The Plant Cell, Vol. 5, 1157–1165, October 1993 © 1993 American Society of Plant Physiologists

Determination and Cell Interactions in Reproductive Meristems

Eva Huala[1] and Ian M. Sussex

Department of Plant Biology, University of California, Berkeley, California 94720

INTRODUCTION

The plant body develops from groups of dividing cells, the meristems, that function as permanent stem cell populations and enable the plant to increase in size continuously throughout its lifetime. Although all meristems function as sources of new cells for differentiation, each type of meristem is capable of producing only certain structures; for example, the shoot meristem gives rise to leaves, axillary buds, internodes, and flowers, whereas the root meristem produces only roots. The choice between shoot and root identity occurs very early in the establishment of a new meristem (Christianson and Warnick, 1983), but how a particular meristem acquires and maintains its identity is unknown. Although meristem identity can be stable for long periods of time, it is capable of changing in some circumstances. One easily observed change in meristem identity occurs when plants shift from vegetative to reproductive growth. Before this transition, cells derived from the shoot meristem differentiate as leaves, internodes, and axillary buds. After the transition to reproductive growth, floral bracts, inflorescence branches, and flowers are formed. By studying the transition from vegetative to reproductive growth, we hope to understand how a meristem acquires and stably propagates an identity and what role a meristem plays in the developmental fate of its derivative cells.

DETERMINATION OF MERISTEM IDENTITY

Determination can be defined experimentally as a change in developmental fate that is induced in response to a set of conditions and that persists when those conditions no longer exist. If conditions required for a change in fate can be removed without causing reversion, then determination has occurred. Conditions that may be required for the determination of an inflorescence meristem include those operating within a plant, such as the age of the plant or the number of vegetative nodes, and external conditions, such as an appropriate daylength or temperature regime. Species that flower in response to environmental cues are easily tested for determination by removal of the required environmental stimulus. In most cases, transfer to noninductive conditions does not cause reversion to vegetative growth, indicating that the plant is stably determined for reproductive development. Species that flower in response to endogenous signals can be tested for determination by physical separation of the terminal or axillary buds from the mature leaves and the roots, which can be sources of signals to flower or to remain vegetative (see Bernier et al., 1993, this issue). The excised buds can then be grown in culture or grafted to a vegetative plant to test whether the bud was determined for reproductive development at the time of its removal from the original plant.

Determination for reproductive development is a widespread phenomenon that occurs in both environmentally responsive and unresponsive species (McDaniel et al., 1992). In a day-neutral cultivar of tobacco, the shoot apical meristem normally produces a uniform number of vegetative nodes before flowering. However, if the apical bud is excised before it becomes determined and is allowed to form new roots, it will not become determined for reproductive development but will continue to produce vegetative nodes. To assess at what point in its development the tobacco shoot apex becomes determined to form a terminal flower, Singer and McDaniel (1986) excised and rooted apical buds from plants of different ages and measured the number of vegetative nodes they produced after rooting. Apical buds that were excised before the plant had produced ~37 of 41 vegetative nodes produced new plants with approximately the same number of vegetative nodes as seed-derived plants, whereas apical buds excised and rooted just after this point formed approximately four vegetative nodes before flowering (Singer and McDaniel, 1986; McDaniel et al., 1987). These studies suggest that determination of the tobacco apical bud to form a terminal flower occurs just before the last four leaves are initiated. However, determination may occur much earlier in sunflower (Haberman and Sekulow, 1972).

In some species that flower in response to a photoperiodic signal, determination in response to an inductive photoperiod can be quite rapid. In the long-day plant *Lolium temulentum*, a single inductive long day followed by a return to noninductive short days is sufficient to induce flowering (Evans, 1958). The floral stimulus is produced in the leaves in response to the inductive photoperiod. Removal of the leaves at various times after induction has demonstrated that by 6 hr after the

[1] To whom correspondence should be addressed.

minimum inductive photoperiod of 16 hr, enough of the floral stimulus has been exported from the leaves to bring about flowering (Evans and Wardlaw, 1966; McDaniel et al., 1991). Similarly, excision and culturing of apices at various times after induction has shown that 10 hr after the minimum inductive photoperiod, approximately half of the apices tested have received enough of the floral stimulus to differentiate as inflorescences in culture.

