Phase Change and the Regulation of Shoot Morphogenesis in Plants

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The shoot system of higher plants passes through several different phases during its development. Each of these phases is characterized by a unique set of morphological and physiological attributes. The intermediate character of the structures produced during phase changes and the phenotypes of mutations that affect this process demonstrate that these phases are specified by independently regulated, overlapping developmental programs. Transitions between phases appear to be initiated by factors extrinsic to the shoot apical meristem; the ability of the shoot to respond to such factors and to remain in a particular phase of development is regulated by factors intrinsic to the meristem. The possibility that developmental phases are maintained by epigenetic cell states and the role of DNA methylation in this process are discussed.

ALL ORGANISMS PROGRESS THROUGH AS SERIES OF DISTINCT developmental phases during their growth. In higher animals, each phase represents a different episode in the life of a single organism. In higher plants, on the other hand, these developmental phases are episodes in the life of a part of an organism, the shoot apex. The shoot apex of higher plants passes through three more or less distinct phases during its post-embryonic development: a juvenile vegetative phase, an adult vegetative phase, and a reproductive phase. The juvenile phase of shoot development starts when the shoot meristem begins to initiate a stem, true leaves, and axillary buds. This phase may last for a few days or many years in different species and is distinguished by a variety of unique vegetative traits and by the absence of reproductive structures. The adult phase that follows is characterized by a different set of vegetative traits and is usually also defined by the ability of the shoot to undergo sexual reproduction. The transformation of the shoot apex into a reproductive structure, such as an inflorescence, flower, or cone, marks the end of its growth and involves particularly dramatic changes in its differentiation. In some plants reproduction is the last phase in the life of the shoot; in other types of plants the growth of the shoot is perpetuated by a lateral vegetative meristem after the terminal meristem becomes reproductive, whereas in some species the primary meristem remains permanently vegetative and only lateral shoots form reproductive structures. Even within the reproductive phase, position-related transitions can occur, such as a change in flower form.

These and other patterns of shoot morphogenesis raise many fundamental questions about the regulation of pattern formation in plants (*1*). How are changes in the phase of the shoot initiated, and how are they maintained? When is the fate of the shoot determined? Is the fate of lateral organs specified by the apical dome or by other parts of the shoot? How are contrasting developmental patterns integrated by the shoot? And what are the genetic and biochemical mechanisms of these processes? In this brief review I will discuss some of what we know about the regulation of post-embryonic phases of shoot development, focusing in particular on genetic approaches to these questions.

The way in which developmental phases are expressed during shoot growth creates some terminological difficulties. Because the primary axis of the shoot elongates by the addition of new structures at one pole, structures formed early in development are located at the base of the shoot and structures formed later are located in more apical positions. Structures formed during a specific phase of shoot growth retain morphological and physiological features characteristic of that phase even after the shoot has entered a new phase. Consequently, phases of shoot development are permanently recorded as variation in the character of structures along the axis of the shoot. This phenomenon is known as heteroblasty (*2*) and is the most obvious result of a change in the developmental phase of the shoot. This polar pattern of development makes it extremely difficult to distinguish temporal, spatial, and quantitative factors in shoot development. As the shoot develops, its apical meristem increases in age, changes its position in relation to previously formed parts of the shoot, and produces an ever increasing number of structures (such as cells, leaves, and internodes). To discern which of these factors is responsible for a change in the state of the shoot is difficult—a fact that is often obscured by the terminology used to describe a phenomenon. The use of the terms juvenile and adult for different phases of shoot growth implies that these phases are regulated by temporal factors, but it would be just as reasonable to describe these as basal and apical patterns of development, in which case spatial or enumerative factors would be implicated. Similarly, some of the terms used to describe aberrations in shoot development (such as homeosis) imply spatial regulation, when temporal or enumerative factors may be involved instead.

Features of Phase Change in Plants

Phase changes in woody and herbaceous species. The transition from vegetative development to reproductive development is abrupt and involves unmistakable changes in the character of the shoot, particularly in flowering plants. In contrast, the transition from a juvenile to an adult phase of vegetative growth usually occurs gradually and may involve rather subtle changes in shoot morphology and physiology. These differences are most obvious in woody species because

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of their prolonged juvenile and adult phases, but are also apparent in herbaceous plants (*1, 2*). Several traits differentiate juvenile and adult states of shoot development in English ivy, a woody species, and maize, a herbaceous plant (Table 1). In both woody and herbaceous species, leaf shape is one of the most conspicuous signs of the vegetative phase of the shoot. Juvenile shoots are usually characterized by leaves that are smaller and simpler in structure than those of adult shoots, although in some cases the opposite is true. In various species, juvenile and adult phases of shoot development may also be distinguished on the basis of their phyllotaxis, leaf retention, the growth habit of lateral branches, thorniness, adventitious root production, chemical composition, photosynthetic efficiency, disease and insect resistance, and many other traits (*3–5*). As mentioned earlier, these vegetative traits are usually also correlated with the reproductive capacity of the shoot. There are, however, many exceptions to this rule, and it is unlikely that vegetative and reproductive aspects of shoot development are both regulated in exactly the same way (*6*).

