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Cosuppression, Flower Color Patterns, and Metastable Gene Expression States

Richard A. Jorgensen

In plants, transgenes often induce rapid turnover of homologous endogenous transcripts. This "cosuppression" of homologous genes is an extremely nonlinear response to small increases in gene expression or dosage, inversely amplifying them into dramatic phenotypic alterations. Pigment transgenes elicit metastable cosuppression patterns organized by flower morphology. Pattern organization and metastability reflect regulatory states (probably transgene transcription states) that respond to morphological features and are labile to physiology and development. Shifts between regulatory states can be highly ordered; for example, a shift may be imposed on a population of cells defining a meristem, which then stably maintains and transmits the new state throughout growth.

When plant biologists began to investigate the control of gene expression and to overexpress genes and their products by introducing transgenes into the plant genome, they encountered a great deal of variability in transgene expression that was at first attributed to inferred "position effects." However, it was soon learned that at least two kinds of trans effects contribute to this variability: one that appears to cause transcriptional silencing and another that acts posttranscriptionally. These effects are collectively termed "homology-dependent, or repeat-induced, gene silencing." Transcriptional silencing is usually a directional outcome whereby one gene silences another, as in the phenomenon of paramutation, known also as epigene conversion (*1*). Posttranscriptional silencing is usually reciprocal, resulting in the "cosuppression" of expression of homologous genes (*2*). Insights into the mechanisms behind cosuppression and epigene conversion have come from analyses of many plant genes, and this body of work has been extensively reviewed (*3*). Cosuppression is common in plants but has yet to be reported in fungi or animals. Possible mechanistic relations between epigene conversion and various transcriptional silencing phenomena in fungi and animals have been widely discussed (*3–5*).

This paper discusses cosuppression mainly in the context of physiological and developmental controls that can influence the cosuppression outcome. The purpose is to show how a hierarchy of mechanisms can explain the organization, diversity, and plasticity of cosuppression-based phenotypes and how this hierarchy can behave as a dynamic regulatory system, capable of orderly shifts in regulatory states, as in normal physiology and development. The emphasis is on a petunia flower color gene, chalcone synthase (CHS), because its visible phenotype allows us to monitor gene expression on a cell-by-cell basis, and loss of flower pigments has no pleiotropic effect on petal development. An important feature of petunia is its herbaceous, freely branching growth habit, which allows us to monitor the influence of physiology on gene expression and so to explore not only individual mechanisms but also the ensemble of mechanisms that together create complex ordered patterns of control.

CHS is necessary for the biosynthesis of anthocyanin pigments that are responsible for the blue, purple, and red coloration of many flowers. Introduction of a chimeric CHS transgene (in which a sense-oriented CHS coding sequence is driven by a heterologous promoter) results in white flowers that exhibit cosuppression of endogenous and introduced CHS transcripts (*6, 7*). The cosuppression state is defined by contrast with the "coexpression" of these transcripts in purple "revertant" flowers retaining the transgene, which shows that endogenous gene suppression is not caused simply by a high steady-state concentration of transgene transcript. Cosuppression is often referred to as "sense suppression" to avoid confusion with unrelated methods exhibiting similar outcomes (*8*). The mechanism of sense suppression is still unknown. The possibility of feedback regulation by the overexpressed enzyme or its biochemical product (*7*) has been ruled out in several systems, including CHS, by demonstration of the fact that truncated nonfunctional genes are sufficient to induce cosuppression (*9–11*). Organisms control transcript concentration by modulating transcript production and degradation. Through analysis of transcripts in isolated nuclei, sense suppression was found to occur posttranscriptionally (*12, 13*). Also, transcript turnover depends on transcription of the endogenous gene (*9*) and on transgene dosage (*12, 14*). In eukaryotes, transcription is controlled not only by the action of trans-acting factors on promoter elements but also by local effects of DNA sequence organization patterns and chromatin states, as in position-effect variegation (PEV) (*15*). Plant transgenes frequently exhibit variable expression patterns reminiscent of PEV that could account for the metastability of some cosuppression phenotypes (*16*).