Determination Is a Property of Individual Buds Rather than Whole Plants

Plants generally continue to flower even if the conditions required to initiate flowering are removed. However, there are a few examples of plants that will return to vegetative growth after beginning inflorescence development (Battey and Lyndon, 1990). These can be subdivided into cases of inflorescence or floral reversion and cases of partial flowering. In inflorescence or floral reversion, an individual meristem begins to develop in an inflorescence or floral pattern and then reverts to vegetative development. An extreme example of floral reversion is found in *Impatiens balsamina*, in which reversion to vegetative growth can occur at any stage of flower development if the plants are shifted from inductive short days to noninductive long days (Krishnamoorthy and Nanda, 1968; Battey and Lyndon, 1990). Flowers of *Impatiens* are capable of reverting even after carpels have been initiated and have begun to form ovules, resulting in the outgrowth of a vegetative shoot that originates from inside the carpels. Although most species will not revert to vegetative growth after they have been fully induced, in many cases, some degree of inflorescence or floral reversion has been observed following a suboptimal induction (Battey and Lyndon, 1990).

In partial flowering, some meristems that would otherwise form inflorescences or flowers develop vegetatively, but no individual meristems revert to vegetative growth. Partial flowering is observed in many photoperiodic species that are exposed to a suboptimal number of inductive photoperiods or to photoperiods of suboptimal length for induction and are then returned to noninductive conditions. In some cases, the terminal bud or the youngest axillary buds show no evidence of determination following a suboptimal induction, but slightly older axillary buds respond to the induction by developing as flowers. This has been observed in the obligate short-day plant *Pharbitis nil* (Japanese morning glory) after exposure to a single inductive 14-hr dark period (Larkin et al., 1990). Terminal buds that included six leaf primordia were excised and grafted onto uninduced plants at various times after the end of the dark period, and the occurrence of flowers at axillary and terminal positions on the graft was recorded. The axillary buds situated at nodes 1 and 2 (numbered from the base of the plant), which had already begun to initiate leaf primordia at the time of induction, were unresponsive to the inductive signal and continued to develop vegetatively. The majority of the axillary buds located at nodes 3 and 4 were determined within 1 to 2 hr after the end of the inductive dark period, whereas the apical bud and the axillary buds located at nodes 5 and 6 were not determined in the majority of plants until 4 to 8 hr after induction. Similarly, in *Glycine max* (soybean), only axillary buds at a particular stage of development will develop as flowers in response to a suboptimal induction, whereas buds that are either older or younger fail to respond to the induction and continue to develop vegetatively (Borthwick and Parker, 1938).

In contrast to the examples above, in other plant species the apex may become determined to form a terminal flower, while the axillary buds retain the ability to develop as vegetative shoots. In *Kalanchöe blossfeldiana*, plants given a suboptimal inductive signal can form a terminal flower, yet previously initiated axillary buds develop as vegetative shoots rather than as inflorescence branches (Harder, 1948). Smith and McDaniel (1992) found that in both the day-neutral tobacco cultivar Hicks and the short-day mutant Hicks *maryland mammoth*, ~30% of the inflorescences produced by rooted axillary buds form a terminal flower but develop vegetative shoots in place of inflorescence branches when assayed in noninductive long days. Determination of the axillary buds occurs ~5 to 9 days later than determination of the terminal bud in day-neutral tobacco when the developmental state is assayed by rooting (Singer and McDaniel, 1986).

Determination of Inflorescence Meristems and Flower Primordia Are Separate Events

Some evidence suggests that the primordia initiated by the apical meristem of a florally determined bud are determined in a separate event after, rather than at, the time of initiation. A study of the development of the maize tassel has demonstrated that determination of the shoot apex and its derivatives can be experimentally separated (Irish and Nelson, 1991). Maize shoot tips consisting of an apical meristem with one or two attached leaf primordia were cultured at different stages of growth, spanning initiation of the last five leaves, of the tassel branches, of the spikelet pairs, of the spikelets, of the florets, and of the floral organs. Determination of the apex occurred at approximately the time of the initiation of the last leaf primordium. Apices cultured before this stage reiterated the vegetative portion of the plant before forming a tassel, whereas apices cultured slightly later formed determinate structures without lateral organs or with vegetative shoots in place of tassel branch primordia and spikelet pairs. Although the apex lost the ability to continue vegetative growth and became committed to form a determinate structure, the spikelet primordia initiated after determination of the apex were still capable of developing as vegetative shoots, demonstrating that a determined apex does not always give rise to determined derivatives. Spikelet primordia initiated by apices cultured at a still later stage were committed to reproductive development. This experiment

suggests that establishment of inflorescence meristem identity and flower primordium identity are separate events that occur sequentially in the maize tassel. Determined buds of *Nicotiana sylvestris* can also produce undetermined derivatives. Rooted axillary buds of *N. sylvestris* taken from the three nodes immediately below the inflorescence are determined to make a terminal flower, but axillary buds derived from the meristem of this determined bud develop vegetatively (Dennin and McDaniel, 1985).