The phase of the primary shoot axis is also recorded in the developmental behavior of axillary buds located in various positions along the shoot. The character of these lateral shoots depends on their position on the shoot and on the phase of the shoot at the time they arose (*3, 7*). Lateral buds produced during the vegetative phase of development are usually vegetative, whereas lateral buds produced during the reproductive phase of development form flowers or other reproductive structures. In the same way, branches from the juvenile portion of a shoot express juvenile traits, while adult phase shoots produce adult phase branches.

The extent to which the development of lateral shoots is influenced by the primary shoot is illustrated by the behavior of axillary buds in tobacco. When the primary shoot of *Nicotiana tabacum* cv. Wisconsin 38 is decapitated at various points along its length, axillary buds below the point of decapitation are released from apical dominance, produce a certain number of vegetative nodes, and then flower. The number of nodes produced by the bud immediately below the point of decapitation is related to its position on the shoot (*8*). Buds at successively higher positions on the stem produce successively fewer vegetative nodes than axillary buds located in more basal positions on the stem. Axillary buds produced before the primary shoot becomes florally determined only express this position-dependent behavior in situ. However, buds produced by florally determined shoots form the same number of nodes whether they are grown in situ or excised and rooted (*9*). Thus, the behavior of axillary buds in tobacco is specified both by positional information provided by the primary axis of the shoot and by the developmental phase of the shoot at the time the bud was initiated.

Although it is convenient to describe the development of the shoot in terms of discrete, stable developmental phases, this is clearly an oversimplification. In addition to discrete transitions in developmental patterns, the shoot also undergoes a gradual aging process represented by, among other aspects, a reduction in growth rate, the loss of apical dominance, and reduced flower production (*10*). This loss of vigor can be distinguished from the phenomenon of phase change because it can be readily reversed by grafting the shoot to a new root stock or by a change in nutrition. Furthermore, although the developmental fate of a structure is largely specified by the phase of the shoot during the development of that structure, in some cases changes in the developmental potential of a structure can occur long after it has matured (*9, 11*).

A combinatorial model of shoot development. A schematic diagram of the way in which juvenile, adult, and reproductive traits are expressed during the post-embryonic growth of a determinate shoot is presented in Fig. 1. This model implies that the development of the shoot is specified by a series of independently regulated, overlapping programs that modify the expression of a common set of processes required for shoot growth. Evidence that the morphology of the shoot is determined in a combinatorial fashion rather than by a series of mutually exclusive developmental programs comes from many different sources. Some evidence for this conclusion is provided by the intermediate character of the structures produced during phase transitions. In maize, for example, leaves produced during the transition from juvenile to adult growth have a combination of juvenile and adult cell types and express a variety of other traits in a quantitatively intermediate fashion (*12*). Axillary buds produced during this transition also express a range of juvenile and adult traits (*13*). In woody plants, the transition from a juvenile to an adult phase of development is also accompanied by the production of intermediate patterns of shoot development that combine cellular and morphological traits from each phase (*6*).

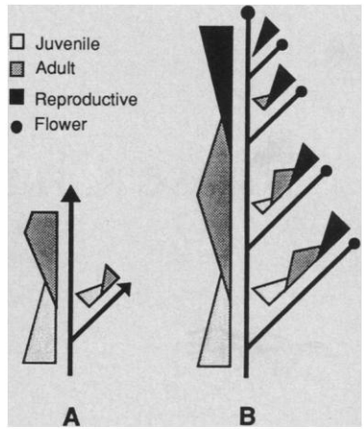

Fig. 1. A schematic representation of the expression of juvenile vegetative, adult vegetative, and reproductive traits in an immature (**A**) and mature (**B**) shoot of a determinate plant. Processes required during all phases of development for the growth of the shoot meristem and the initiation of later organs are illustrated by a black line. Juvenile, adult, and reproductive developmental programs may be regulated by separate developmental programs with intermediate phases being the consequence of the overlap between these programs.

Intermediate developmental patterns are common during the transition from vegetative to reproductive development as well. During the early phase of the transition to reproductive growth in *Arabidopsis thaliana*, for example, the shoot produces rudimentary leaves and elongated lateral branches similar to those produced by rosette nodes. Later in the development of the inflorescence, leaf development is suppressed and solitary flowers are formed in place of branches. Within a flower, the production of reproductive organs

Table 1. Features that distinguish juvenile and adult phases of English ivy (*Hedera helix*) and maize (*Zea mays*) (*3–5, 10*).

Traits	Juvenile	Adult
	Hedera helix	
Leaf shape	Entire	Lobed
Leaf thickness	230 μm	330 μm
Phyllotaxy	Alternate	Spiral
Plastochron	1 week	2 weeks
Growth habit	Plagiotropic	Orthotropic
Anthocyanin	Present	Absent
Aerial roots	Present	Absent
Rooting ability	Good	Poor
Flowers	Absent	Present
	Zea mays	
Cuticle thickness	1 μm	3 μm
Epidermal cell shape*	Circular	Rectangular
Epicuticular wax	Present	Absent
Aerial roots	Present	Absent
Epidermal hairs	Absent	Present
Bulliform cells	Absent	Present
Lateral buds	Tiller-like	Ears or absent
Anthracnose resistance	Poor	Good

*Transverse section.

