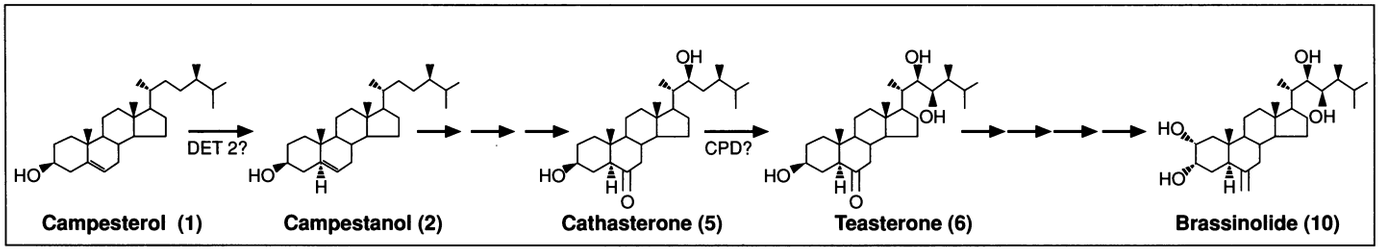

Fig. 2. The biosynthetic pathway of brassinolide. Genetic evidence in *Arabidopsis* suggests that the *DET2* and *CPD* gene products catalyze the indicated reactions in the multistep pathway leading to brassinolide. [Courtesy J. Chory, Salk Institute]

steroids termed brassinosteroids, and that these steroids may play a role in the regulation of gene expression by light.

A conclusive demonstration of this role is provided by Szekeres *et al.* (*4*). These authors show that a pleiotropic phenotype arising from mutation of the *Arabidopsis* *CPD* gene, which includes disregulation of light-responsive genes, dwarfism, male sterility, and activation of stress-response genes, can also be traced to a defect in brassinolide synthesis. The DNA sequence of the *CPD* gene reveals it to be a member of the cytochrome P450 class of enzymes, with distinct sequence identity to P450s involved in mammalian steroid hormone biosynthesis. Because steroid intermediates in the brassinolide pathway with a 23-hydroxyl group successfully reverse the phenotypes arising from mutation of the *CPD* gene, whereas those that lack this modification do not, it seems likely that the CPD enzyme catalyzes the 23-hydroxylation of cathasterone to form teasterone, which is thereafter converted into brassinolide. Szekeres *et al.* (*4*) go on to show that brassinolide will overcome the pathologies associated with at least five other *Arabidopsis* mutations (*det1*, *cop1*, several *fus* mutations, *dim1*, and *axr2*), each of which lead to disruptions in light-mediated development. These results implicate brassinolide as a major player in the growth and differentiation of *Arabidopsis*.

The studies of Li *et al.* (*1*) and Szekeres *et al.* (*4*) reveal the tip of an iceberg that will affect disciplines ranging from botany to biochemistry to endocrinology. We can look forward to the elucidation of several processes including the mechanism of intracellular signaling through which brassinosteroids work, the role of steroid reductases and P450 monooxygenases in the biosynthesis of this widely distributed class of compounds, and the cross-talk that must occur between light-activated regulatory systems and those that respond to steroid hormones, and perhaps we will also gain insight into nonreceptor-based actions of steroid hormones, as it is rumored that plants lack DNA sequences common to mammalian steroid hormone receptors (*5*). Finally, the development of therapeutic inhibitors of steroid 5α-reductase is a thriving area in the pharmaceutical industry (*3*). At least some of these inhibitors come from plant sources (*6*), and it is conceivable that by acting on DET2-like enzymes, or other enzymes in the pathway, they serve as endogenous regulators of brassinosteroid synthesis. The seeds of the *Arabidopsis* findings will sprout much future research!