If the primordia initiated by the meristem of a florally determined apex are capable of developing as vegetative structures, then it becomes necessary to measure the determination of an inflorescence meristem by characteristics other than the identity of its derivatives. In the case of the cultured maize apex, determination was apparent in the loss of its ability to reiterate the vegetative growth pattern, a change in the size and shape of the meristem, the initiation of a limited number of nodes, and the arrangement of derivative primordia in the pattern typical of a tassel. It may therefore be necessary to define an inflorescence meristem by its phyllotaxy, by a determinate growth pattern, or by the formation of a terminal flower rather than by a shift in the identity of the primordia it produces, because conditions may exist that cause the determination of the shoot apical meristem but not its derivatives.

Determination of Flower Primordia and Floral Organ Primordia Are Separate Events

The distinction between the identity of a meristem and the identities of its derivatives may be seen most clearly in the case of the flower primordium itself. The identity of the organs initiated by a flower primordium can be altered by genetic or environmental manipulations without greatly affecting the functioning of the floral meristem, as indicated by the whorled arrangement of the floral organs. Mutations in several different genes, including *APETALA2*, *AGAMOUS* (*AG*), *PISTILLATA* (*PI*), *APETALA3*, and *SUPERMAN*, alter floral organ identity in Arabidopsis (Komaki et al., 1988; Bowman et al., 1989, 1992; see Coen and Carpenter, 1993, this issue; Okamuro et al., 1993, this issue). Although floral organ identity is altered in each of these mutants, the whorled arrangement of floral organs and the shortened internodes typical of flowers are maintained. In addition, reversion of floral organs to leaflike organs without affecting their arrangement can be accomplished in some species by transferring plants from inductive to noninductive conditions (Krishnamoorthy and Nanda, 1968; Fisher, 1972; Battey and Lyndon, 1990).

Other evidence indicates that at least for some epidermal cell types, organ identity is not fixed until quite late in development of the floral organ. Mutations in the *deficiens* (*def*) gene of Antirrhinum cause the development of sepal-like organs rather than petals in the second whorl and carpels rather than stamens in the third whorl (Carpenter and Coen, 1990; Schwarz-Sommer et al., 1990). Patches of petal epidermal cells caused by excision of a transposable element from the *def* gene are sometimes seen in the second whorl floral organs of plants carrying an unstable *def* allele. These patches, which have sharp boundaries and are surrounded by normal sepal epidermal cells, can consist of as few as four cells, suggesting that the identity of these epidermal cells is not fixed until as late as the last rounds of cell division (Carpenter and Coen, 1990).

Location of the Cells Determined for Reproductive Development

Culturing and grafting experiments have demonstrated that at least some of the determined cells in a plant are located in terminal and axillary buds that contain the apical meristem and several young leaves (Wetmore et al., 1959; Haberman and Sekulow, 1972; Dennin and McDaniel, 1985; Singer and McDaniel, 1986; McDaniel et al., 1987; Larkin et al., 1990; Smith and McDaniel, 1992). Determination to form a tassel has been demonstrated in isolated maize apices consisting of the meristem and only one or two leaf primordia (Irish and Nelson, 1991). In another experiment, axillary meristems from the third node from the base of *Pisum sativum* (pea) were cultured without any leaf primordia but with 0.2 to 0.4 mm of subjacent tissue. These isolated meristems produced callus that was capable of regenerating florally determined shoots that flowered after 4 to 11 nodes rather than after the 16 to 17 vegetative nodes produced by intact plants, demonstrating that cells in the meristem itself are determined for reproductive development (Ferguson et al., 1991).

Other studies have demonstrated determination for reproductive development in the absence of an organized meristem. Determination has been demonstrated in whole stem segments of tobacco and in explants consisting of the external three to six cell layers (Aghion-Prat, 1965; Konstantinova et al., 1969; Tran Thanh Van, 1973; Singer and McDaniel, 1987). In these experiments, the ratio of florally determined shoots to vegetative shoots regenerated was highest for callus derived from the uppermost internodes of flowering plants and declined gradually with distance from the terminal flower; callus derived from the most basal internodes produced mainly vegetative shoots. In addition, the number of leaves formed before flowers were produced was lowest for explants taken from the uppermost internodes. These experiments suggest that determination occurs not only in the meristem but also in other tissues of the plant.