Sense suppression of CHS in petunia produces, besides white flowers, an array of flower color patterns (*6, 17, 18*). However, unlike PEV, these patterns are organized according to three primary features of corolla morphology: veins, junctions between petals, and corolla edges (Fig. 1). Boundaries between white and purple sectors are many cells wide and exhibit steep gradients of pigment intensity from zero to wild-type

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amounts (*19*). In each flower, sector boundaries in upper and lower epidermis are coincident, which indicates that patterns are organized three dimensionally (*20*). Thus, cosuppression of CHS appears to follow fields of positional information generated by petal edges and veins.

Napoli *et al.* (*6*) recognized two classes of flower color patterns: "wedge" (Fig. 1, D to F) and "Cossack Dancer" (Fig. 1C). Wedge patterns are determined by petal junctions (fused edges of adjacent petals) and are now called "junction" patterns (*17*). These patterns vary widely in sector size (Fig. 1, D to F). Cossack Dancer patterns are determined by three corollar features: petal junctions, petal edges (outer, nonfused), and petal veins. This class of patterns varies considerably with respect to the orderedness of patterns, some versions being highly disordered (*6, 18*). Similar patterns ("star" and "picotee"; Fig. 1, A and B) were produced in 1838 by early plant hybridizers who created horticultural petunias from crosses among wild species (*21*). Unlike organ- and tissue-specific pigmentation patterns, this mode of pattern elaboration is not mediated by transcriptional control of the entire anthocyanin pathway. Rather, the breeders' patterns, like sense suppression patterns, exhibit transcriptional activation of the entire anthocyanin pathway throughout the corolla, then produce white sectors by posttranscriptional control of only CHS genes (*22*). This second stratum of control over gene expression increases the potential complexity and diversity of patterns elaborated during corolla differentiation.

Mechanism of Sense Suppression

Threshold-induced turnover and dynamic regulatory networks. Dougherty and co-workers (*23*) contributed the observation that transgenes expressing RNA homologous to cytoplasmically located viral RNAs are suppressed along with viral RNA. Consideration of this fact in light of the sense suppression phenomenon led them to propose the existence of a cytoplasmic threshold-sensitive system for destroying foreign transcripts produced in excessive quantities. They suggested that such a generalized turnover system might be made sequence specific by means of RNA-dependent RNA polymerases that would produce antisense RNA copies of the sense RNA.