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Histone Deacetylase: A Regulator of Transcription

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Histones, nuclear proteins that interact with DNA to form nucleosomes, are essential for both the regulation of transcription and the packaging of DNA within chromosomes. The stability and positioning of chromatin structures determine whether nucleosomes repress (*1*) or activate transcription (*2*). Mutation of individual histones in vivo alters the general organization of chromatin throughout the eukaryotic nucleus, yet the concomitant changes in gene expression are highly selective (*3*). This selectivity appears to depend on the assembly of specific nucleoprotein architectures. Within these structures, transcriptional regulators make direct contact with specific domains of individual core histones (*4*, *5*). Targeting of these "chromatin organizers" to particular genes, through protein-protein or protein-DNA interactions, can account for the selective impact of histone mutations on gene expression. This conceptual framework only provides a static image of gene regulation. In reality, events are much more dynamic, as highlighted in a report in this week's issue of *Science* (*6*) describing the cloning of one of these organizers, histone deacetylase.

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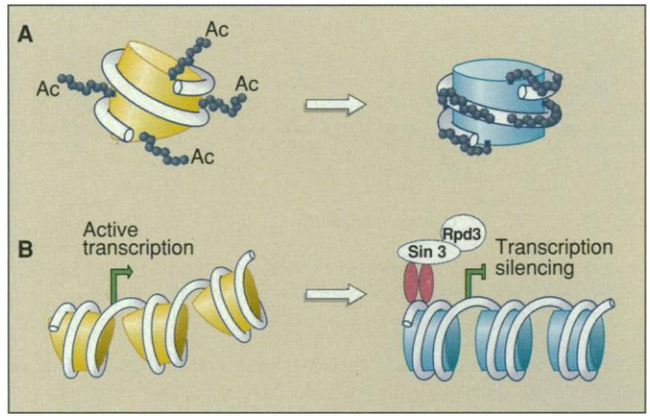
A major source of dynamic variation in chromatin structure in vivo is histone acetylation. Acetylated lysine residues in the NH_2-terminal tail domains of nucleosomal histones serve as landmarks for transcriptionally active chromatin within the chromosome (*7*). Hyperacetylation of the histones reduces their ability to constrain the path of DNA within chromatin, resulting in allosteric changes in nucleosomal conformation (see figure, part A) (*8*), destabilization of internucleosomal contacts (*9*), and an increase in the accessibility of nucleosomal DNA to transcription factors (*10*). The elimination of histone acetylation is correlated with transcriptional silencing (*11*). The amount of histone acetylation is determined by an equilibrium between histone acetyltransferases and deacetylases. Thus, chromatin structure could be reversibly modulated to activate or silence transcription by targeting histone acetyltransferases or deacetylases to a particular gene. This model now receives strong support from the recent purification and molecular characterization of a human deacetylase with remarkable identity (~60%) to the *Saccharomyces cerevisiae* transcriptional regulator Rpd3p (*6*).

Rpd3p is a global transcriptional regulator required for target genes to achieve maximal transcriptional efficiency (*12*). Without Rpd3p, both the activation and

repression of regulated genes is less effective. Thus, the range of transcriptional regulation achieved in vivo is amplified by Rpd3p. Although they display dramatic evolutionary conservation, neither the human deacetylase nor Rpd3p has recognizable functional motifs. Rpd3p does not bind to DNA, nor does it have biochemically defined interactions with other proteins. However, genetic experiments do indicate a close functional relation with another global transcriptional regulator, Sin3p (*12*, *13*). *RPD3* and *SIN3* participate in the same transcriptional regulatory functions and appear to be components of one pathway. Both genes are required for the regulation of inducible genes responding to external signals (*PHO5*), cell differentiation (*SPO11* and *SPO13*), and cell type (*HO*, *TY2*, and *STE6*). Sin3p is not itself a DNA binding protein but contains paired amphipathic helices and tetratricopeptide repeats that are known motifs for protein-protein interactions. Sin3p is proposed to interact with bona fide yeast DNA binding proteins (*14*). Targeting of Sin3p by fusion to a DNA binding domain will direct transcriptional repression (*15*). Thus, Rpd3p and Sin3p function alternately as components of co-activator or co-repressor complexes that are targeted to particular genes by DNA binding transcription factors. How might these contrasting functional requirements be realized?