(the stamens and the pistil) is preceded by the production of two whorls of organ, the sepals and petals, that have a combination of vegetative and nonvegetative traits.

By itself, intermediacy does not demonstrate that the development of the shoot is regulated by the simultaneous expression of overlapping developmental programs. Nevertheless, it is hard to avoid this conclusion when faced with all variant forms of shoot development that occur naturally or can be experimentally induced. Developmental patterns reflecting a shift in the time or locus of expression of phase-specific traits are common in nature and often have a genetic basis (*14*). A familiar example of this type of shift is the lily flower, where sepals and petals are replaced by structures (tepals) that have both sepal and petal characteristics. Many other interesting shifts in developmental fates are described in the classical literature on teratologies (*15*). Mutations that cause spatial transformations during flower development are particularly common and are described by Schwarz-Sommer *et al.* (*16*).

Aberrant combinations of traits from different phases of development can be produced experimentally as well as genetically. Vegetatively transformed inflorescences arise when photoperiodic species are prematurely shifted from an inductive to a noninductive photoperiod (*17*). In woody plants, shoots that have a combination of juvenile and adult vegetative traits may be produced by hormonal treatments (*18*) or by grafting shoots in one phase of development to plants in a different developmental phase (*19*). The character of the structures produced by these experiments strongly suggests that the morphology of the shoot is determined by additive effects of several independently regulated developmental programs rather than by a single regulatory scheme.

This model of shoot development has important evolutionary implications because of the way in which changes in the relative timing or locus of expression of different developmental processes can affect the morphology and reproductive biology of the plant. Variation in the timing of a developmental process is known as heterochrony, whereas variation in the locus of expression of a process is known as homeosis or heterotopy (*20, 21*). In many animals these phenomena can be distinguished because the process of pattern formation is confined to a limited period of time and is not associated with growth. As a consequence, processes affecting the regulation of spatial patterns can usually be distinguished from those involved in the subsequent elaboration of these patterns. As pointed out above, this distinction is more difficult to draw in plants because of their continuous, polar growth, so the use of these terms to describe changes in the character of the shoot is somewhat arbitrary. Heterochrony may be distinguished from homeosis by comparing the relative rate of development of different parts of an organism, but this distinction does not carry mechanistic implications. In other words, changes in the relative timing of two processes and changes in the spatial expression of these processes may be regulated by the same mechanism (*22*).

The development of a comprehensive framework for the analysis of heterochrony (*23*) and the identification of mutations with heterochronic phenotypes in both animals (*24*) and plants (*25*) have provided new opportunities for the analysis of this phenomenon. In a review of the evidence for heterochrony in plants, Lord and Hill (*18*) point out that although botanists have often used heterochronic concepts such as developmental arrest and neoteny to interpret patterns of development and evolution, the role of heterochrony in plant biology has never been fully explored. Takhatajan (*26*) postulated that herbaceous plants evolved from woody plants by the acceleration of reproductive development relative to vegetative development, a hypothesis that accounts for the absence or reduced amount of secondary growth in herbaceous species and for the brevity of their juvenile phase. Early in this century Goebel (*27*) invoked the idea of developmental arrest to explain heteroblastic patterns of development, attributing variation in leaf shape to the cessation of development at different points along a single developmental pathway. This specific hypothesis has not been supported by more recent analyses of heteroblastic leaf series where the shift in leaf form is dramatic (*28*). In these cases differences in organ form occur at inception of the primordium and so they express divergent developmental pathways. When mature organs in a heteroblastic series are not highly divergent, Goebel's concept of arrest holds more applicability, as evidenced by the studies on some cleistogamous species (*29*).

Stephens (*30*) explicitly addressed the role of heterochrony in cotton evolution in his classic study of the genetic regulation of leaf shape in this genus. In cotton, leaves become progressively more lobed until the shoot begins to flower, at which point the shoot adopts a constant "climax" leaf shape. By introducing different alleles of a locus that controls the degree of leaf lobing into early and late flowering backgrounds, Stephens (*31*) showed that the phenotype of these alleles was correlated with flowering time. In an early flowering background, the developmental change in the phenotype conditioned by a particular allele was accelerated, but this change in leaf shape was arrested by the precocious flowering of the shoot before the phenotype typical of that allele in a late flowering background was attained. Furthermore, it was possible to transform the climax shape of one allele into that of another by experimentally prolonging the vegetative phase of development. Stephens concluded that much of the interspecific variation in leaf shape in cotton is a consequence of the modification of a few basic patterns of vegetative development by the reproductive habit of a plant. More recently, Lord and her colleagues (*29*) have used the heterochronic models developed by Alberch *et al.* (*23*) to evaluate the developmental origin of closed and open flowers in several cleistogamous species. Similar studies have been performed on two *Delphinium* species with morphologically distinct flowers (*32*). In both studies, the morphological differences between flower types could be explained, at least in part, by a shift in the rate or duration of growth of either the entire flower primordium or of different parts of the flower primordium relative to one another. The ability to regulate different processes in development independently and to integrate these processes functionally provides the shoot with a vast repertoire of morphogenetic patterns. The problem confronting developmental biologists is to discern how this regulation is accomplished.