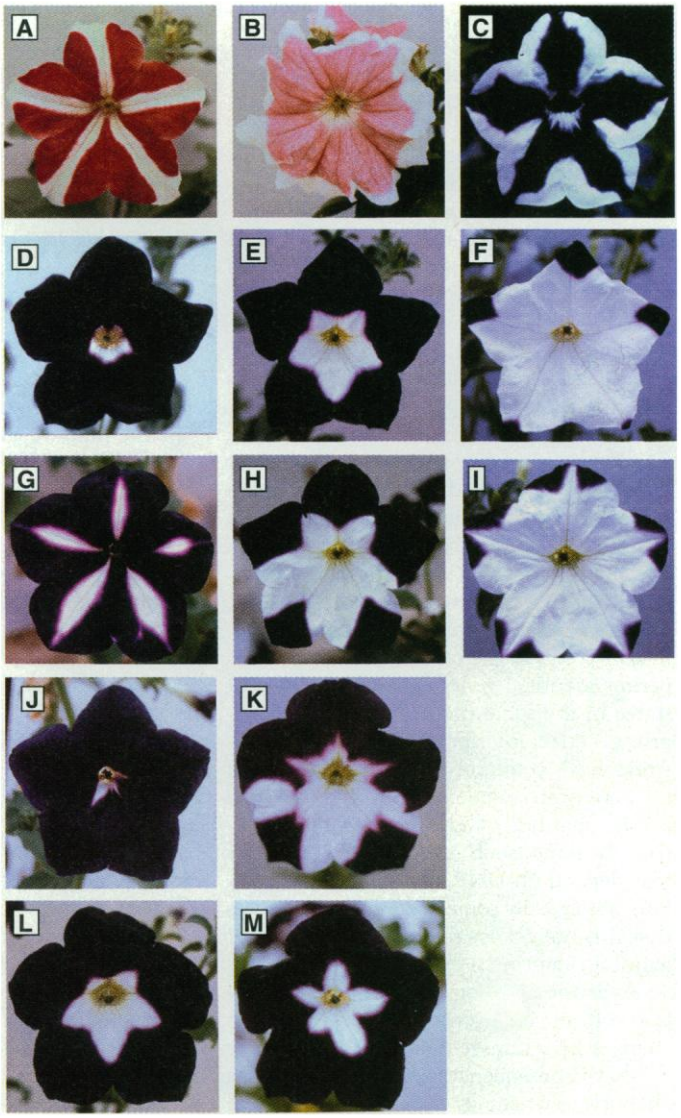

Fig. 1. Morphological control of the CHS cosuppression state. (**A** and **B**) are common patterns available from breeders. (**C** through **P**) result from CHS sense suppression. (D through F) are sexual progeny of the original hemizygous, white CHS41 plant backcrossed to its nontransgenic parent, V26. (G through I) were produced by regeneration (*43*) of a plant homozygous for the (putative) P epiallele of the transgene (epigenotype designated as 41 P/P). (J) was produced by a basal axillary branch of A667.3, a sexual progeny of CH541, hemizygous for the transgene locus. (K through M) were produced on plants from rooted cuttings of A667.3. [(C) is reprinted from (*6*)]

A more complex mechanism is that of dynamic regulatory networks, whose involvement in sense suppression was suggested by Meins and Kunz (*24*). Such networks can exist in multiple, stable regulatory states and can shift between states in response to internal variables (for example, concentrations of the system's components) or outside variables (such as physiological or developmental events). In the case of sense suppression, Meins and Kunz (*24*) propose a dynamic regulatory network based on positive autoregulation of coding sequence–specific turnover of homologous transcripts. A diffusible activator molecule, whose function is to maintain stability of the transcript, is assumed to be degraded in a first-order process; thus, the balance between synthesis and degradation of activator molecules determines concentration. This positive feedback loop, in which an activator is derived from the transcript that it stabilizes, produces multiple stable states of activator concentration and corresponding states of transcript concentration.

Whereas the Dougherty laboratory's model rests on a simple threshold response to transcript concentration and predicts only two turnover states ("on" and "off"), that of Meins and Kunz allows for multiple thresholds and multiple states, such as the "transgene on, endogenous gene off" state reported for CHS sense suppression in rose (*11*). Although the simple threshold model may explain some examples of sense suppression, the dynamic regulatory network model has the potential to explain more. Each model focuses on different aspects of the problem and neither is complete. Both are meant to be heuristic, that is, to stimulate interest as a means of furthering investigation, and are useful to keep in mind when evaluating experiments that address the sense suppression phenomenon.

Dynamics of sense suppression. In order for these models to be developed into testable hypotheses for the molecular mechanism of sense suppression, it will be necessary to consider the dynamics of sense suppression, such as in the context of the physiology and development of the plant. This was first done for the gene encoding polygalactonurase (PG) in ripening tomatoes (*9*). Before induction of the endogenous gene encoding PG during fruit ripening, transcripts from a transgene for PG are present at high steady-state concentrations; but al-

Table 1. Kanamycin-resistant progeny (*31*) of original white and purple states of CHS41. BC$_2$ and BC$_3$ are second- and third-generation backcrosses of CH541 to its nontransgenic parent V26. A white BC$_1$ plant was the female parent of BC$_2$. Nine PW and 13 PP plants from BC$_2$ were used as female parents to produce BC$_3$ populations, Data from BC$_3$ populations derived from PW parents were summed (ΣPW), as were data from progeny PP parents (ΣPP). SR indicates spontaneous revertant (Fig. 2A); TCR indicates tissue culture revertant (Fig. 1I). Intercrossing of 41 W and 41 P is described in (*36*).

