation use a factor similar to that produced by the *nodAB* genes (i.e., mitosis stimulating, hydrophobic, heat stable)?

GENETIC ANALYSIS OF MERISTEM DEVELOPMENT

Genetic approaches have begun to answer questions about how the apical meristem forms the plant. Various mutants provide tools to address the complexities of apical meristems. For example, lateral organs initiated from vegetative meristems are typically formed as a unit (called a phytomer) that consists of a lateral organ (leaf), a node, the axillary meristem, and an internode. By contrast, the floral meristem simply initiates compressed nodes and a lateral organ (e.g., sepal or petal). A tomato mutant, Sidebranchless, reportedly initiates units from the vegetative apical meristem without evidence of axillary meristems (Clements and Guard, 1958). If so, then the formation of complete phytomers from the vegetative meristem may depend on a simple genetic signal.

The mutants in corn, rice, and Arabidopsis, among others, provide tools to examine the various ideas on apical meristem functions (Nagato et al., 1989; Clark and Sheridan, 1991; Medford et al., 1992). The corn mutants represent one of the most extensive collections of vegetative meristem mutants to date (Clark and Sheridan, 1991). Analysis of these mutants will be extremely valuable, although it will be challenging because the corn apical meristem is active during embryogenesis. Hence, these studies will be complicated by the fact that the analysis done with material from corn kernels will have potential maternal effects.

Related to the shoot meristem mutants is an impressive collection of Arabidopsis mutants affecting body organization (Mayer et al., 1991). To date, none of the mutants is attributed to a lesion in the vegetative shoot apical meristem, although it seems likely that some will be related. These mutants may define specific compartments of action and may add new insights into the fourth function of the apical meristem, communication with other parts of the shoot, and perhaps also the organization of the shoot (Sachs, 1991).

Some of the Arabidopsis mutants have already suggested ideas about how the shoot apical meristem functions. Evidence from one mutant suggests that at least two meristem functions are directly linked. The *fey* mutation disrupts the proper formation of leaf primordia and maintenance of the shoot apical meristem as a formative region (Medford et al., 1992). Work is in progress to determine how one gene links these two functions. In addition, preliminary evidence suggests that disrupting the formation of leaf primordia may also disrupt primordia positioning (J. D. Callos and J. I. Medford, unpublished data).

CONCLUSION

A distinctive feature of plant development is that it is continuous. Development of the above-ground portion of the plant can be traced to a small meristem in the shoot apex. The shoot apical meristem is a distinct part of the shoot apex and a functional unit. It can be defined by the exclusion of some molecular probes, whereas other molecular probes define specific zones within the apical meristem.

The shoot apical meristem makes the plant through at least four functions: initiating organs, initiating tissues, communicating signals, and maintaining itself as a formative region. Advances toward understanding how the apical meristem

functions will take place through careful examination of gene expression in the apical meristem rather than a broadly defined shoot apex. Because of the small size of the apical meristem, these studies will be difficult. However, studies that distinguish between the apical meristem and the shoot apex are essential if the remarkable functions of apical meristems are to be understood. One way in which molecular analysis of the apical meristem will be helped is through the use of the polymerase chain reaction.

Some of the molecular analysis done to date may provide a clue to at least one of the four functions: how the meristem maintains itself as a formative region. Two mechanisms are worth considering: that the apical meristem lacks an inducer(s), a positive signal for certain types of gene expression, or that gene expression in the apical meristem is repressed by a negative signal(s). Perhaps some of the best evidence for a repressor comes from the work of Fernandez et al. (1991) and Maiti et al. (1991), which showed that at least certain types of gene expression are repressed in the apical meristem. Many other genes could also be repressed in the meristem. A close examination of gene expression in the apical meristem, as opposed to the shoot apex, may provide much-needed data.

At this time, it is premature to conclude that a repressor is the sole type of regulator in apical meristems. For example, the genes examined by Fernandez et al. (1991) and Maiti et al. (1991) could be expressed in the apical meristem but at reduced levels. This possibility is difficult to eliminate because studies looking directly at the apical meristem via in situ hybridization or GUS localization are only semiquantitative. A further possibility is that the apical meristem uses both repression and lack of an inducer to remain formative. Because studies on the Fey mutant (Medford et al., 1992) suggest that the ability of a meristem to remain formative is linked to its ability to initiate leaf primordia, a dual type of regulation may exist in shoot apical meristems. Bringing developmental genetics to bear on the study of meristems has provided, and will continue to provide, insight into the remarkable features of apical meristems.

