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REVIEW ARTICLE

The Initiation and Determination of Leaves

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INTRODUCTION

Leaves are initiated sequentially in precisely ordered patterns throughout the vegetative phase of shoot development by the apical meristem, which maintains itself in the process as an organized unit of cells whose fates within the shoot are not yet determined. Leaves originate relatively uniformly as simple peg-like outgrowths, and subsequently exhibit divergent patterns of determinate growth. The leaf has been characterized as a developmental "ground state," an identity that is modified through the action of homeotic genes to generate floral organs (Coen and Meyerowitz, 1991). Yet leaves themselves are diverse, not only among different species of plants, but also within an individual. Plants frequently exhibit some degree of heterophylly, producing different leaves on the same shoot system. Alternative leaf forms on a single plant are often sufficiently different with respect to morphology and anatomy that they could be said to have different "identities" in the same sense that leaf and petal are different identities. Furthermore, leaves are subdivided into regions of differing identities, such as the petiole and blade of a typical dicot leaf or the sheath and blade regions of a grass leaf. Thus, leaf determination, the process by which the characteristics of the leaf are fixed, is a complex problem in its own right. How is a leaf initiated, and how are its characteristics determined? Here, we explore these questions by reviewing selected aspects of early leaf development in angiosperms, discussing where possible the underlying mechanisms of control such as the roles played by genes and hormones. Excellent reviews with similar themes have been written previously (for example, Halperin, 1978; Lyndon, 1983).

LEAF INITIATION

Leaves are initiated by groups of cells within the organogenic region on the flanks of the shoot apical meristem. The group of cells that initiates a leaf spans all the histogenic layers of the meristem, the tunica layer(s) (L1 in monocots and both L1 and L2 in dicots) and the corpus (L3 in dicots, which have two tunica layers). Periclinal chimeras, in which individual meristem layers are of different origins and can be distinguished by means of morphological markers, have demonstrated that each layer of the meristem makes a predictable contribution to the formation of leaves. In dicots, the L1 layer contributes only to the epidermis, whereas the L2 and L3 layers contribute to the internal tissues of the leaf (Satina et al., 1940; Satina and Blakeslee, 1941). The contributions of individual layers are not strictly lineage dependent, however. A cell from one layer occasionally invades a neighboring layer, where it contributes to lateral organs in a manner characteristic of the new layer rather than the original layer, demonstrating its lack of commitment (Dermen, 1953; Stewart and Burk, 1970; Stewart and Dermen, 1975). Clonal analyses of the size and number of leaf sectors arising from single cells in the meristem have led to the estimate that approximately 100 to 200 cells on the flank of the meristem give rise to a leaf primordium in both tobacco and maize (Poethig, 1984a, 1984b).

Leaf initiation is achieved through coordinated changes in the polarity and rate of cell division and expansion within a group of leaf founder cells on the flank of the shoot apical meristem. The earliest morphologically recognizable event in the initiation of a leaf is a new pattern of cell division. In pea, for example, leaf initiation begins with an increase in the frequency of periclinal cell divisions (i.e., new cell wall parallel to the surface of the apex) in the prospective leaf-initiating region of the apical meristem (Lyndon, 1972; Cunninghame and Lyndon, 1986). This change in the relative frequency of periclinal divisions becomes apparent about half a plastochron before the primordium begins to emerge from the apex, a plastochron being a unit of time that separates the initiation of two successive leaf primordia. A modest increase in the rate of cell division in this region can also be observed (Lyndon, 1970). The meristem cells that give rise to leaves thus seem to emerge from the apex by dividing increasingly in the orientation characteristic of the future leaf. However, irradiation of wheat seedlings with a dose of x-rays sufficient to inhibit cell division did not prevent the initial protrusion of leaf primordia from the next prospective leaf site, as illustrated in Figure 1. In the absence of cell division, the initial protrusion formed by

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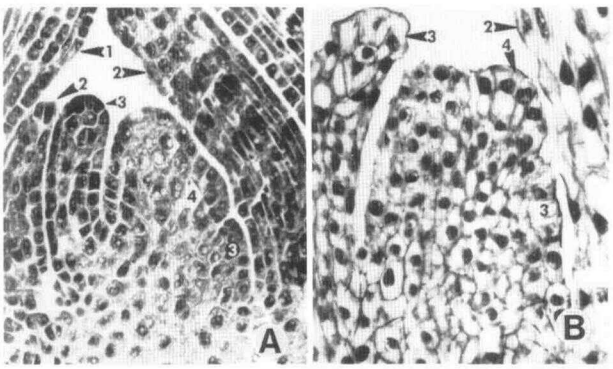

Figure 1. Initial Protrusion of Leaf Primordia in Normal and Irradiated Wheat Seedlings.

(A) Sagittal section (longitudinal side view) of a normal embryo having four leaf primordia in an ungerminated "Monon" wheat grain. The primordia are numbered 1 to 4 successively from the oldest to the youngest.

(B) Sagittal section through the shoot apex of a "gamma plantlet" 11 days after heavy gamma irradiation of an embryo that had initiated three leaf primordia at the time of irradiation.

Figure reprinted from Foard (1971).

polarized cell expansion (Foard, 1971). This observation suggests that the primary morphogenetic event in leaf initiation is a regional shift in the polarity of cell expansion, which is normally accompanied by—but not caused by or dependent on—a change in the plane of cell division.