The Mechanism of Phase Change

When is the fate of lateral organs determined? The character of intermediate structures produced during phase transitions can indicate how contrasting developmental phases are specified and integrated within individual organs during shoot growth. In maize, leaves produced during the transition from a juvenile to adult phase of vegetative growth have juvenile traits, such as epicuticular wax and bulbous epidermal cells, at the tip of the leaf and adult traits, such as epidermal hairs and cuboidal epidermal cells, at the base of the leaf (*12*). Because the tip of the leaf matures before the base of the leaf, this suggests that the fate of the leaf is determined gradually after it is initiated, while the shoot is changing phase. Experimental analyses of the origin of intermediate structures in *Impatiens balsimina* (*33*) and *Hippuris vulgaris* (*34*) provide considerable support for this conclusion. When the short day plant, *I. balsimina*, is returned to a noninductive photoperiod after being exposed to an inductive photoperiod for 5 days, the shoot produces structures that have both leaf and petal characteristics (*33*). Histological analysis reveals that these traits are coexpressed in both tissues and in individual

cells. In intermediate structures the mesophyll has some of the histological organization of a leaf, but is the thickness of a petal; epidermal cells within this region express anthocyanin—a petal trait—but have the shape of epidermal cells in the leaf. Primordia are capable of developing as intermediate structures until they are about 750 μm long, and generally produce intermediate tissue in broad regions at the base of the structure rather than in clonal sectors. This is consistent with the fact that base of a leaf or petal primordium is the last part of the primordium to mature and shows that cells in a primordium acquire their fate after the primordium is initiated.

Intermediate structures are also formed during the transition from aquatic to aerial leaf types in heterophyllous aquatic plants. When shoots of *Hippuris vulgaris* are transferred from conditions that specify one leaf form to conditions that specify the alternate form, leaf primordia present at the time of this transition develop as intermediate structures (*34*). As in the case of the intermediate structures in maize and *I. balsimina*, these intermediate forms possess distinct regions with different developmental patterns. The apex of an intermediate leaf develops according to the initial set of conditions, whereas the base of the leaf develops the form specified by the new conditions. In all three of these species, therefore, the fates of lateral primordia are determined gradually after they are initiated, with the fate of different regions of the primordium being determined at different times in development. That intermediacy can also be histologically and cytologically apparent suggests that different attributes of a primordium are also determined at different times in development.

The transition from vegetative to reproductive development. If the character of an organ is determined late in development and can be modified by factors that act directly on the primordium, then it is reasonable to ask whether the phase of the shoot is regulated by the shoot apical meristem or by factors extraneous to the meristem. Not surprisingly, both appear to occur. The nature of the factors that regulate phase changes is best understood in the case of the transition from vegetative to reproductive development. In most species it is possible to define two discrete steps in this process: the transition from a reproductively incompetent (juvenile) to a reproductively competent (adult) phase of development, and the actual initiation of reproductive development (*35*). This distinction is easiest to make when the initiation of reproductive development is triggered by a well-defined set of environmental conditions, as in photoperiodic plants, because in these cases the juvenile to adult transition can be operationally separated from the actual initiation of reproductive development. In those cases in which reproductive development occurs spontaneously when the plant reaches a certain age or size, it is more difficult to distinguish these two aspects of reproductive development (*18*). However, because mutations can be found that make the initiation of reproductive development sensitive to photoperiod without eliminating the juvenile phase in many of these species, it is reasonable to assume that these two aspects of reproductive development are regulated separately in most, if not all, species.

Genetic analyses of flowering in peas by Murfet and Reid have provided the most detailed picture we now have of the factors and complex interactions involved in the initiation of reproductive development in plants (*36*). Six major genes regulating reproductive behavior, *Veg*, *Lf*, *Sn*, *Dne*, *Hr*, and *E*, have been identified either as induced mutations or as spontaneous mutations in cultivated varieties (Table 2). Together, various alleles of these loci generate a spectrum of reproductive pheotypes ranging from (i) types with an extremely short juvenile phase to those that fail to flower under any conditions, (ii) types that flower under any photoperiod to those that require long days, and (iii) types whose flowering is unaffected by temperature to those that respond strongly to a cold treatment.

Table 2. Phenotype and sites of action of the dominant alleles of genes involved in regulating flower initiation in peas (*33*).

Gene	Phenotypic effect	Site of action
Veg	Required for flower initiation	Shoot apex
Lf	Specifies minimum flowering node	Shoot apex
Sn	Delays flowering; promotes photoperiodic response	Leaves and cotyledons
Dne	Delays flowering; promotes photoperiodic response	Leaves and cotyledons
Hr	Enhances effect of *Sn* and *Dne*	Leaves
E	Enhances effect of *Sn* and *Dne*	Cotyledons

Flower initiation in peas can therefore be made to mimic many patterns of flower initiation found in nature, making this an excellent model system for the analysis of the transition from vegetative to reproductive growth.