ACKNOWLEDGMENTS

I thank Jill Deikman and members of the lab (Fred Behringer, Bibo Xu, Joe Callos, Bruce Link, and Dina Pharye) for their helpful comments and Laurie Smith for references on corn mutants. This work is supported by Grant No. DCB 90-060208 from the National Science Foundation.

Received April 24, 1992; accepted July 10, 1992.

REFERENCES

Buvat, R. (1952). Structure, evolution et fonctionnement du meristeme apical de quelques dicotyledones. Ann. des Sc. Nat. Bot. **13,** 199–300.

Catesson, A.-M. (1953). Structure, evolution et fonctionnement du point vegetatif d'une monocotyledone: *Luzula pedemontana.* Ann. des Sc. Nat. Bot. **14,** 253–291.

Clark, J.K., and Sheridan, W.F. (1991). Isolation and characterization of 51 *embryo-specific* mutations of maize. Plant Cell **3,** 935–951.

Clements, J.N., and Guard, A.T. (1958). An anatomical study of side-branching in the 'Rutgers' and the 'sideshootless' tomato plants. Am. J. Bot. **48,** 527.

Clowes, F.A.L. (1961). Apical Meristems (Oxford: Blackwell Scientific Publications).

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interaction controlling flower development. Nature **353,** 31–37.

Colasanti, J., Tyers, M., and Sundaresan, V. (1991). Isolation and characterization of cDNA clones encoding a functional $p34^{cdc2}$ homologue from *Zea mays.* Proc. Natl. Acad. Sci. USA **88,** 3377–3381.

Cutter, E.G. (1965). Recent experimental studies of the shoot apex and shoot morphogenesis. Bot. Rev. **31,** 7–113.

Dobres, M.S., and Thompson, W.F. (1989). A developmentally regulated bud-specific transcript in pea has sequence similarity to seed lectins. Plant Physiol. **89,** 833–838.

Esau, K. (1977). Anatomy of Seed Plants, 2nd ed. (New York: John Wiley and Sons).

Fahn, A. (1982). Plant Anatomy, 3rd ed. (New York: Pergamon Press).

Fernandez, D.E., Turner, F.R., and Crouch, M.L. (1991). In situ localization of storage protein mRNAs in developing meristems of *Brassica napus* embryos. Development **111,** 299–313.

Gasser, C.S., Gunning, D.A., Budelier, K.A., and Brown, S.M. (1990). Structure and expression of cytosolic cyclophilin/peptidyl-prolyl cis-trans isomerase of higher plants and production of active tomato cyclophilin in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **87,** 9519–9523.

Green, P.B. (1985). Surface of the shoot apex: A reinforcement-field theory for phyllotaxis. J. Cell Sci. Suppl. **2,** 181–201.

Green, P.B. (1986). Plasticity at the stem apex: A biophysical view. In Plasticity in Plants, D.H. Jennings and A.J. Trevawas, eds (Cambridge, UK: Company of Biologists), pp. 211–232.

Hanstein, J. (1868). Die Scheitelzellgruppe im vegetationspunckt der phanerogamen. Festschr. Niederrhein Ges. Natur. u. Heilkunde 109–143.

Kelly, A.J., Zagotta, M.T., White R.A., Chang, C., and Meeks-Wagner, D.R. (1990). Identification of genes expressed in the tobacco shoot apex during the floral transition. Plant Cell **2,** 963–972.

Kohler, S., Coraggio, I., Becker, D., and Salamini, F. (1992). Pattern of expression of meristem-specific cDNA clones of barley (*Hordeum vulgare* L.). Planta **186,** 227–235.

Koning, A.J., Tanimoto, E.Y., Kiehne, K., Rost, T., and Comai, L. (1991). Cell-specific expression of plant histone H2A genes. Plant Cell **3,** 657–665.

Kosugi, S., Suzuka, I., Ohashi, Y., Murakami, T., and Arai, Y. (1991). Upstream sequences of rice proliferating cell nuclear antigen (PCNA) gene mediate expression of PCNA-GUS chimeric gene in meristems of transgenic tobacco plants. Nucl. Acids Res. **19,** 1571–1576.

Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Prome, J.C., and Denarie, J. (1990). Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. Nature **344,** 781–784.

Liang, X., Dron, M., Schmid, J., Dixon, R.A., and Lamb, C.J. (1989). Developmental and environmental regulation of a phenylalanine

ammonia-lyase-β-glucuronidase gene fusion in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA **86,** 9284–9288.

Lloyd, J.C., Raines, C.A., John, U.P., and Dyer, T.A. (1991). The chloroplast FBPase gene of wheat: Structure and expression of the promoter in photosynthetic and meristematic cells of transgenic tobacco plants. Mol. Gen. Genet. **225,** 209–216.

Lopriore, A. (1895). Regeneration gespätener sprosspitzen. Ber. dt. bot. Ges. **12,** 410–414.

Lyndon, R.F. (1990). Plant Development: The Cellular Basis (Winchester, MA: Unwin Hyman Inc.).

Maiti, D., List, A., and Dobres, M.S. (1991). Histo-developmental analysis of a putative vegetative-lectin transcript in pea (*Pisum sativum*). Penn State Summer Symposium in Molecular Biology **10,** 93 (abstract).

Mayer, U., Ruiz, R.A.T., Berleth, T., Misera, S., and Jurgens, G. (1991). Mutation affecting body organization in the *Arabidopsis* embryo. Nature **353,** 402–407.

Medford, J.I., Elmer, J.S., and Klee, H.J. (1991a). Molecular cloning and characterization of genes expressed in shoot apical meristems. Plant Cell **3,** 359–370.

Medford, J.I., Xu, B., Behringer, F.J., and Callos, J.D. (1991b). Molecular genetics of shoot apical meristems. Penn State Summer Symposium in Molecular Biology **10,** 46 (abstract).

Medford, J.I., Behringer, F.J., Callos, J.D., and Feldmann, K.A. (1992). Normal and abnormal development in the Arabidopsis vegetative shoot apex. Plant Cell **4,** 631–643.

Melzer, S., Majewski, D.M., and Apel, K. (1990). Early changes in gene expression during the transition from vegetative to generative growth in the long-day plant *Sinapis alba.* Plant Cell **2,** 953–961.

Nagato, Y., Kitano, H., Kamijima, O., and Satoh, H. (1989). Developmental mutants showing abnormal organ differentiation in rice embryos. Theor. Appl. Genet. **78,** 11–15.

Nougarede, A., and Rembur, J. (1978). Variations of the cell cycle phases in the shoot apex of *Chrysanthemum segetum* L. Z. Pflanzenphys. **90,** 379–389.

Owttrim, G.W., Hofmann, S., and Kuhlemeier, C. (1991). Divergent genes for translation initiation factor eIF-4A are coordinately expressed in tobacco. Nucl. Acids Res. **19,** 5491–5496.

Pilkington, M. (1929). The regeneration of the stem apex. New Phyt. **28,** 37–53.

Poethig, R.S., and Sussex, I.M. (1985). The developmental morphology and growth dynamics of the tobacco leaf. Planta **165,** 158–169.

Pri-Hadash, A., Hareven, D., and Lifschitz, E. (1992). A meristem-related gene from tomato encodes a dUTPase: Analysis of expression in vegetative and floral meristems. Plant Cell **4,** 149–159.

Pyke, K.A., Marrison, J.L., and Leech, R.M. (1991). Temporal and spatial development of the cells of the expanding first leaf of *Arabidopsis thaliana* (L.) Heynh. J. Exp. Bot. **42,** 1407–1416.

Ravnikar, M., and Gogala, N. (1990). Regulation of potato meristem development by jasmonic acid in vitro. J. Plant Growth Reg. **9,** 233–236.

Rembur, J., and Nougarede, A. (1977). Duration of cell cycles in the shoot apex of *Chrysanthemum segetum* L. Z. Pflanzenphys. **81,** 173–179.

Richards, F.J. (1948). The geometry of phyllotaxis and its origin. Symp. Soc. Exp. Biol. **2,** 217–245.

Ruth, J., Klekowski, E. J., Jr., and Stein, O.L. (1985). Impermanent initials of the shoot apex and diplontic selection in a juniper chimera. Am. J. Bot. **72,** 1127–1135.

Sachs, T. (1991). Pattern Formation in Plant Tissues (New York: Cambridge University Press).