Insight into the mechanisms governing the polarity of cell expansion in the shoot apical meristem and its reorientation during leaf initiation has come from the analysis of cytoskeletal and cell wall architecture. As illustrated in Figure 2A, cell wall cellulose microfibrils within the L1 layer of the shoot apical meristem are aligned in parallel arrays that encircle the shoot apex, forming "hoops of reinforcement" perpendicular to the growth axis that presumably constrain the direction of cell growth. As shown in Figures 2B and 2C, leaf initiation is accompanied by a localized reorientation of cellulose microfibrils on the flanks of the meristem to produce a new array that encircles the incipient leaf primordium. This reorientation is thought to be necessary to permit the change in growth polarity with which leaves are initiated (Hardham et al., 1980; Green and Lang, 1981; Jesuthasan and Green, 1989). Wall cellulose microfibril arrays parallel the microtubule arrays within the adjacent cell cortex, and the shift in the orientation of cellulose microfibrils that accompanies leaf initiation is believed to result from a corresponding shift in the orientation of cortical microtubules (Hardham et al., 1980; Gunning and Hardham, 1982). The mechanism by which cortical microtubules determine the orientation of cellulose deposition in the neighboring cell wall is not understood, but it has been proposed that microtubules guide the translocation of cellulose synthetase molecules through the plasma membrane, trailing cellulose microfibrils along behind them (Gunning and Hardham, 1982).

If the cytoskeleton indeed orchestrates an essential reorientation of cellulose deposition, then whatever mechanisms coordinate leaf initiation must operate in part at the level of

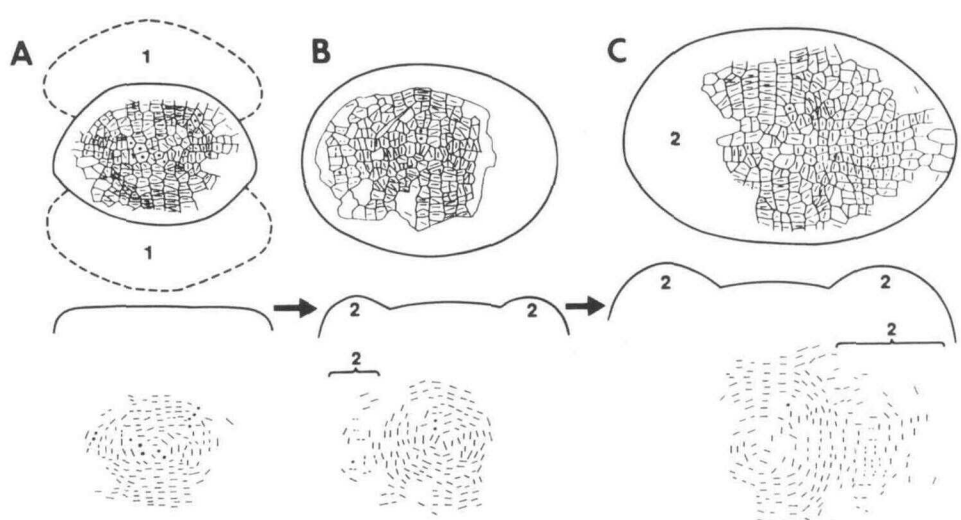

Figure 2. Alignment of Cellulose Microfibril Arrays in the L1 Layer of the Shoot Apex of *Vinca major.*

(A) to **(C)** Series shows the reorientation in cellulose alignment that occurs as a leaf (2) is initiated. Top figure in each section shows the identifiable cells on the apical dome. The bases of the first pair of leaves (1) are shown dotted in **(A)**. Each cell is given a bar showing the alignment of cellulose microfibrils. If the alignment was weak, the bar is dashed; if alignment was not detectable, the cell is given a dot. Central figure in each section is a profile of the apical dome. Bottom figure in each section shows cellulose alignment only. Figure reprinted from Jesuthasan and Green (1989).

the cytoskeleton. Green (1985) has proposed that the growth of existing leaf primordia produces stretching that causes the reorientation of cortical microtubules at leaf initiation sites in the meristem. However, leaves can be initiated by the shoot apical meristem in the absence of existing leaf primordia (Ball, 1951), as is the first leaf on a new shoot. Thus, if the reorientation of microtubule and cellulose microfibril arrays is essential, it does not depend absolutely on the presence of existing leaf primordia.

Surgical experiments have suggested that the site of leaf initiation and the determinate nature of the leaf (i.e., its limited potential for growth) are both established during the plastochron preceding its emergence from the apex. Vertical incisions bisecting prospective leaf sites on the apical meristem of *Lupinus* caused the displacement of leaves initiating from those sites only if they were made more than one half plastochron before emergence; after this time, leaf initiation sites were apparently fixed (Snow and Snow, 1933). Tangential incisions isolating prospective leaf sites on the apical meristems of *Solanum* and *Epilobium* during the plastochron prior to emergence resulted in the production of determinate lateral organs with abnormal "centric" morphology, suggesting that cells in this region of the apex had already lost their potential to develop as primary meristem but were not yet completely determined to form a dorsiventral leaf (Sussex, 1955; Snow and Snow, 1959). Thus, at the same time that a new pattern of cell division and growth begins within a group of leaf founder cells on the flank of the shoot apical meristem, these cells apparently become committed in some sense to the production of a determinate lateral organ, although the final fate of the organ may not be determined at that time.