Sexual cross	P (%)	PP (%)	IM (%)	PW (%)	Progeny (no.)	Populations (no.)
			41 W backcrosses to nontransgenic V26			
BC$_2$	0.3	45	0.3	54	294	15
BC$_3$ ΣPW	0	39	0.7	60	148	9
BC$_3$ ΣPP	0.4	36	4	60	242	13
BC$_3$ total	0.3	37	3	60	390	21
			Progeny of 41 P/P and revertants			
41 P/P × V26	100	0	0	0	418	10
SR × V26	44	13	10	33	70	2
TCR × V26	0	44	0	56	36	1
			Intercross of 41 W and 41 P			
W/− × V26	1	41	2	55	1243	56
P/− × V26	95	5	0	0	113	6
W/P × V26	68	26	1	4	807	37
W/P corrected	42	47	3	8	403	5

most immediately upon induction of the endogenous gene, both genes' transcripts are cosuppressed.

The simple threshold model would suggest that the concentration of the PG transgene's transcript happens to be poised just below the threshold, close enough that a small amount of additional transcript from the endogenous gene would be sufficient to trigger the suppression state. The dynamic regulatory network model would make a similar suggestion but is more flexible in that it predicts multiple stable states, including intermediate states that may make progressive changes in state possible. Meins and Kunz (*24*) cite the metastability and multiple states exhibited by the CHS sense suppression system as being consistent with their model.

Gene dosage experiments. The induction of cosuppression at the onset of expression of the endogenous gene encoding PG suggests sensitivity to small inputs. Another way to test this is to alter transgene dosage. For glucanase and chitinase transgenes in tobacco, it was found that both the transgene and the endogenous gene are expressed in plants hemizygous for the transgene, whereas progeny homozygous for the transgene exhibit cosuppression, which suggests a sharp threshold (*12*, *14*). The effect of transgene dosage on CHS sense suppression in petunia was tested by crossing of a line that exhibits predominantly purple flowers (fully purple corolla limbs, white junction-based sectors restricted to the corolla tube, and white anthers) with four other lines, each carrying a different transgene locus and exhibiting a fully purple corolla. Progeny carrying two loci were invariably pure white, whereas all progeny carrying one locus resembled the parent (*25*). Thus, one additional copy of the CHS transgene was sufficient to cause the system to shift into the cosuppression state in all cells of the corolla limbs, which indicates that the coloration phenotype is highly sensitive to small quantitative changes in gene dosage and perhaps in gene expression levels.

Thresholds and boundaries in physiology and development. The concept that shallow gradients and thresholds produce sharp boundaries in a developing biological system (*26*) has received a great deal of attention. In animals, there is now strong evidence that cells can respond differentially to as little as twofold variations in signal concentration (*27*). CHS sense suppression patterns have very steep boundaries, and the gene dosage experiment demonstrates a dramatic inverse amplification of a small relative change. It is conceivable that factors directly or indirectly influencing the cosuppression state vary spatially in a shallow concentration gradient defined by fields of positional information and that the steep pigmentation gradient reflects the extreme nonlinearity of the cosuppression response.

Diverse Metastable States of CHS Expression

Plant transgenes are frequently found to be epigenetically unstable and can exist in a multiplicity of transcription states of varying expression and stability (*16*). Two types of mechanisms for maintenance and transmission of transcription states, termed "steady-state systems" and "chromatin-marking systems" by Jablonka *et al.* (*28*), have been distinguished. Steady-state systems are the transcriptional regulatory networks proposed by Monod and Jacob (*29*). Chromatin-marking systems (also known as "imprints") are based on patterns of DNA methylation or metastable protein-DNA (chromatin) complexes or both (*4*, *30*). Chromatin imprints differ from transcriptional regulatory networks in that they are higher order structures that determine regulatory states at the chromosomal level and permit identical genes to exist in different states in the same nucleus. Chromatin imprints and transcriptional regulatory networks have common ground in the case of transcription complexes that are stabilized in the manner of chromatin states. (Although some such chromatin complexes may depend on DNA methylation, the latter's absence in some eukaryotes indicates that it is not always required to establish or maintain imprints.)