COORDINATION OF PATTERN IN THE INFLORESCENCE MERISTEM

The shoot apical meristem of many angiosperms consists of three cell layers, designated L1, L2, and L3, that give rise to separate cell lineages (Satina et al., 1940; Sussex, 1989). These

cell layers are distinguished by their positions and patterns of cell division. Cells of the L1 layer divide anticlinally throughout development and form the epidermis of the plant. Cells of the L2 layer also divide anticlinally within the meristem but in other planes during differentiation. Cells of the deepest layer, L3, divide in all planes. Both the L2 and L3 layers contribute to the body of the plant in proportions that vary in different organ types. The L2 layer is generally the source of the germ cells (Stewart, 1978). This layered organization, illustrated in Figure 1, persists after the transition from a vegetative meristem to an inflorescence meristem. However, in most cases, the cytological zonation present in the vegetative meristem is not apparent in the inflorescence meristem.

Although the lineages produced by each meristem layer usually contribute to distinct regions within each organ in a more or less predictable way, many instances of aberrantly oriented divisions that cause the invasion of one layer by the derivatives of another layer have been observed. The consistent differentiation of the invading cells in accordance with their new position suggests that cells in the different layers are not restricted in their developmental fate. Many other experiments have confirmed that the developmental fate of plant cells is decided by position rather than by cell lineage (McDaniel and Poethig, 1988; Jegla and Sussex, 1989; Furner and Pumpfrey, 1992; Irish and Sussex, 1992). Although the cells of each layer are not limited in their developmental potential, they may be functionally distinct. Evidence for this can be found in the different orientation of cell divisions in different layers and in the expression patterns of some genes that appear to reflect the layered organization of the meristem (Pri-Hadash et al., 1992; Shahar et al., 1992; Fleming et al., 1993; Meeks-Wagner, 1993, this issue).

The anticlinal divisions of the L1 and L2 layers of the meristem and the divisions in all planes in the L3 layer must be coordinated both in the inflorescence meristem, which maintains its size and shape over long periods, and in the flower primordium, which undergoes drastic changes in size and shape in the process of developing into a mature flower. How this is accomplished is not clear; however, general considerations suggest that some signaling process must exist that is capable of coordinately regulating cell growth in all the layers to achieve a complex multicellular pattern. Genes that participate in generating such signals are not likely to be cell autonomous, whereas genes that function to recognize and interpret signals are likely to be cell autonomous.

Evidence for signaling between cell layers can be seen in a *Camellia* chimera (*Camellia* + "Daisy Eagleson") that was generated from a *C. japonica* scion grafted onto a *C. sasanqua* stock (Stewart et al., 1972). *C. sasanqua* has single flowers with a single whorl of petals and normal stamens and carpels. The *C. japonica* cultivar used in the graft is a double-flowered form that produces sepals and many whorls of petals but no stamens or carpels. The chimera consists of an L1 layer derived from *C. sasanqua* and L2 and L3 layers derived from the double-flowered *C. japonica* cultivar. Flowers of the chimera resemble those of the *C. sasanqua* line in epidermal characteristics such as petal color, fragrance, and presence of hairs. Interestingly, although the double flowers of the *C. japonica* line never produce any stamens and carpels, possibly due to a mutation analogous to *ag* in Arabidopsis or *plena* (*ple*) in Antirrhinum, the flowers of the chimera contain both stamens and carpels, and the pollen, derived from the L2 layer, resembles that of single-flowered *C. japonica*. The ability of the chimera to form stamens and carpels, which except for the epidermis consist entirely of L2- and L3-derived cells, suggests that a signal supplied in the L1 layer is capable of moving between cell layers to correct the developmental defect in the *C. japonica*–derived L2 and L3 layers and to coordinate the growth and differentiation of the flower.