Very little is known about the mechanisms that bring about these coordinated changes within leaf founder cells on the flanks of the shoot apical meristem. A number of mutants have been described that fail to initiate leaves, but all of these mutants have defects in the formation or maintenance of the shoot apical meristem itself (Caruso, 1968; Clark and Sheridan, 1986, 1988; Sheridan and Thorstenson, 1986; Mayer et al., 1991). The genes identified by these mutants may thus provide valuable insights into the control of meristem formation and maintenance, but they are not directly implicated in the control of leaf initiation. An unexpected clue to the genetic control of leaf initiation has come from the analysis of the expression pattern of the maize gene *KNOTTED-1* (*KN1*), which is known for the effects of its dominant mutant alleles on leaf development (Gelinas et al., 1969; Freeling and Hake, 1985). In wild-type plants, the *KN1* gene product is present in nuclei of vegetative and floral shoot apical meristems, as well as in other relatively undifferentiated cells in the shoot apex region, but is undetectable in leaf and floral organ primordia. Interestingly, KN1 protein is also absent from leaf founder cells on the flank of the vegetative apical meristem prior to the emergence of a leaf primordium, as illustrated in Figure 3 (Smith et al., 1992). To our knowledge, *KN1* is the first gene whose expression is correlated with the earliest known events in leaf initiation.

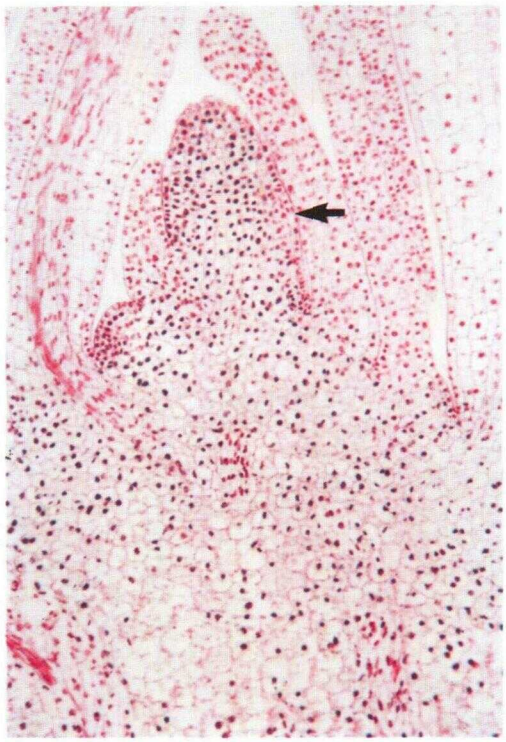

Figure 3. Median Longitudinal Section Through the Shoot Apex of a Vegetative Maize Seedling Labeled with Antibody to KN1 Protein.

Labeled nuclei are black; unlabeled nuclei are pink. KN1 protein is present in nuclei throughout the apical meristem as well as the underlying ground meristem but is absent from leaf primordia and from the leaf founder cells on the flank of the meristem in the position where the next leaf will be initiated (arrow).
Figure reprinted from Smith et al. (1992).

It is possible that the downregulation of *KN1* at leaf initiation sites in the apical meristem is important for the coordinated changes in cell division, growth, and developmental potential that occur within leaf founder cells during the plastochron prior to emergence of a leaf primordium. Indeed, the idea that the *KN1* gene product opposes the determination process is supported by the observation that its ectopic expression in developing mutant leaves apparently interferes with the determination of cell fates (Smith et al., 1992; also discussed in the final section of this review).

PHYLLOTAXIS

How are leaf initiation patterns, i.e., phyllotaxis, controlled? A number of different phyllotactic patterns are commonly found among angiosperms. Leaves can be initiated singly, separated by 180° ("distichous"), or in a spiral pattern in which they are separated by angles approaching the "Fibonnaci angle" of

137.5°. Whorled patterns result from the simultaneous initiation of two or more leaves. For example, in the "decussate" whorled arrangement, an opposite pair of leaves is initiated simultaneously that is offset by 90° from the previous pair. Several models have been proposed to explain how sites of leaf initiation are determined. Relatively recent reviews of phyllotaxis have described these models and the relevant evidence in depth (for example, Erickson, 1983; Schwabe, 1984; Jean, 1989); some of these ideas will be described here only briefly.

Classic surgical experiments performed over 40 years ago established that the initiation of leaves by the shoot apical meristem does not depend on the presence of existing leaf primordia or other, more mature tissues, but that existing primordia can influence the positions of new primordia (Snow and Snow, 1931, 1933, 1935; Ball, 1951). Because these surgical manipulations could have changed the space available for leaf formation on the apical meristem, these and other experiments have led some authors to adopt the view proposed as early as 1907 (Iterson, 1907) that a leaf primordium is centered on the first space within the organogenic region on the flanks of the apical meristem that becomes available as the shoot apex grows (Snow and Snow, 1962; Sachs, 1991). An alternative interpretation of the surgical studies is that the incisions interrupted the transmission of substances inhibitory to leaf initiation that are produced by existing leaf primordia. This interpretation is consistent with models proposing that a morphogenetic field exists within the shoot apex that favors the initiation of leaves at points most distant from existing leaf primordia (for example, Richards, 1951; Thornley, 1975; Mitchison, 1977; Steeves and Sussex, 1989).