As is the case for developmental induction of an endogenous gene, epigenetic changes in a transgene's transcription state will have consequences for sense suppression phenotypes whenever they cause the system to cross a sense suppression threshold (*23*). For systems close to a threshold, even a small change in transcription could be amplified into a major phenotypic change. The sensitive CHS sense suppression system behaves metastably in certain lines and produces a diverse series of expressed states, some qualitatively distinct and some germinally transmissible. Different states can interact in paramutation-like fashion, with one causing a heritable change in the other. Changes in state may arise in an orderly fashion in response to physiological and environmental information.

Genetic analysis of heritable states of CHS *expression.* The most interesting example of complex regulatory dynamics shown by plants that are subject to sense suppression of CHS is provided by somatic sports and sexual progeny of a transgenic petunia line called CHS41, which in its original state (W) produced pure white flowers (*6*), but over time and generations has yielded a wide variety of different flower color patterns (Fig. 1, D to M).

One variant of the white state of CHS41 is a predominantly purple (PP) version of the junction pattern (Fig. 1D), which arises at high frequency in sexual progeny (Table 1). This pattern is relatively stable during plant growth, especially along a branch; but new axillary branches occasionally switch to a new state in which they produce predominantly white (PW) flowers (Fig. 1F). Intermediately pigmented (IM) flowers (Fig. 1E) are less common. PW plants rarely revert to the PP state during normal plant growth but frequently do so through sexual reproduction. Both PP and PW plants yield sexual progeny of both types at similar frequencies, which indicates that these states are interconvertible when passed through

Table 2. Inheritance of induced epigenetic states of plant A667.3.

Sexual cross	P (%)	PP (%)	IM (%)	PW (%)	Progeny (no.)	Populations (no.)
Upper branches × V26	16	22	23	39	162	4
Basal branch A × V26	70	26	3	0.6	159	4
Basal branch B × V26	60	29	6	5	188	5
Basal branch C × V26	80	20	0.5	0	187	5
Basal branch A-vp* × V26	99	0	0	1	75	2
Basal branch B-vp × V26	4	38	0	58	206	5
Basal branch C-vp × V26	99	1	0	0	344	6

*vp indicates vegetatively propagated.

the germ line (*31*) (Table 1), though the degree of interconvertibility can decrease over generations (*32*).

Plants with white or predominantly white flowers occasionally produce a different type of variant that is fully purple (P) (*6*). The P state is somatically stable and germinally transmissible (Table 1), but occasionally reverts to the white state (Fig. 2A) and is changeable into other states (Fig. 1, G to I). Derivatives include the junction-pattern (Fig. 1H) pure white flowers, a midvein-based "star" pattern (Fig. 1G), and a similar pattern in which the star pattern is superimposed on a junction pattern (Fig. 1I). The P state and revertant states are heritably distinct (Table 1).