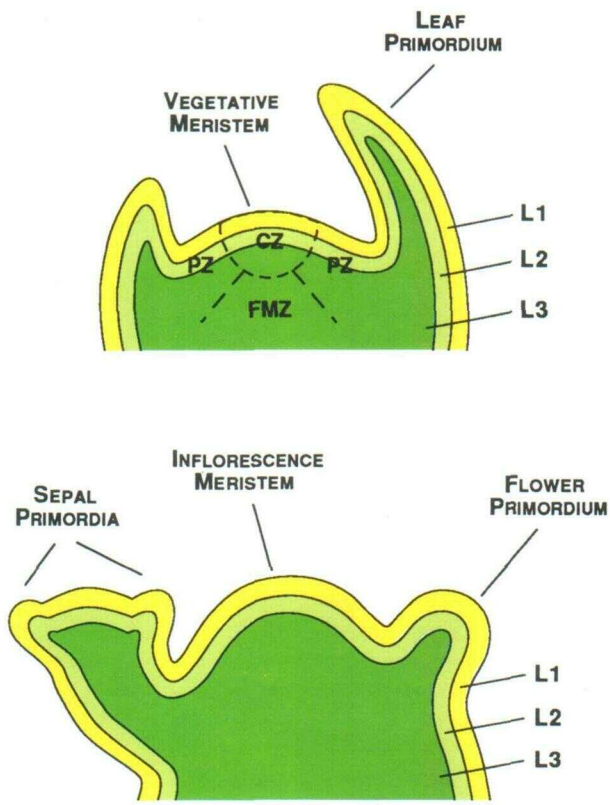

Figure 1. Organization of Vegetative and Inflorescence Shoot Apices.

CZ, central zone; PZ, peripheral zone; FMZ, file meristem zone.

Cell Layers Interact in Antirrhinum Flower Development

Genetic analysis of flower development in Antirrhinum has yielded additional information on signaling between cell layers during reproductive development. Unstable mutations caused by insertion of a transposable element are readily isolated in this plant. Reversion of such a mutation due to excision of the

transposon results in a sector of wild-type cells in an otherwise mutant plant. Such chimeras afford an opportunity for studying the interactions of adjacent cells located within a single cell layer or within different layers.

Mutations in either *def* or *globosa* (*glo*) result in Antirrhinum flowers with sepals rather than petals in the second whorl and carpels rather than stamens in the third whorl (Carpenter and Coen, 1990; Schwarz-Sommer et al., 1990). Both *def* and *glo* contain a MADS box sequence, suggesting that they may function as transcription factors (Sommer et al., 1990; Tröbner et al., 1992; Coen and Carpenter, 1993, this issue). Examination of unstable alleles of *def* and *glo* has shown that wild-type sectors of petal epidermal cells occur in the sepaloid second whorl organs (Carpenter and Coen, 1990; Schwarz-Sommer et al., 1990; Sommer et al., 1991; Tröbner et al., 1992). The cells underlying several such epidermal sectors were green and resembled sepal mesophyll cells rather than unpigmented subepidermal petal cells, suggesting that the reversion of the unstable *def* allele took place in the L1 layer (Carpenter and Coen, 1990). These epidermal sectors have sharp boundaries, suggesting that, at least for the differentiation of neighboring petal epidermal cells, the *def* and *glo* gene products are cell autonomous. However, no such wild-type sectors of stamen epidermis appear on the carpelloid third whorl organs of the unstable *def* mutant. This could be due to an earlier requirement for the *def* gene product in stamen epidermal development (Sommer et al., 1991).

In addition to the small revertant sectors discussed above, a periclinal chimera made up of both *def* and wild-type cell layers has been described (Sommer et al., 1991). All of the flowers of this chimera carry second whorl organs that are almost entirely petaloid except for a narrow green rim. Crosses between this chimera and a *def*/+ heterozygote suggest that a reversion event has occurred in the L2 layer (Z. Schwarz-Sommer, personal communication). Satina (1944) demonstrated that cells derived from the L1 layer can form a small amount of subepidermal tissue along the margins of petals in some species. The presence of a green rim around the petals suggests that the chimera consists of a *def* mutant L1 layer, which forms the epidermis and the subepidermal cells along the petal margins, and a wild-type L2 layer, which forms most or all of the remaining subepidermal tissue of the petal. The sharp boundary dividing the green rim from the rest of the petal further supports the idea that *def* is cell autonomous. Interestingly, the mutant epidermal cells overlying the petaloid parts of the second whorl organs develop as petal epidermis, as indicated by the presence of pigment (Z. Schwarz-Sommer, personal communication). This suggests that although *def* is cell autonomous with regard to adjacent cells within a layer, it may play a role in generating a signal that moves from subepidermal layers to the epidermal cells and determines their pattern of differentiation. Thus, it appears that although expression of *def* in either epidermal or subepidermal tissue can cause epidermal cells to differentiate as petal epidermis, expression of *def* in an epidermal cell cannot influence the developmental pattern of its neighbors. A similar lack of cell autonomy between cell layers has been observed for *PI*, the cognate homolog of *glo* in Arabidopsis (V. Irish, personal communication).