Although the application of plant hormones and hormone synthesis or transport inhibitors to the shoot apex has in several cases been reported to produce stable changes in phyllotactic patterns, these treatments also altered the growth of the shoot apex itself, and a direct role for any hormones in the suppression or stimulation of leaf initiation cannot be deduced (Schwabe, 1971; Maksymowych and Erickson, 1977; Meicenheimer, 1981; Marc and Hackett, 1991). Careful analyses of the effects of hormone or antagonist treatments on the growth of the shoot apex have suggested that the critical factor in the phyllotactic switch is not the amount of space on the apical dome but rather the rate of radial and vertical displacement of existing primordia from the leaf-forming region of the meristem (Schwabe, 1971; Meicenheimer, 1981; Marc and Hackett, 1991). The molecular basis of the apparent inhibiting effects of existing leaf primordia remains unknown.

Thus, in spite of a large body of theoretical and experimental work, the problem of how sites of leaf initiation are determined is still largely unsolved. Mutations altering phyllotaxis may contribute insights into this problem in the future. For example, the "ABPHYL syndrome" in maize changes the normally distichous phyllotactic pattern to a decussate or spiral pattern (Greyson and Walden, 1972). The shift to a spiral leaf initiation pattern in ABPHYL plants is associated with an increase in apical meristem size (Greyson et al., 1978). Recessive *clatavata-1*, *fasciata-1*, and *fasciata-2* mutations of Arabidopsis produce irregular alterations in phyllotaxy, as well as stem fasciations and bifurcations; all three mutant phenotypes are associated with enlarged, morphologically abnormal shoot apical meristems (Leyser and Furner, 1992). These mutants again illustrate the close relationship between the geometry of the shoot apex and leaf initiation patterns. The products of the wild-type alleles of these genes may play a role in the determination of leaf initiation sites. Alternatively, these mutations may perturb phyllotactic patterns as an indirect result of their effects on the growth of the shoot apex.

DETERMINATION OF LEAF IDENTITY

Heteroblasty

The timing and mechanisms of leaf determination can be studied in heterophyllous plants producing alternative leaf forms, such as those exhibiting heteroblasty, a condition in which the juvenile phase of shoot development is distinct from the adult phase by a number of criteria, including leaf morphology (Goebel, 1900; Allsopp, 1967). Juvenile leaves are usually smaller and simpler than their adult counterparts and may differ in many other respects as well. For example, juvenile maize leaves are not only shorter and narrower than adult leaves, but they have epicuticular waxes not present on adult leaves, their epidermal cells are of different shapes than those of adult leaves, and they lack the hairs present on adult leaves (Poethig, 1990).

Although juvenile leaves were initially thought to be developmentally arrested forms of adult leaves (Goebel, 1900), comparative developmental analyses of the two leaf types in several species have demonstrated that juvenile leaf primordia are usually smaller and morphologically distinct from their adult counterparts at, or shortly after, inception (Foster, 1935; Kaplan, 1973, 1980; Franck, 1976). The transition from the juvenile to the adult phase of shoot development is also marked by the transformation of the shoot apical meristem to a larger, morphologically distinct adult form (Abbe et al., 1941; Stein and Fosket, 1969; Kaplan, 1973; Franck, 1976; Greyson et al., 1982), and this correlation has led many authors to the view that juvenile versus adult leaf identity is determined at inception by the developmental state of the meristem itself. However, the correlation between meristem size and leaf size is not universal among plants exhibiting heteroblasty. For example, in *Muehlenbeckia platyclados*, the transition from juvenile to adult shoot development is accompanied by an increase in meristem size, but adult leaves are smaller and less complex than juvenile leaves and their primordia are correspondingly smaller at inception (Bruck and Kaplan, 1980). Thus, the dependence of juvenile versus adult leaf characteristics on the apical meristem is unclear.

One approach to understanding the determination of juvenile versus adult leaf types is the analysis of a group of semidominant mutations in maize that prolong the expression of juvenile characteristics throughout shoot development (Poethig, 1988a). All of these mutations display a "whole plant"

phenotype that includes the production of leaves that are narrower than normal adult leaves and have other juvenile characteristics, the production of a larger number of vegetative nodes, and the development of leaflike structures in the inflorescences. These mutations may cause abnormal expression of genes that are normally involved in the regulation of juvenile development. Interestingly, the *Teopod-1* (*Tp1*) mutant phenotype is not cell autonomous in genetic mosaics. Thus, *Tp1* apparently controls the production or distribution of a diffusible substance conditioning juvenile traits (Poethig, 1988b).