Grafting studies, leaf removal experiments, and analyses of the photoperiodic requirements of various genotypes suggest that these six genes operate either by regulating the production of a flower promoter and flower inhibitor in leaves, cotyledons, and stem, or by affecting the sensitivity of the meristem to these factors. Flowering is thought to occur when the balance between the promoter and inhibitor exceeds a threshold determined by the sensitivity of the shoot meristem (*36*). The products of both the *Lf* and *Veg* loci are believed to be involved in the perception of flowering stimuli by the shoot apex. Alleles of the *Lf* locus determine the minimum node at which the shoot will flower and can therefore be considered to regulate the length of the juvenile phase. Grafting experiments indicate that expression of *Lf* is confined to the shoot apex, and it is believed that this locus determines the sensitivity of the shoot meristem to the ratio of promoter to inhibitor. The *Veg* locus is defined by a recessive mutation that completely blocks flower initiation in all genotypes and under all conditions that have been tested. This mutation is expressed autonomously by the shoot apex, which suggests that it does not affect a factor that promotes flowering. In addition, it is epistatic to alleles of *Lf*; that is, none of the vegetative effects of these alleles are visible in *veg* plants. This result suggests that *veg* regulates the perception of floral stimuli rather than the actual differentiation of the flower because factors involved in flower differentiation would not be expected to be epistatic to *Lf*. *Sn* and *Dne* control the production of a graft-transmissible inhibitor in the cotyledons and leaves. Recessive alleles of these loci condition early flowering and eliminate the long-day photoperiodic requirement characteristic of plants that carry dominant *Sn* and *Dne* alleles. Assuming that recessive alleles represent loss-of-function mutations, this result suggests that inductive photoperiods act by suppressing the activity of *Sn* and *Dne*, thereby increasing the ratio of promoter to inhibitor at the shoot apex. Dominant alleles of two other loci, *E* and *Hr*, appear to regulate the expression of *Sn* and *Dne*. *E* suppresses the effects of *Sn* and *Dne*, and results in early flowering. *Hr*, on the other hand, enhances the effects of *Su* and *Dne*, and makes plants almost strictly photoperiodic. Both genes appear to be organ-specific, with *E* acting primarily in cotyledons and *Hr* acting primarily in foliage leaves. Loci involved in the production of a flower promoter have proved to be somewhat elusive; however, the phenotype of the recently described *gi* mutation suggests that it may have this function (*37*).

The interaction between factors that regulate vegative growth and those involved in reproductive development is illustrated by the pleiotropic effects of the *Sn* and *Dne* products. In addition to inhibiting flower initiation, these loci delay the senescence of the shoot, prolong juvenile leaf morphology, increase the length of the flower peduncle, increase the life-span of the flower, and delay fruit

development. Thus, the products of these genes have the general effect of enhancing vegetative growth. Whether this effect is hormonally mediated or a consequence of a change in the basic metabolism of the plant is unknown. The specific response to *Dne* and *Sn* depends on a third gene, *Lf*, the expression of which appears to be regulated independently of *Dne* and *Sn*. Each allele of *Lf* specifies a different period of vegetative growth. The genotype of the *Dne* and *Sn* loci modifies the duration of this vegetative period, but does not change the relative effects of *Lf* alleles. Thus, certain allelic combinations produce plants that have the same reproductive phenotype but different vegetative phenotypes.

The genetic experiments described above provide a great deal of insight into the nature of the factors that regulate reproductive development, but do not reveal why the expression of these factors changes during shoot growth. This question has not been answered in peas, but research on other species indicates that the size, rather than the age of the shoot, is of primary importance. Shoots of a variety of species will flower when they reach a certain size, independent of how long it takes to reach this size. In black currant (*38*), tobacco (*39*), and several other species (*40*), this effect appears to be due primarily to factors produced by the root system, as flowering is inhibited by rerooting shoots before they reach the critical size, grafting shoots to short stocks, and by inducing root formation on apical sections of the stem. In all cases, leaf removal had no effect on the number of nodes produced before the transition to reproductive growth. Rerooting experiments suggest that proximity to the root system may also be important in regulating reproductive development in maize (*41*). These studies show that the root system influences the ability of the shoot to undergo the transition from vegetative to reproductive growth, but it is not known how this is accomplished.

The identity of the diffusible factor that promotes flower initiation is the Holy Grail of plant physiology. Considerable effort has been invested in the search for this elusive factor and much has been learned about its properties along the way, but the discovery of the floral stimulus does not appear to be imminent. In part, this is because many different factors have significant effects on flower initiation and it is difficult to determine whether these factors act directly or indirectly on this process. The problem faced by investigators in this field is illustrated by the work on gibberellic acid (GA) in peas. This hormone is thought to participate in flower initiation because exogenous applications of GA delay flower initiation and shoot senescence, whereas conditions that induce flowering are associated with a reduction in the GA content of the shoot (*42*). Yet mutations that block early steps in GA biosynthesis have only minor effects on flowering behavior and do not modify the expression of the mutations described above (*43*). This result suggests that GA is not the primary regulator of flower initiation in peas. The identity of the factors involved in regulating the transition to reproductive growth will probably have to await the molecular analysis of mutations that specifically affect this process.