In contrast to *def* and *glo*, another gene required for flower development in Antirrhinum shows no evidence of cell autonomy, consistent with the possibility that it plays a role in generating a signal rather than responding to one. Mutations in *floricaula* (*flo*) cause flowers to be replaced by leafy shoots (Carpenter and Coen, 1990; Coen et al., 1990; Coen and Carpenter, 1993, this issue). Plants carrying an unstable allele of *flo* sometimes produce a few wild-type flowers in addition to many flowers with the *flo* phenotype, but no sectored flowers have been reported, suggesting that *flo* is not cell autonomous. Analysis of the progeny of these revertant flowers has shown that some produce wild-type progeny whereas others do not, suggesting that restoration of gene function in either the L2 layer or one or both of the other two layers can restore wild-type flower development (Carpenter and Coen, 1990; Coen et al., 1990, 1991).

Similar results have been obtained for plants carrying mutations in *ple*, in which third whorl organs are petaloid, fourth whorl organs show variable identity, and several extra whorls of petaloid organs develop internally to the fourth whorl (Carpenter and Coen, 1990). Occasional revertant wild-type flowers appearing on the mutant plants are probably due to the restoration of gene function in the L2 layer, because some wild-type progeny are produced by these flowers and the L2 layer is the source of the germ cells (Carpenter and Coen, 1990). No distinct sector boundaries have been reported within revertant flowers, suggesting that *ple* is not cell autonomous.

L3 Layer Controls Several Aspects of Pattern Formation in Tomato Inflorescences and Flowers

In the fortuitous chimeras described above, it is usually difficult to determine the genotype of each of the three cell layers, complicating interpretation of the results. To avoid this problem, periclinal chimeras with a variety of genetic markers have been deliberately created to study interactions between cell layers (Szymkowiak and Sussex, 1992). Incorporation of genetic markers visible in each cell layer makes it possible to identify the genotype of each layer in a chimera. The layer controlling a particular element of the developmental pattern can then be determined by analyzing periclinal chimeras having each possible arrangement of the contributing genotypes, as shown in Figure 2.

This approach has been used to study control of carpel number and the positioning of the pedicel abscission zone in tomato. Chimeras derived from two *Lycopersicon esculentum* (tomato) lines, one carrying a *fasciated* mutation that causes extra carpels to be formed and one without the *fasciated* mutation, were used to demonstrate that the genotype of the L3 layer determines the number of carpels and the size of the floral meristem in tomato. The L3 layer was also found to control carpel number in a second set of chimeras, in this case

derived from *L. peruvianum* and tomato. Another set of experiments used chimeras derived from either *L. pennellii* or *L. peruvianum*, and wild-type or *jointless* (lacking the abscission zone) tomato to examine the control of abscission zone position on the pedicel. The L3 layer was found to determine the position of the abscission zone in each chimera (Szymkowiak and Sussex, 1989; Szymkowiak, 1990). Because the L1, L2, and L3 layers must differentiate in a coordinated manner to form both carpels and the pedicel abscission zone, the L3 layer in these incipient organs must somehow signal the adjacent cells of the L1 and L2 layers to differentiate in the appropriate way.

Layer interactions can also be demonstrated in the initiation of tomato petal primordia. A chimera with an L1 layer derived from a tomato line carrying a *lateral suppressor* (*ls*) mutation and L2 and L3 layers derived from a tomato line wild type for *Ls* developed normal petals, although the *ls* mutant fails to initiate petal primordia (Szymkowiak and Sussex, 1993). These results indicate that *Fasciated*, *Jointless*, and *Lateral suppressor* all belong to a class of genes that are not cell autonomous and that participate in directing pattern formation.

Although in the chimeras described above, cell layers with different genotypes were able to coordinate their development to form normal structures, chimeras exhibiting abnormal inflorescence development have also been observed. In chimeras containing cells from both tomato and *Solanum nigrum*, only plants with an L1 layer derived from tomato and L2 and L3 layers derived from *S. nigrum* produced normal flowers (Szymkowiak, 1990). Other layer combinations produced normal vegetative structures, but flower meristems were arrested after sepal initiation in some chimeras and after stamen initiation in others. Examples of two such chimeras are shown in Figure 3. In some chimeras, breaks in the epidermis were observed at the base of the inflorescence (Figure 3B). These may represent cases in which signaling between the cell layers failed and development of the individual layers was not coordinated. Similar aberrations in flower development were observed in some *L. peruvianum*/tomato chimeras, but flowers of similar chimeras made using a different tomato line developed normally, suggesting that incompatibility between layers could be due to differences at relatively few genetic loci (E.J. Szymkowiak and I.M. Sussex, unpublished results).