Do *Teopod* mutations alter the development of adult leaves by acting at the shoot apex, or do they act directly on developing leaves? This question has been addressed recently by a clonal analysis of cell lineages in *Tp2* plants (Dudley and Poethig, 1991). The results showed that the *Tp2* mutation does not alter the extent and distribution of clonal sectors within the first 16 nodes of the plant that arose from single cells in the embryonic shoot apical meristem. The apparent number of embryonic meristem cells giving rise to each leaf–internode unit (phytomer) is also unaltered. These results indicate that the *Tp2* mutation does not prolong the expression of juvenile characteristics in the leaf by altering patterns of growth and cell division within the shoot apical meristem. In particular, *Tp2* leaves are not narrower simply because they are initiated by a smaller number of leaf founder cells. Bassiri et al. (1992) have shown that *Tp2* mutant shoot apices are smaller (more juvenile-like) than wild-type apices, but only from the 12th plastochron onward. The effects of *Tp2* are evident in leaves initiated earlier than the 12th plastochron and thus cannot be the consequence of the effects of *Tp2* on meristem size. Both of these results support the conclusion that the *Tp2* mutation acts on the apical meristem to delay its increase in size and the transition to floral development, and also on leaf primordia after initiation to promote the development of juvenile leaf characteristics.

Gibberellic acid (GA) has been implicated as an important regulator of juvenile development in woody angiosperms and thus in the determination of juvenile leaf characteristics (reviewed by Hackett, 1985; Zimmerman et al., 1985). In English ivy (*Hedera helix*), for example, juvenile shoot tips contain higher levels of gibberellin-like substances than their adult counterparts (Frydman and Waering, 1973), and treatment of adult shoots with GA causes a reversion to juvenile development (Robbins, 1957; Rogler and Hackett, 1975; Wallerstein and Hackett, 1989). However, inhibitors of gibberellin biosynthesis do not usually cause premature maturation of juvenile shoots, and it is likely that a reduction in endogenous gibberellin levels is necessary but not sufficient for the transition to adult shoot development in woody angiosperms (Hackett, 1985). Feldman and Cutter (1970a, 1970b) addressed the question of whether GA_3 influences juvenile leaf characteristics in yellow star thistle (*Centaurea solistitialis*) indirectly via its effects on the shoot apex, or directly by acting on leaf primordia. They found that GA_3 treatment of adult shoots produces smaller meristems and smaller, simpler leaves characteristic of juvenile shoots. Furthermore, leaf primordia excised from adult shoot apices at plastochrons 5 through 10 (i.e., the fifth through tenth leaf primordia from the apical dome) are directly influenced by GA_3 to develop more juvenile-like morphology in culture. These experiments demonstrate that adult leaf characteristics were not irreversibly determined at the time of explantation by the adult state of the meristem and that GA_3 can act directly on the leaf even at relatively late stages of leaf development to induce juvenile characteristics.

In summary, the determination of juvenile and adult leaf characteristics is subject to both genetic and hormonal influences. Investigation of these influences has suggested that the determination of juvenile or adult identity is not complete at the earliest stages of leaf development, despite the early morphological divergence of the two leaf types that has been described for several species.

Environmentally Induced Heterophylly

Heterophyllous aquatic plants, which produce leaves of strikingly different morphology and anatomy depending on whether the shoot apex is in an aerial environment or submerged underwater, provide another opportunity to investigate the timing and mechanisms of leaf determination. In contrast to the age-related differences in shoot development described above, shoot apical meristems and young leaf primordia (200 to 600 μm in length) of submerged shoots are morphologically indistinguishable from those of aerial shoots for several different species (Jones, 1955; Bostrack and Millington, 1962; England and Tolbert, 1964; Schmidt and Millington, 1968; Deschamp and Cooke, 1985; Goliber and Feldman, 1990). Environmental shift experiments have demonstrated clearly that the determination of leaf type in these species is a gradual process that begins at about the same time their morphological divergence begins but is not complete until relatively late in organ development. Leaf primordia shifted during the time interval within which determination occurs develop intermediate characteristics (McCully and Dale, 1961; Bostrack and Millington, 1962; Schmidt and Millington, 1968; Deschamp and Cooke, 1984). For example, submerged and aerial leaf types of *Callitriche heterophylla* begin to diverge morphologically when they reach the 400-μm stage (Deschamp and Cooke, 1985). Leaves shifted before the 500-μm stage develop entirely according to their new environment, whereas those shifted at the 500-μm stage or later develop intermediate characteristics. Developing leaves do not completely lose the ability to respond to the environmental shift until they are almost fully expanded (Deschamp and Cooke, 1984).

Abscisic acid (ABA) apparently plays an important role in the determination of leaf identity in heterophyllic aquatic species (reviewed in Goliber, 1989a). ABA treatment of submerged shoots induces aerial leaf development in several species, thus mimicking the effect of shifting to an aerial environment (Anderson, 1978; Mohan Ram and Rao, 1982; Deschamp and Cooke, 1984; Young and Horton, 1985; Kane and Albert, 1987). For example, in a detailed series of studies on the effects of ABA on leaf development in the aquatic buttercup, Young and colleagues have shown that ABA-treated submerged leaves

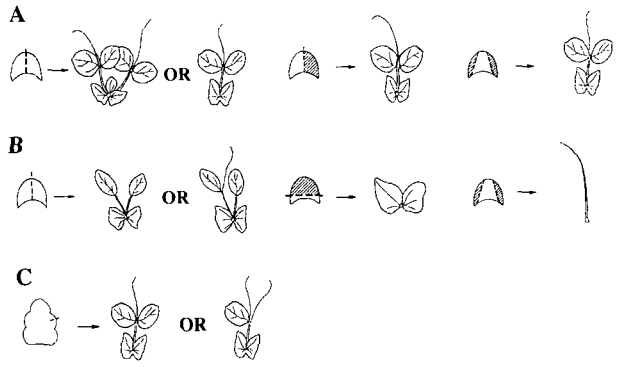

Figure 4. Regeneration Experiments on Early Pea Leaf Primordia.