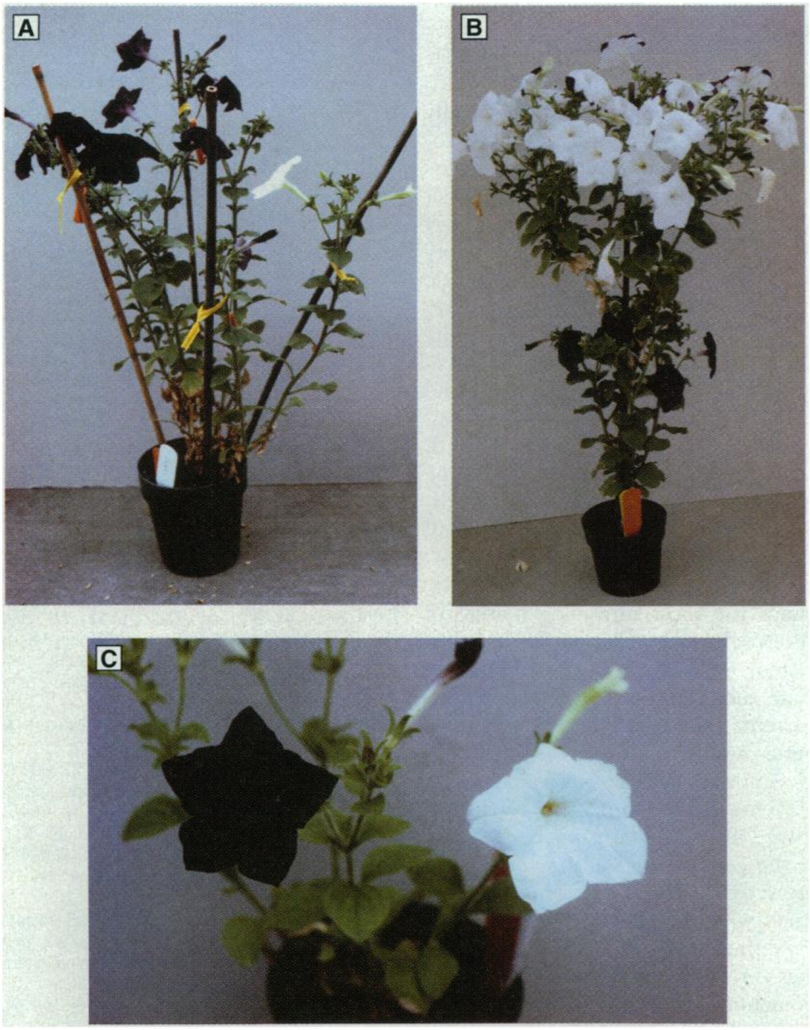

Fig. 2. Plants exhibiting somatic changes in state of cosuppression. **(A)** is a homozygous plant derived by selfing the P state of CHS41. The white-flowered branch is a heritable somatic revertant to the original white state (Table 1). **(B)** is plant A667.3. All upper branches produced PW flowers. Three basal branches, A, B, and C, produced purple or patterned flowers. At the stage shown, each of these three basal branches has produced one purple or predominantly purple flower. Two of the branches are on the lower right and one is on the lower left of the central stem of A667.3. **(C)** is a PP plant in which a nonclonal change to the PW state has occurred.

The complex and progressive origin of some P variants is illustrated by a PW plant (A667.3) that gave rise to new states in each of three adjacent basal axillary branches (in contrast to axillary branches from upper nodes, which still produced PW flowers) (Fig. 2B) (*33*). Two basal axillary branches, A and C, exhibited a new vein-based pattern (Fig. 1J), whereas basal branch B gave fully purple flowers. Most sexual progeny of these branches were in the P state or a new PP state with vein-based patterns (*31*, *34*) (Table 2). This new vein-based state was unstable, being changeable into both purple and junction states (Fig. 1L) as well as another state marked by a different vein-based pattern (Fig. 1M) (*35*). Thus, changes in state can be both reversible and heritable, both somatically and germinally. Some changes appear to be progressive, as in the transition of A667.3 from the junction-based pattern to vein-based patterns and then to the fully purple state.

These states are not known to be chromatin based, but their germinal transmission would suggest that they are. The differences between some states of CHS41 can be qualitative in that they elicit patterns based on different morphological inputs. Imprinting of transgenes is often thought to occur stochastically and to lead to gene inactivation (allowing an on and an off state, with perhaps intermediate states that differ only quantitatively). The qualitative changes in state in the CHS system indicate the existence of a series of functional on states.

Paramutation-like interactions between states. Intercrossing of the original white state of CHS41 with its derived purple state produces paramutation-like interactions between these states whereby the white state becomes altered toward the purple state (Table 1) (*36*). One explanation for such an outcome is a merging of dynamic regulatory states (*24*). However, in several well-characterized examples of transgene paramutation, a strict correlation with DNA methylation has been observed, which suggests a chromatin-based mechanism (*3*). As noted, there is common ground between these mechanisms in the form of promoter-localized, chromatin-based transcription states.

Consideration of transvection in flies and paramutation in plants has led to the suggestion that chromatin-based transcription states can be transmitted between alleles or homologous genes through homology-sensing interactions that permit the exchange of factors that determine chromatin-based transcription states (*37*). Several examples of paramutation have been shown to require promoter homology (*3*), which is consistent with the proposal that active chromatin states can be promoter based (*4*). If so, it is easy to see how a series of active chromatin states could be responsible for the diversity of qualitatively distinct phenotypic states of CHS expression (Fig. 1, D to M). States could differ by the incorporation of different transcription factors into the transgene's transcription complex, each state being based on a factor controlled by a particular gradient of positional information, and so generate a particular pattern.