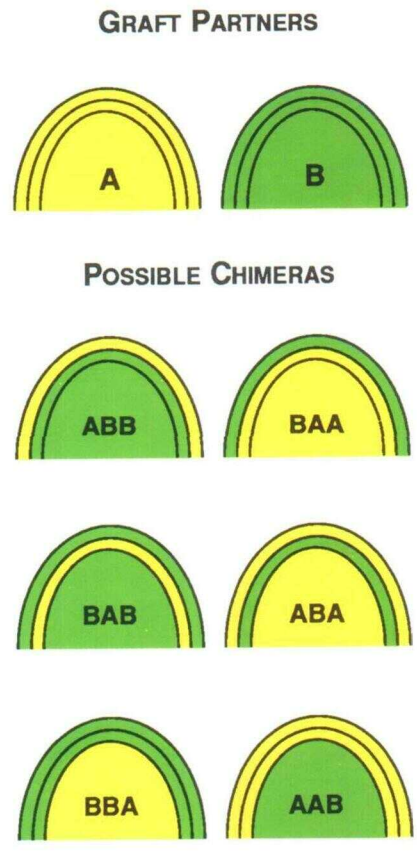

Figure 2. Possible Layer Arrangements in a Periclinal Chimera.

Diagrammatic representation of the six possible periclinal chimeras that result from combining cell layers of two genetically different plants that have three-layered shoot apical meristems. Top row: Two plants, one of genotype A and the other of genotype B, are grafted together as stock and scion. Subsequently, the graft region is cut through, and chimeric shoots regenerate, as described in Szymkowiak and Sussex (1992). Second row: The L1 of each chimera is genetically different from the L2 and L3. Third row: The L2 of each chimera is genetically different from the L1 and L3. Fourth row: The L3 of each chimera is genetically different from the L1 and L2. In the left column of the chimeras, the genetically different cell layer is of genotype A, and in the right column, the genetically different layer is of genotype B.

PROSPECTS

We still have much to learn about the establishment and functioning of the inflorescence meristem. A question of central importance is how determination for reproductive development occurs at a molecular level. Investigations of genes controlling floral meristem identity and floral organ primordium identity are proceeding and promise to contribute to an understanding of the genetic mechanisms that control the establishment and maintenance of meristem identity (Meeks-Wagner, 1993, this issue). Our understanding of the physiological processes that take place during the transition from vegetative to reproductive development is also being extended by ongoing research (Bernier, 1993, this issue).

The role of the meristem in determining the identity of its derivatives is an area that requires more investigation. Because in some cases, a meristem determined for reproductive development can give rise to vegetative derivatives, it would be useful to know whether the determination of flower primordium or floral organ primordium identity depends on signals from outside the inflorescence apex. Some evidence bearing on