(A) Results of surgical manipulations performed on leaf primordia early in plastochron 1 when the next oldest primordium was about 70 μm long. **(B)** Results of surgical manipulations performed on leaf primordia late in plastochron 1 when the leaf primordium was about 70 μm long. **(C)** Results of surgical manipulations performed on leaflet primordia early in plastochron 2 when the leaf primordium was about 100 μm long. Figure adapted from Sachs (1969).

are indistinguishable from aerial leaves both morphologically and anatomically, although ultrastructural analysis revealed minor differences (Young and Horton, 1985; Young et al., 1987, 1990). Consistent with the hypothesis that endogenous ABA regulates aerial leaf development, ABA was found to be present in aerial shoots but undetectable in submerged shoots of mare's tail (*Hippuris vulgaris*; Goliber and Feldman, 1989). Aerial leaf development in mare's tail can also be induced in submerged shoots by high light levels and high osmolarity, conditions that also cause an increase in endogenous ABA levels within submerged shoots (Goliber, 1989b; Goliber and Feldman, 1989). It is thought that when a shoot emerges from water into the air, the resulting osmotic stress and higher light levels induce ABA production, which then regulates the development of aerial leaves (Anderson, 1978; Goliber, 1989a).

Many authors have proposed that leaf determination can be separated into an early phase (coinciding approximately with organ initiation) in which the basic identity of the leaf as a whole is determined (leaf versus petal, for example) and a later phase in which the details of its morphology and anatomy are determined (aerial versus submerged leaves, for example). This idea is contradicted directly by recent studies on the determination of lateral organs in garden balsam (*Impatiens balsamina*). When vegetative shoots of this species are partially induced to flower by brief exposure to inductive short days, lateral organ primordia of the smaller size and phyllotactic arrangement characteristic of petals are initiated, but when the shoots are returned to long-day conditions, these primordia develop into leaves. Thus, although these organs apparently begin their development as petals, they are not irreversibly determined as petals at the time the plant is shifted back to long days (Battey and Lyndon, 1984). Daylength shift experiments demonstrated that the identity of these lateral organs remains sensitive to shifts in daylength until they reach the 750-μm stage; up to this stage, shifts in daylength result in the differentiation of mosaic organs with patches of both petal and leaf tissue (Battey and Lyndon, 1988). Although *Impatiens balsamina* may be a special case, these studies suggest the general conclusion that determination of leaf identity is not an early event that is separable from the determination of leaf morphology and anatomy. Rather, all characteristics that give a lateral organ its unique identity appear to be subject to a gradual determination process that occurs progressively during early stages of organ development.

DETERMINATION OF REGIONAL IDENTITY WITHIN THE LEAF

Leaflets and Tendrils in the Pea Leaf

The compound leaf of pea provides an excellent system to study the timing and mechanisms of determination of regional identity within the leaf. A normal pea leaf is composed of a pair of basal stipules and a rachis bearing two or more pairs of lateral leaflets, two or more pairs of tendrils, and a terminal tendril (see Figure 5A below). A surgical study (Sachs, 1969) suggests that the morphology of the pea leaf is determined progressively during early stages of leaf development. As illustrated in Figures 4A and 4B, bisection or surgical removal of portions of the leaf primordium early in the first plastochron of pea leaf development (before the 30-μm stage) leads to the regeneration of leaves with normal morphology, but by the end of plastochron 1 (the 70-μm stage), leaf primordia have lost this ability to regenerate. However, leaflet primordia cut shortly after their initiation, when the leaf primordium is at the 100-μm stage (early in plastochron 2), most often regenerate normally (Figure 4C). This study suggests that the overall architecture of the pea leaf is determined during plastochron 1 but that the morphology of leaflets is determined later, during plastochron 2.

A fascinating collection of pea leaf mutants demonstrates that the determination of leaflet and tendril identity in pea leaves is under genetic control (Marx, 1977, 1987). Plants homozygous for the recessive *afila* (*af*) mutation produce no lateral leaflets but, instead, produce a highly branched system of lateral tendrils, as shown in Figure 5B. In contrast, homozygous *tendrilless* (*tl*) mutants produce leaflet pairs instead of tendrils (Figure 5C). *Tendrilled acacia* homozygous recessive mutants produce a distal pair of subterminal tendrils but a terminal leaflet instead of a terminal tendril (not shown). Double mutants homozygous for both *af* and *tl* have a novel phenotype consisting of a highly branched system of very small leaflets and no tendrils (Figure 5D). Studies of the developmental morphology of *af* and *tl* mutants have shown that no morphological differences from wild-type plants are observed in their

shoot apical meristems or in leaf primordia during the first two plastochrons, but differences in the morphology of lateral primordia become apparent in the *af* mutant during plastochron 3 and in the *tl* mutant during plastochron 6 (Meicenheimer et al., 1983; Gould et al., 1986). Thus, the wild-type *AF* gene must begin to function no later than plastochron 3, and the *TL* gene no later than plastochron 6, to influence leaflet and tendril identity; the surgical study described earlier suggests that their functions are probably critical even earlier, during plastochron 2.