Nonclonal origin of somatic changes in state. The junction pattern's PW state frequently arises in basal axillary branches of plants in the pattern's PP state (Fig. 2C). Hundreds of these somatic events have been observed and none have given any indication of clonal sectors, which indicates a nonclonal origin for these events. Thus, the induction of these events appears to involve the entire meristem. But only a minority of meristems impose these changes of state and so some other factor, perhaps an environmental one, must play a role as well. Support for an environmental role comes from the observation that the frequency of PW plants differs significantly between plantings of seed from single fruits (*38*). Whether a progeny plant adopts the PP or PW state appears to be determined after germination when the apical meristem is already fully formed. The simplest interpretation may be that shoot meristems can exist in two physiological states, and that the epigenetic state producing the PP pattern is labile to one meristem state and stable to the other.

The events in basal axillary meristems of plant A667.3 (Fig. 2B) are also nonclonal events, encompassing an entire meristem at each of three adjacent nodes on different sides of the stem. It is tempting to speculate that the state that induces these nonclonal events might be the juvenile phase of development, and that adult-phase meristems are less capable of inducing these events. The plasticity and reversibility so often observed in plant growth and development are largely based on the capacity to produce new meristems in axillary (and adventitious) positions and to reversibly establish states of meristem potential. The observation that a transgenically elicited expression state can be responsive to the environment of a meristem is both surprising and potentially useful in exploring the nature of shoot meristems.

Summary and Perspectives

At least three different molecular mechanisms require elucidation before we will fully understand the diversity of outcomes in a typical sense suppression experiment: (i) the induction of transcript turnover, (ii) the establishment and maintenance of epigenetic gene expression states, and (iii) paramutation-like interactions among gene expression states of homologous transgenes that heritably alter epigenetic states. Because of this complexity, careful investigation of phenotypes in the whole organism under a variety of conditions is an important complement and guide to appropriate molecular investigations of sense suppression.

The organization, diversity, and plasticity of the patterns produced by sense suppression of CHS are based on a surprisingly complex, dynamic regulatory system comprised of multiple interfacing mechanisms, probably along the following lines: (i) Morphologically controlled transcription factors subtly influence transgene transcription to produce a shallow gradient in transcription rate varying spatially according to corolla morphology. (ii) Threshold-sensitive induction of a state of high transcript turnover is triggered in cells that exhibit excessive production of CHS transcripts, inversely amplifying the transcription gradient where the threshold is reached. (iii) The epigenetic state of the pigment transgene discriminates among the available morphologically controlled factors, determining which features of corolla morphology influence transgene transcription and thereby the turnover state. (iv) Certain changes in physiology and development produce new regulatory environments that reformat epigenetic states and thereby alter a transgene's subsequent responsiveness to morphogenetically determined factors. For any such system that is far from chemical equilibrium and comprised of multiple nonlinear reactions, unpredictability is to be expected (*39*). However, such systems can potentially be stabilized with appropriate feedback mechanisms and sensitively controlled with small inputs, exhibiting great flexibility in performance (*40*).

Perhaps the most intriguing feature of the pattern-eliciting, CHS sense suppression system is the degree to which these plants are subject to highly ordered, nonclonal events that heritably reprogram the epigenetic state of the plant, perhaps through imprints at the transgene locus. These events recall Brink's (*41*) concept, put forward in 1960, of a paragenetic function of the genome in plant development. In contrast to the genetic function, which is concerned with heredity and embodied in the DNA, the paragenetic function of chromosomes is to receive, record, and transmit developmental information and is embodied in what Brink referred to as "parachromatin" (*42*). Because parachromatin replicates in phase with DNA, he suggested that it is a quasigenetic material. As "the meeting ground of the genetic and epigenetic functions of chromosome," it effects communication between the DNA and the constituents of the nucleus outside of chromatin. It was an idea soon eclipsed by the compelling simplicity of the regulatory network hypothesis of cellular differentiation advanced by Monod and Jacob (*29*), and by the accessibility of bacteria to genetic investigations of the mechanisms of gene regulation. However, viewed from a modern perspective informed by advances in molecular genetics, Brink's concept of parachromatin can be seen as a further elaboration on the Jacob-Monod hypothesis, one that recognizes the dynamic, promoter-based regulatory complexity of active chromatin states and their potential as a chromosomally based information storage system for physiology and development.