Satina, S., Blakeslee, A.F., and Avery, A.G. (1940). Demonstration of the three germ layers in the shoot apex of Datura by means of induced polyploidy in periclinal chimeras. Am. J. Bot. **27,** 895–905.

Sattler, R. (1988). Homeosis in plants. Am. J. Bot. **75,** 1606–1617.

Schmidt, J., Wingender, R., John, M., Wieneke, U., and Schell, J. (1988). *Rhizobium meliloti* nodA and nodB genes are involved in generating compounds that stimulate mitosis of plant cells. Proc. Natl. Acad. Sci. USA **85,** 8578–8582.

Schmidt, J., John, M., Wieneke, U., Stacey, G., Rohrig, H., and Schell, J. (1991). Studies on the function of *Rhizobium meliloti* nodulation genes. Adv. Mol. Gen. Plant-Microbe Interactions **1,** 150–155.

Schoute, J.C. (1913). Beitrage zur Blattstellungslehre. Rec. Trav. Bot. Neerl. **10,** 153–325.

Shahar, T., Hennig, N., Gutfinger, T., Hareven, D., and Lifschitz, E. (1992). The tomato 66.3-kD polyphenoloxidase gene: Molecular identification and developmental expression. Plant Cell **4,** 135–147.

Shivji, M.K.K., Kenny, M.K., and Wood, R.D. (1992). Proliferating cell nuclear antigen is required for DNA excision repair. Cell **69,** 367–374.

Smith, R.H., and Murashige, T. (1970). In vitro development of the isolated shoot apical meristem of angiosperms. Am. J. Bot. **57,** 562–568.

Smith, J.A., Krauss, M.R., Borkird, C., and Sung, Z.R. (1988). A nuclear protein associated with cell divisions in plants. Planta **174,** 462–472.

Snow, M., and Snow, R. (1931). Experiments on phyllotaxis. I. The effect of isolating a primordium. Phil. Trans. **221,** 1–40.

Snow, M., and Snow, R. (1933). Experiments on phyllotaxis. II. The effect of displacing a primordium. Phil. Trans. **222,** 353–400.

Snow, M., and Snow, R. (1951). Minimum areas and leaf determination. Proc. R. Soc. Lond. Ser. B **139,** 545–566.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, 2nd ed. (New York: Cambridge University Press).

Sterk, P., Booij, H., Schellekens, G.A., Van Kammen, A., and De Vries, S.C. (1991). Cell-specific expression of the carrot EP2 lipid transfer protein gene. Plant Cell **3,** 907–921.

Sussex, I.M. (1951). Experiments on the cause of dorsiventrality in leaves. Nature **167,** 651–652.

Sussex, I.M. (1952). Regeneration of the potato shoot apex. Nature **170,** 755–757.

Sussex, I.M. (1964). The permanence of meristems: Developmental organizers or reactors to exogenous stimuli? In Meristems and Differentiation, J.P. Miksche, W.S. Hillman, R.M. Smillie, H.H. Smith, M.E. Koshland, and H.J. Curtis, eds. (Washington, DC: Office of Technical Services, Department of Commerce), pp. 1–12.

Thielke, C. (1948). Beitrage zur entwicklungsgeschichte und zur physiologie panaschierter blätter. Planta **36,** 2–33.

Ursin, V.M., Irvine, J.M., Hiatt, W.R., and Shewmaker, C.K. (1991). Developmental analysis of elongation factor-1α expression in transgenic tobacco. Plant Cell **3,** 583–591.

Wardlaw, C.W. (1949). Experiments on organogenesis in ferns. Growth (Suppl.) **13,** 93–131.

Wardlaw, C.W. (1950). Experimental and analytical studies of pteridophytes. XVI. The induction of leaves and buds in *Dryopteris artistata* Druce. Ann. Bot. **14,** 435–455.

Wardlaw, C.W. (1957). On the organization and reactivity of the shoot apex in vascular plants. Am. J. Bot. **44,** 176–185.

Wardlaw, C.W. (1965). Organization and Evolution in Plants (London: Longmans, Green and Co.), pp. 17–54.

Williams, M.E., Mundy, J., Kay, S.A., and Chua, N.-H. (1990). Differential expression of two related organ-specific genes in pea. Plant Mol. Biol. **14,** 765–774.