Young (1983) has proposed that the identity of lateral appendages in the pea leaf is determined by primordial size at a critical stage of development; primordia below the size threshold would develop as tendrils and those above it as leaflets. Consistent with this model, surgical cuts bisecting or removing portions of lateral leaflet primordia during plastochron 2 sometimes cause a tendril to form instead of a leaflet (Figure 4C; Sachs, 1969). Young (1983) hypothesizes that the *af* and *tl* mutations alter the critical thresholds rather than altering primordial size at the critical time, a proposal consistent with the lack of morphological differences between mutant and wild-type leaf primordia during the first two plastochrons of pea leaf development (Meicenheimer et al., 1983; Gould et al., 1986). Molecular analysis of these genes and their products would likely provide invaluable information concerning the control of regional identities within the pea leaf.

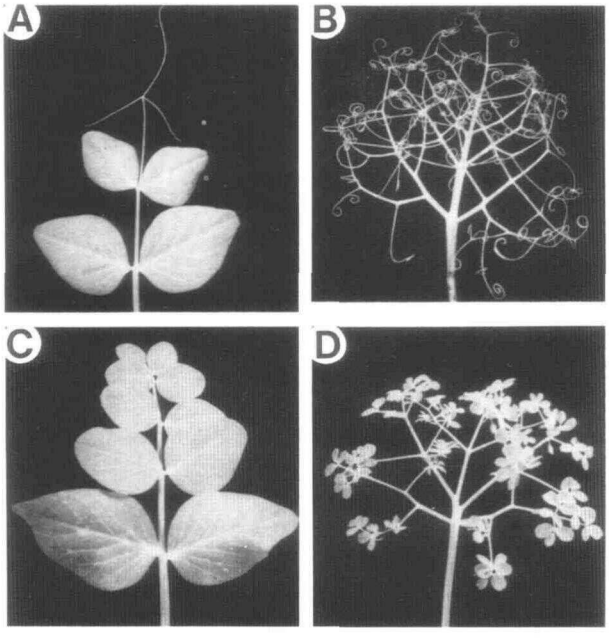

Figure 5. Wild-Type and Mutant Pea Leaves.

(A) Wild-type leaves.
(B) *af/af*, "*afila*" mutant.
(C) *tl/tl*, "*tendrilless*" mutant.
(D) *af/af tl/tl*, "*parsley-leaved*" mutant.
Figure reprinted from Young (1983).

Sheath and Blade Domains of the Maize Leaf

The maize leaf also provides an opportunity to study the determination of regional identities. It is composed of a basal sheath domain and a distal blade domain, separated by an auricle and a ligule, a flap of epidermally derived tissue located on the adaxial surface of the leaf. Sheath and blade domains of the maize leaf are quite distinct, differing in many respects such as venation pattern, internal anatomy, epidermal cell shape, and epidermal hair patterns (Russell and Evert, 1985; Sylvester et al., 1990). A recent developmental scanning electron microscopy analysis of the leaf surface has shown that the divergence in sheath and blade surface characteristics (cell size and shape), as well as the demarcation of these two domains by a distinctive "pre-ligular band," first becomes evident at plastochron 3, suggesting that sheath and blade domains might be determined by this time (Sylvester et al., 1990).

Several dominant mutations have been described that alter determination patterns within the maize leaf, causing sheath and auricle characteristics to develop in a variety of patterns within the blade, as illustrated in Figure 6 (Hake et al., 1985; Freeling et al., 1992). The most extensively characterized of these are the *Knotted-1* (*Kn1*) mutations, whose effects are focused on lateral (major) veins, altering cell division and differentiation in all layers of the leaf (Gelinas et al., 1969; Freeling and Hake, 1985). The *KN1* locus was cloned by transposon tagging (Hake et al., 1989), and the cDNA sequence revealed that *KN1* encodes a homeodomain protein, a class of transcriptional regulators associated with the control of animal development (Vollbrecht et al., 1991). All dominant *Kn1* mutations map to noncoding portions of the gene and appear to alter its regulation (reviewed by Hake, 1992).

As described earlier, analysis of *KN1* expression revealed that in wild-type plants, *KN1* is expressed at high levels in vegetative and floral shoot apices. Whereas KN1 protein is undetectable immunohistochemically at all stages of wild-type leaf development, it is localized ectopically in developing lateral veins of *Kn1-N2* mutant blades from plastochron 5 onward (Smith et al., 1992). Previous analysis of genetic mosaics demonstrated that the *Kn1-N2* mutation acts in the innermost tissue layer of the leaf, which consists primarily of vascular bundles (Sinha and Hake, 1990), strongly supporting the conclusion that this ectopic expression is the cause of the mutant phenotype. Thus, although there is no evidence that the *KN1* gene product normally plays a role in the determination of sheath characteristics, its ectopic expression in the developing blade apparently results in the acquisition of sheath characteristics. We have proposed that *KN1* delays the progression of cells in the blade to their final fates, causing them to respond to sheath-determining signals they would not normally perceive. It is perhaps surprising that ectopic expression of *KN1* beginning in plastochron 5 can have this effect even though the development of sheath and blade domains starts to diverge at least as early as plastochron 3. This result seems to illustrate once again a plasticity in the determination of leaf