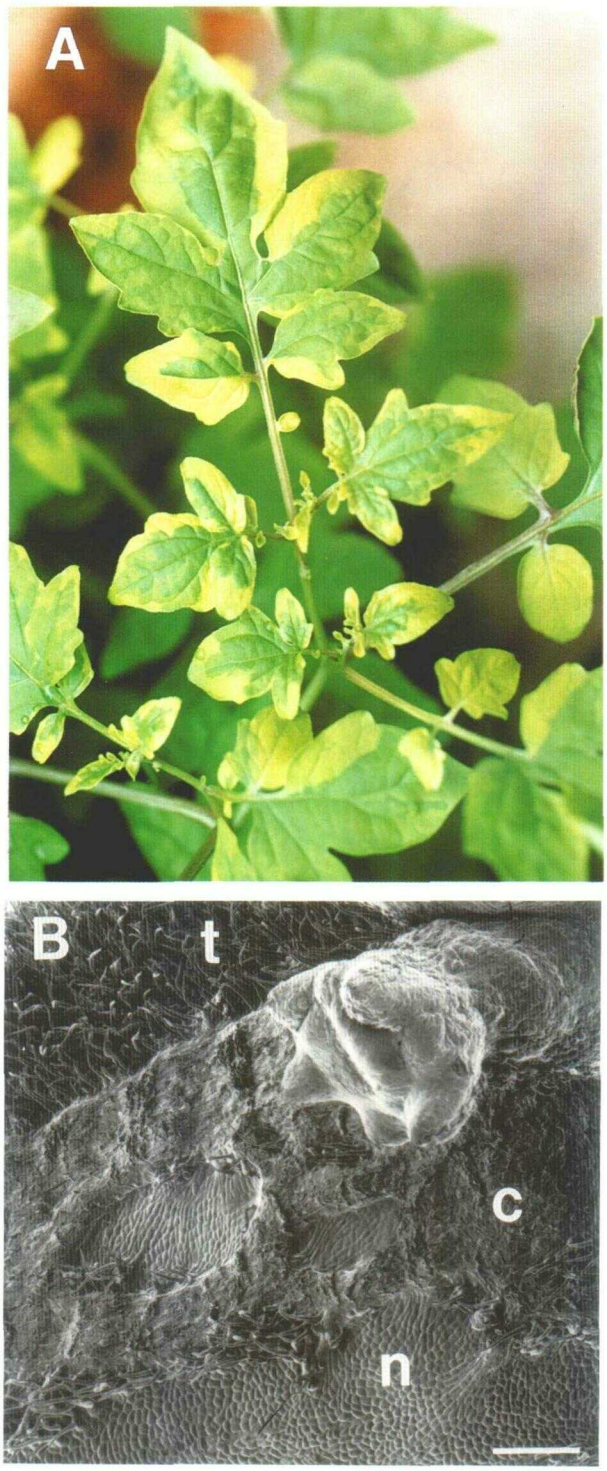

Figure 3. Developmental Incompatibility in *Solanum nigrum*/Tomato Periclinal Chimeras.

(A) An *S. nigrum*/tomato periclinal chimera; L1 and L3 are *S. nigrum*, and L2 is tomato. In the center of the leaf, the dark green *S. nigrum* cells derived from the L3 layer are visible underneath the yellow tomato cells. Although vegetative development is normal in all *S. nigrum*/tomato periclinal chimeras, the flowers of this chimera arrest after sepal initiation.

(B) The stem at the base of an inflorescence from a different *S. nigrum*/tomato periclinal chimera; L1 is *S. nigrum*, and L2 and L3 are tomato. Cracking of the *S. nigrum* epidermis was followed by differentiation of new epidermis derived from internal tomato tissue along the margins of the cracked area. The flower visible at the top has arrested after sepal initiation. The trichomes are characteristic of the tomato graft partner. n, *S. nigrum* epidermis; t, tomato epidermis with trichomes; c, callus that formed in the cracked area. Bar = 300 μm.

this issue comes from the study of determination in the maize tassel (Irish and Nelson, 1991). Culturing the shoot apex at early stages of tassel development can result in the replacement of inflorescence branches and spikelets by vegetative shoots, suggesting that a signal from outside the apex is required for the determination of these structures.

As we come closer to understanding the molecular basis of determination, it is important to bear in mind that the outcome of functional tests for determination are strongly dependent on the organizational unit being assayed. For example, if a photoperiodic plant is shifted to noninductive conditions immediately after photoinduction, it will flower, demonstrating that the plant is determined for reproductive development. However, if the same apical bud that flowered on the intact plant is removed and cultured immediately after induction, it may develop vegetatively because insufficient time has passed for the signal to reach the apex. Likewise, the apical bud may be determined in the operational sense before the meristem itself is determined, assuming that the site of determination is in the meristem. Plants that do not require external signals to initiate flowering are, by the operational definition, always determined at the level of the whole plant, because no change in growth conditions can delay flowering. Nevertheless, when an apical bud from such a plant is assayed by grafting or culturing, it is apparent that determination at the level of the bud can occur late in development. Determination defined in this way is not an absolute quality, and it may have somewhat different meanings at a mechanistic level for the whole plant, the apical bud, the meristem, and the individual cell.

The organization of a group of cells into a coherent developmental unit such as the inflorescence meristem requires communication between cells. Further investigation of signaling between cell layers in the meristem and developing organs through the construction of appropriate chimeras will greatly aid our understanding of this phenomenon. In addition, chimeras derived from lines carrying different alleles of genes that may be required for meristem identity may reveal whether determination can occur at the level of an individual cell.

ACKNOWLEDGMENTS

We would like to thank Vivian Irish (Yale University) and Zsuzsanna Schwarz-Sommer (Max-Planck-Institut für Züchtungsforschung) for communicating unpublished results and Gene Szymkowiak for supplying Figure 3. Our research on meristem identity is supported by United States Department of Agriculture Grant No. 1-440376-22472 and National Science Foundation Grant No. DCB-8916945.

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