The transition from juvenile to adult growth. Much less is known about the regulation of juvenile and adult phases of vegetative development. In part this is because completely different aspects of this phenomenon have been studied in woody and herbaceous species. In woody species, vegetative phases of development are usually defined in terms of the reproductive competence of the shoot. In herbaceous species, on the other hand, phase change is usually studied as it relates to leaf morphogenesis. Unfortunately, it is not clear how either of these phenomena is related to the vegetative phase of the shoot. The complex relationship between reproductive and vegetative development has already been described. The relationship between leaf shape and the phase of the shoot is complicated by the remarkable plasticity of leaf morphogenesis. Nevertheless, because treatments that modify heteroblastic patterns of leaf development never completely eliminate the progression of shapes that occurs during shoot development, it is reasonable to assume that this type of heteroblasty reflects a general change in the state of the shoot (*2*). Much of the research on the regulation of reproductive development in woody species is probably also relevant to the regulation of vegetative development because reproductive and vegetative phases of the shoot are closely related in these species (*44*).

As in the case of reproductive development, changes in the vegetative phase of the shoot appear to be initiated by diffusible factors that arise outside the shoot apical meristem. Thus, rejuvenation of adult shoots in herbaceous species can be accomplished simply by rerooting the apex of the shoot, whereas adult phase shoots of woody species will sometimes revert to a juvenile phase when grafted to juvenile shoots (*2, 44*). Unfortunately, research on the regulation of the juvenile to adult transition has been complicated by the wide variety of conditions that affect the expression of these phases. In general, conditions that retard growth, such as poor mineral or carbohydrate nutrition, water stress, defoliation, low light, and low temperature, prolong juvenile growth or cause rejuvenation of adult shoots. In contrast, conditions that encourage vigorous growth accelerate the transition to an adult phase. In many species, various forms of GA will induce adult phase shoots to revert to a juvenile phase (*18, 45*). Although this observation may suggest that GA functions as a juvenile hormone, there is no evidence that a reduction in the amount of endogenous GA accelerates adult development. Consequently, the function of GA in the regulation of vegetative phase change, if any, is not clear (*44*).

Genetic analysis of vegetative phase change is complicated by the fact that species with stable, distinctive vegetative phases are not readily amenable to genetic analysis because of their long life cycles. In these respects, maize has several advantages as an experimental system. Aside from its excellent genetics and relatively short life cycle (compared to woody plants) maize has the advantage of possessing juvenile and adult phases of vegetative development that are distinguished by a large number of obvious morphological, cellular, and biochemical traits (Table 1). Because shoot growth terminates with the initiation of a male inflorescence (the tassel), factors that affect reproductive development can be distinguished from factors that affect vegetative development by their effect on the duration of shoot growth. Three genes thought to be involved in regulating juvenile development have been identified in maize (*25*). These genes are defined by semidominant, gain-of-function mutations—*Tp1*, *Tp2*, and *Tp3*—whose pleiotropic phenotype appears to reflect the imposition of a juvenile vegetative program on an otherwise normal pattern of shoot growth (Fig. 2). This conclusion is supported by the observation that these mutations do not affect the rate or duration of shoot growth or the time of tassel determination (*45*). Additional support for this conclusion is provided by the intermediate character of mutant organs. In *Tp* plants, leaves in what is normally the adult part of the shoot possess both juvenile and adult cell types and are morphologically and anatomically intermediate between juvenile and adult leaves (*12*). Reproductive structures (the tassel and ear) of mutant plants possess both leaves and flowers. This phenotype demonstrates that the juvenile phase of development in maize is regulated, at least to some extent, independently of adult and reproductive phases.

The phenotypic similarity of *Tp1* and *Tp2* and the way they interact with various genetic modifiers, suggests that these genes have closely related functions (*25*). *Tp1* is believed to act via a diffusible factor because it is expressed nonautonomously in genetic mosaics (*47*). Several genes that may be involved in the same pathway as *Tp1* and *Tp2* have been identified on the basis of their

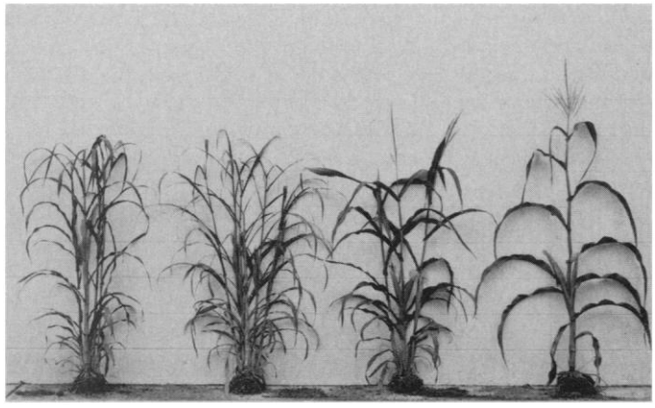

Fig. 2. The phenotype of, from left to right, *Tp1*/+, *Tp2*/+, *Tp3*/+, and wild-type maize plants in an Oh51a inbred background.

phenotype and their interaction with these two mutations (*48*). The best characterized of these is *teosinte branched* (*tb*). Recessive alleles of this gene have a highly tillered phenotype resembling that of the *Tp* mutations. A duplication of the wild-type *Tb* allele suppresses the tillered phenotype of *Tp1* and *Tp2*, whereas recessive *tb* alleles interact synergistically with *Tp1* and *Tp2* to enhance tillering. The identification of other loci that modify the expression of the *Tp* mutations should make it possible to define the components of the system regulating phase change in maize and will further the genetic and biochemical analysis of this phenomenon.