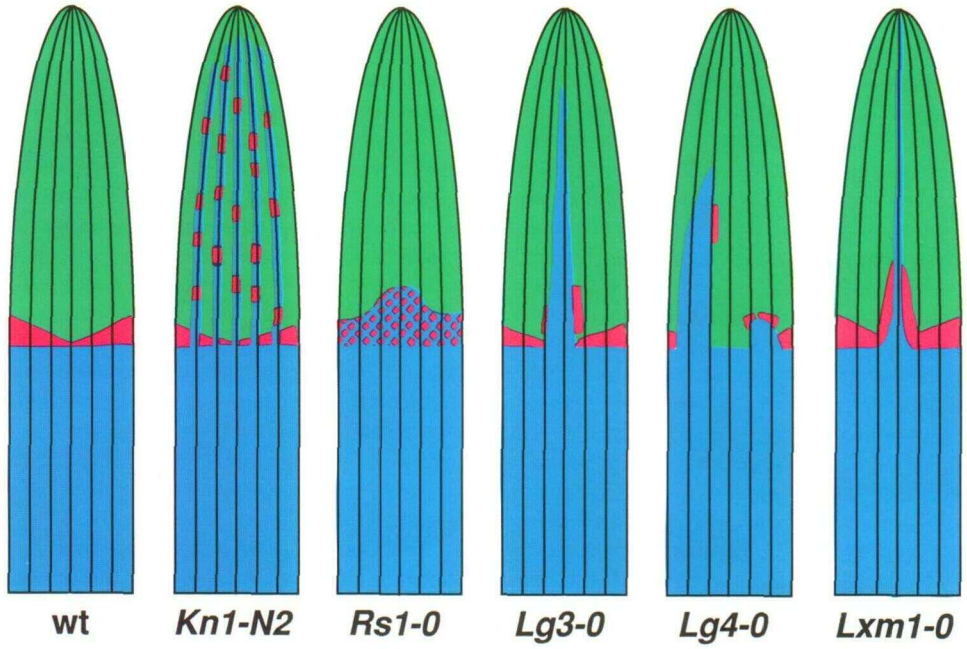

Figure 6. Adult Leaves of Wild-Type Maize and Five Dominant Mutants Illustrating Altered Patterns of Blade, Sheath, and Auricle Development in the Leaf.

Blue, sheath tissue; green, blade tissue; pink, auricle tissue. wt, wild type; *Kn1-N2*, *KNOTTED-1* mutant; *Rs1-0*, *ROUGH SHEATH–1* mutant; *Lg3-0*, *LIGULELESS-3* mutant; *Lg4-0*, *LIGULELESS-4* mutant; *Lxm1-0*, *LAX MIDRIB-1*, mutant.
Figure adapted from Freeling et al. (1992).

characteristics persisting throughout the early stages of leaf development. The *ROUGH SHEATH–1* (P. Becraft, S. Hake, and M. Freeling, unpublished data) and *LIGULELESS-3* (J. Fowler and M. Freeling, unpublished data) genes, whose dominant mutant phenotypes are related to the *Kn1* mutant phenotype, producing sheath or auricle-like characteristics in the blade (Figure 6), have also been found to encode homeodomain proteins. It will be interesting to learn whether the developmental basis of these mutant phenotypes is similar as well.

CONCLUDING REMARKS

The development of a leaf begins with a regional shift in the polarity and rate of cell division and expansion within a group of leaf founder cells on the flank of the shoot apical meristem. How leaf initiation is stimulated to occur in a precise, repeated pattern as the apical meristem grows remains largely unknown. The determination of a leaf is a progressive process that is subject to both genetic and hormonal influences. Although the process may begin within the shoot apical meristem with the acquisition of determinate growth potential, it is not clear that any of the final characteristics of a leaf are completely determined within the meristem. Rather, the determination process apparently continues through early stages of leaf development as the final fate of the leaf becomes gradually more restricted. In this respect, the determination of leaves is comparable to the determination of floral organs, a process regulated by the coordinated activities of homeotic genes. For example, the wild-type Antirrhinum *DEFICIENS* (*DEF*) gene, and its Arabidopsis homolog, *APETALA-3* (*AP3*), are required for the determination of petal and stamen identity. In the absence of wild-type gene activity, sepals develop in the second whorl in place of petals, and carpels develop in the third whorl in place of stamens (Bowman et al., 1989; Schwarz-Sommer et al., 1990). When wild-type *DEF* gene activity is restored just prior to the last few cell divisions in mutant second whorl organs by transposon excision from an insertional *def* allele, a clone of cells with petal characteristics forms within an otherwise sepal-like organ (Carpenter and Coen, 1990). Likewise, temperature shifts throughout early stages of flower development affect the development of carpel versus stamen characteristics in temperature-sensitive *ap3* mutant third whorl organs (Bowman et al., 1989). Thus, although *DEF* and *AP3* are activated in petal and stamen primordia at the time of their initiation by the floral apical meristem (Jack et al., 1992; Schwarz-Sommer et al., 1992), these genes must act to direct the development of petal and stamen characteristics for an extended period, not completing their function in organ determination until relatively late

in organ development. These and other studies illustrate the power of genetics as a tool for the analysis of floral development. Likewise, the addition of genetics to the collection of tools that can be used to dissect leaf development has already provided new insights and holds promise for the future.

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