An attractive model builds on the known consequences for chromatin structure of histone acetylation. For the vast majority of genes regulated by Sin3p, the regulator acts as a repressor (*15*). Thus, Rpd3p could augment the activity of Sin3p by directing the equilibrium levels of histone acetylation toward the deacetylated state with the concomitant stabilization of nucleosomal and higher order structure (see figure, part B). Histone deacetylation may also facilitate the association of other repressive transcriptional regulators within a chromosomal domain (*4*).



How acetylation and deacetylation may change nucleosome structure and influence transcription. (**A**) (Left) Acetylation (Ac) of the core histone tails (black) releases them from contact with the DNA (white). This process leads to a conformational change in the interactions of core histones (yellow) with DNA, thereby loosening nucleosomal structure. (Right) Deacetylation stabilizes contacts of the histone tails with nucleosomal DNA, which in turn stabilizes the interaction of the core histones (blue) with DNA in the nucleosome. (**B**) (Left) Transcriptionally active chromatin possessing a transcription start site (hooked arrow) contains hyperacetylated histones. Three unstable nucleosomes (yellow) containing acetylated histone tails are shown. (Right) Transcriptional silencing is accomplished by targeted deacetylation of the core histones. DNA binding proteins (red) recruit Sin3p, which together with histone deacetylase or Rpd3p modify the histones in the vicinity of the promoter, thereby generating more stable nucleosomes (blue). Transcription initiation (hooked arrow) is inhibited.

The role of Rpd3p in transcriptional activation is more difficult to explain; however, recognition of the architectural role of histones provides a solution. Once transcription factors are recruited to a particular promoter, the specificity of their interactions might depend on a tightly constrained chromatin environment (*2*, *5*). It is also possible that the influence of Rpd3p might be more indirect: Rpd3p might be required for the general packaging of DNA into restricted chromatin environments. Chromatin structure likely limits the number of sites a regulatory molecule has to search before a productive interaction occurs (*16*). In the absence of histone deacetylation, a larger number of nonproductive interactions likely occurs, diverting transcription factors and RNA polymerase from the gene of interest. Thus, histone deacetylation within the bulk of chromatin that contains many inactive genes could facilitate the selective recruitment of the transcriptional machinery to a specific active gene.

A key point emphasized by these new results is that chromatin structure modulates gene activity. As many in vitro biochemical experiments on naked DNA demonstrate, genes can be regulated in the absence of chromatin. However, the range of regulation achieved in this way is much smaller than that in the chromosomal environment. *RPD3* is not essential for transcriptional activation or repression, but it has a significant impact on regulation of particular genes by amplifying activation and repression mechanisms.

The molecular characterization of the histone deacetylase (*6*) opens a door to understanding how dynamic chromatin structural transitions can be targeted to particular chromosomal regions. Such targeting reflects the functional differentiation of the chromosome. We might anticipate that other enzymatic activities that modify chromatin structure—such as histone acetyltransferases and kinases—will exhibit a comparable functional localization. Communication between architectural proteins, such as the histones, and the transcriptional machinery is an essential component of life in the nucleus.

Enhanced Perspectives

On 9 February, *Science* launched a new feature called Enhanced Perspectives on our World Wide Web site. Going beyond the limits of the printed page, Enhanced Perspectives contain active links to the wider world of Internet resources, including molecular biology databases, image archives, and the National Library of Medicine's Medline service. Each Enhanced Perspective contains Hypernotes, which were created by the cyberlibrarians of Stanford University's HighWire Press and which take the reader directly to WWW sites of immediate relevance to the topic under discussion. The editors of *Science* invite you to examine the Enhanced Perspectives, available at http://sciencemag.aaas.org/science/content/current/e-perspectives/.

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