The Maintenance of Developmental Phases

Changes in phase occur gradually and are characterized by an increasing commitment to the new developmental state. Once the shoot is fully committed to a new phase it can be extremely difficult to induce it to revert to a different phase. Fully induced reproductive meristems do not readily revert to vegetative growth and juvenile and adult tissues coexist stably in the same shoot throughout the life of the plant. This phenomenon raises the question of how developmental phases are maintained in a system that is constantly increasing in size and complexity. Clearly the shoot meristem and its derivatives are semiautonomous entities; in some aspect, their behavior is independent of the remainder of the shoot. What is less obvious is the nature of this control. Is the stability of the shoot maintained at a cellular or supra-cellular level, and what is the molecular and biochemical basis of this stability?

In animals, the regulation of cell fate has been studied by various means. These include transplanting cells to new locations in an organism, testing fates of isolated cells in tissue culture, and combining cells with different developmental fates to assess how they interact. These techniques are more difficult to use in plants because small pieces of plant tissue quickly dedifferentiate or fail to grow when they are excised and experimentally manipulated. As a result, cell states in plants have generally been assessed by indirect parameters such as the growth rate or regeneration potential of a tissue. This problem can be circumvented with the use of genetic mosaics to juxtapose cells that have different developmental or physiological potentials. Mosaic plants often arise spontaneously as a result of somatic mutations and have been generated intentionally or unintentionally by plant biologists for hundreds of years. Genetic mosaics have recently been used to study the cellular and biochemical basis of developmental and physiological phenomena in a variety of species (*47, 49*) and may continue to be exploited in the future. At present, however, much of what we know about the cellular basis of phase change is based on more disruptive techniques.

The maintenance of epigenetic cell states. The tendency of plant cells to dedifferentiate in culture and the ease with which they can be induced to regenerate are the basis for the general view that the developmental fate of plant cells is regulated primarily by the tissue or organ of which they are a part. However, in several different systems, stable differences in cell behavior have been noted in cell cultures derived from shoots or tissues in different developmental states. Callus cultures derived from juvenile- and adult-phase ivy, for example, differ both in growth rate and in their regeneration potential. Juvenile tissue grows at a significantly faster rate than adult phase tissue (*50*), and, in contrast to adult tissue, produces shoots instead of embryos (*51*). In *Nicotiana tabacum* var. Wisconsin 38, cultures initiated from the inflorescence tend to produce flowers when induced to regenerate, whereas cultures initiated from vegetative shoots produce only vegetative shoots upon regeneration (*52*). This difference in developmental potential is maintained for two subcultures and then disappears. Maize cell cultures initiated from immature embryos grow rapidly and remain embryogenic, but cultures initiated from more mature embryos or plumules grow slowly and regenerate poorly or not at all (*53*).

An example of a heritable cell state is the phenomenon of cytokinin-habituation in tobacco (*54*). Cells derived from the pith of the tobacco stem normally require auxin and cytokinin for growth in culture, but on occasion lose their requirement for one or both of these hormones. This phenomenon is termed habituation and represents a epigenetic change in cell behavior in that it is both heritable and reversible. Pith cells become habituated at a rate of about 5×10^{-3} per cell generation and can be clonally propagated in this state. Reversion to a nonhabituated state occurs in response to cold temperature and upon shoot regeneration (*55*). Habituation is relevant to normal development because cells from different parts of the shoot vary in their ability to express this trait. Cells from the cortex of the stem show no requirement for cytokinin and are therefore fully habituated. Leaf cells never become cytokinin habituated, and pith cells exhibit the potential to become habituated (*56*). Thus, cells in a tobacco plant exist in three cell-heritable states: a stable habituated state (cortex), a stable nonhabituated state (leaf), and an unstable intermediate state that can be switched in one direction or the other (pith). Meins (*57*) has discussed a model for the regulation of habituation in which alternate cell states are specified by the state of an autocatalytic feedback loop. On the assumption that cell division factors either directly or indirectly induce their own synthesis, habituation is modeled as a balance between the rate of synthesis and the rate of degradation of these factors. Habituation results when the rate of synthesis exceeds the rate of degradation, so that the concentration of cell division factors increases to a concentration where it is autocatalytically maintained.

Another mechanism for the maintenance of epigenetic cell states is DNA methylation. Nearly 30 years ago Brink called attention to the similarity between phase change in plants and changes in cell state known to occur in a variety of nonplant systems, and, influenced by his work on paramutation in maize, proposed that phases of shoot development might be regulated by reversible changes in chromatin structure (*4*). Since then, DNA methylation has been shown to be responsible for mitotically stable, but reversible patterns of gene expression in many organisms. Reversible changes in the genetic activity and methylation status of transposable elements in maize provide the best evidence that this phenomenon may also regulate phases of shoot development.