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31. All progeny in Tables 1 and 2 are kanamycin resistant. Nontransgenic segregants were removed by selection on kanamycin agar at 200 μg/ml . Kanamycin-resistant seedlings were transplanted to soil and grown to flowering in a greenhouse, with supplementary lighting in winter. V26 is the inbred line into which the CH541 locus was introduced.
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33. A667.3 first produced PW flowers, which were pollinated. After fruit harvest, it was pruned just below the first flowering node (approximately 20 nodes above the base of the plant). In petunia, each node has a single leaf and in the axil of each leaf is a dormant axillary shoot. Pruning releases axillary shoots from dormancy.
34. Backcross progeny of basal branches produced many P and few PW plants. PP plants often exhibited vein-based patterns (Fig. 1J), and IM plants possessed both vein and junction influences (Fig. 1K). Many more progeny of the upper branches were fully purple (Table 2) than are usually observed in CHS41. PW plants that did not exhibit such instability (as in Table 1) and vein-based sectors (as in Fig. 1K) were common, indicating that A667.3, although phenotypically PW, was already in a new state that permitted its further change to new purple and patterned states. These purple and vein-patterned states were transmissible to a second backcross generation (*32*).
35. These changes were observed in plants grown from rooted cuttings of the three revertant branches. Each branch produced a plant with a different pattern. Two distinct classes of vein-based patterns were produced: sharply pointed tips (as in Fig. 1, K and L) by branch A and rounded tips (Fig. 1N) by branch C. The junction-based PP pattern J^{PP} (Fig. 1M) was produced by branch B. Each was stably maintained during subsequent plant growth. Progeny of these two vein-based pattern states were almost exclusively purple, indicating full progression to the purple state, whereas progeny of the J^{PP} pattern state were essentially the same as for any plant exhibiting that pattern, which indicates full reversion to the original interconvertible pair of PP and PW junction pattern states (Table 2).
36. This cross produced three epigenotypes: (i) hemizygous for *T-CHS41*, a transgene deriving from the white parent (W/–); (ii) hemizygous for *T-CHS41*, a transgene deriving from the purple parent (P/–); and (iii) "homozygous" for *T-CHS41*, each parent contributing one allele (W/P). In outcrosses to V26, W/– plants yielded progeny with patterns in the ratios typical of such plants, whereas P/– plants produced mainly purple progeny as well as a few PP progeny, but no PW progeny. W/P "homozygotes" (which were always white-flowered, as might be expected from the gene dosage effect), in an outcross to segregate the two transgene alleles, yielded mostly fully purple progeny, many PP progeny, and few PW progeny. After correction to remove the 50% purple progeny whose transgene derived from the purple grandparent, it was seen that nearly half of the progeny receiving the transgene from the white grandparent were purple and most of the rest had the PP phenotype. The results are consistent with the possibility that the purple state is a stably transmitted epiallele of the transgene and the white state is a paramutable epiallele of the transgene.
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38. In the second and third backcross (BC_2 and BC_3) generations, the predominantly purple state of the junction pattern (J^{PP}) and the predominantly white state of the junction pattern (J^{PW}) were equally likely when averaged over multiple populations (Table 1). However, individual populations deviated substantially from this average. Some populations produced over 90% J^{PW} patterns and some produced over 90% J^{PP} patterns. Different plantings of seed from the same fruit frequently resulted in significantly different frequencies of the two patterns.
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43. Regeneration from tissue explants grown in culture often yields plants with heritable epigenetic changes. Alterations in the 41P/P genotype were induced at high frequency by regeneration of adventitious shoots on leaf explants (*3*).
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