The *Spm* transposable element in maize can exist in three heritable states: a stable active state, a stable inactive state, and a labile programmable state from which the element can shift to a stable

active or inactive state (*58*). These states are correlated with the amount of transcription from the programmable element and are characterized by unique patterns of cytosine methylation in the region surrounding the transcription start site at the 5′ end of the element (*59*). Inactive elements are more highly methylated and transcribed less, whereas active elements have almost no methylation in this region and are transcribed more. The expression of programmable elements varies depending on their location in the shoot. The probability that a programmable element will become inactive is directly proportional to its distance from the base of the plant. Thus, elements inherited from the tassel on the main stalk are more likely to be inactivated than elements inherited from the ear on that stalk, and tiller-derived elements have an even lower probability of being inactivated. This result suggests that programmable *Spm* elements in the shoot meristem become progressively imethylated as the shoot grows, and in some plants this has been shown to occur (*60*).

A progressive increase in the methylation of Robertson's *Mutator* (*Mu*) element during shoot growth has been observed with the help of *hcf106*, a *Mu*-induced mutation that produces a pale green, high fluorescent phenotype when *Mu* is unmethylated (*61*). Methylation of the *Mu* element at the *hcf106* locus is associated with the restoration of a wild-type, dark green phenotype. In plants that are homozygous for *hcf106*, this event produces somatic dark green sectors that serve as a visual marker of the methylation status of the *Mu* elements in that sector. The proportion of dark green tissue increases from the base to the tip of the shoot and at some point between the 3rd and 13th node the shoot becomes completely dark green. Seeds derived from *hcf106*-suppressed plants generally remain suppressed, although the suppressed alleles in these plants can be reactivated by crosses to plants with active *Mu* elements. Recovery of fully mutant seedlings from this cross indicates that reactivation occurs at the time of fertilization. Thus, the behavior of this *Mu*-induced mutation differs from that of phase-related traits only in that *hcf106* is expressed in a variable fashion and is susceptible to sexually transmissible forms of modification. The correlation of this behavior with methylation of the *Mu* element indicates that methylation may be a mechanism for maintaining phases of shoot development.

Cellular determination in shoot morphogenesis. Although the existence of epigenetic mechanisms that predispose cells to respond to certain signals or maintain them in a particular differentiated state has been established, the function of these processes in morphogenesis is still unclear. In particular, there is still some uncertainty about the extent to which individual cells or cell lineages become determined for specific fates early in the development of a primordium. That plant cells can stably express certain differentiated states outside of their normal milieu does not necessarily mean that a plant cell becomes determined for a specific developmental fate prior to the actual expression of that fate. If the character of the shoot were specified early in shoot development in the form of cell-heritable states, then one would expect the cells in particular regions of the meristem to have highly predictable fates. This is not the case. Cell lineage patterns in periclinal and mericlinal chimeras show that although cell lineages from various layers of the meristem are constrained to particular regions of the plant, the growth and fate of these lineages can vary considerably without any effect on morphology (*62*). Clonal analysis of cell lineage in the maize shoot at several different stages of development reveals similar phenomena (*63*). Although various regions along the vertical axis of meristem give rise to predictable domains of the shoot, the fate of individual cells ranges over a wide latitude. Cells that normally give rise to the terminal inflorescence, for example, can also give rise to vegetative nodes if the growth of the shoot is prolonged. Similar patterns of cell differentiation have been described in sunflower (*64*). These results and the observation that the shoot regenerates normally after a variety of disruptive treatments (*65*) demonstrate the regulative properties of the shoot meristem and suggest that patterns of shoot morphogenesis are not specified solely within the cells of the meristem.

Supracellular regulation of shoot morphogenesis. Another way that phases of shoot growth might be maintained is by supracellular interactions between parts of the shoot apical meristem. There is good evidence that position of a new leaf primordium is determined by preexisting primordia (*66*). Whether preexisting primordia also regulate the initiation and character of primordia formed later is more controversial. Several investigators have proposed that the identity of the whorls of organs in a flower is regulated by chemical or physical stimuli originating from previously formed organs (*67*). However, it is difficult to reconcile these models with the existence of mutations that eliminate or change the character of one whorl of organs in a flower without affecting the development of more distal structures (*68*). Such mutations strongly suggest that the specification of organ identity during flower development does not depend on the identity of previously formed organs. The influence of preexisting structures on the fate of vegetative organs has not yet been fully investigated.

Summary

Although the character and responses of different phases of shoot development are often quite distinct, these phases all appear to be variations on a common theme. Each phase represents a modification of a fundamental pattern of shoot development, which can be imposed on other patterns of shoot development. The regulatory mechanism for each pattern interacts with the regulatory mechanism of other patterns in ways that are still poorly understood. Integration of these different phases may be carried out subcellularly by communication between different genetic regulatory factors, or at a higher level by interactions between cells or tissues. To understand this process, one must define the components of this system and selectively modify their expression. Genetic, developmental, and molecular analyses of mutations that affect the expression of particular phases of shoot development are beginning to yield a clearer picture of the regulatory framework of shoot development and will contribute significantly to our understanding of this process in the future.

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