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COMMENTARY

Studying plant development: an alternative to “spray and pray”

In this issue, Klee et al. (1987) report the endogenous overproduction of the plant hormone auxin, and the effect it has on plant development. This is a major advance over previous studies, all of which depend on exogenous hormone application, or disrupting the integrity of the plant. The *in vivo* manipulation of endogenous hormone

levels makes it now possible to characterize the pleiotropic effects of hormones throughout the life cycle of the plant. Whereas animal cells become committed to a specific developmental program at an early stage, plant cells retain an amazing degree of flexibility (Wareing and Phillips 1981). Even differentiated cells can be repro-

grammed in response to hormone levels. For instance, depending on the relative levels of two classes of hormones, auxins and cytokinins, leaf cells can regenerate into roots or shoots (Skoog and Miller 1957). Thus, plant hormones are crucial to the developmental programming of each cell.

Since their discovery in the 1930s auxins have been shown to affect many processes in plants, including cell enlargement, root differentiation, xylem formation, and apical dominance (the capacity of the shoot apex to inhibit the outgrowth of lateral buds). The study of apical dominance provided the first indication that a hormone-like substance was active in plants. The shoot apex manufactures indole-3-acetic acid (IAA, one of several known auxins) that is actively transported downward and, by an unknown mechanism, suppresses lateral growth. Auxins may also induce cell enlargement. When cell enlargement occurs in one cell layer but not in another, changes can occur in the direction of growth. For example, a longitudinally split maize coleoptile responds to auxin application by a dramatic inward curling of the split halves. Curling occurs because the cells of the outer epidermis enlarge more than the inner layers. Antibodies to an auxin-binding protein prominent in epidermal cells inhibit the response (Lobler and Klamt 1985). This is the first case in which a link has been made between a plant hormone receptor and a physiological response.

In most cases the diverse effects of auxins have been studied in disrupted plants, explants, or cell culture. Each of these systems has greatly enhanced our knowledge of individual auxin effects. Yet, the interrelationships among such effects are often difficult to assess. The networking of auxin responses, which is vital to normal development, needs to be studied in the intact plant and throughout its life cycle. In this issue Klee et al. demonstrate that such experiments are now possible. These authors have elegantly harnessed the capacity of the plant pathogenic bacterium *Agrobacterium tumefaciens* to produce IAA from tryptophan. Two *Agrobacterium* genes are involved in this process (Schroder et al. 1984; Thomashow et al. 1986). The *iaaM* gene product converts tryptophan to indole-acetamide (IAM), which the *iaaH* gene product converts to the auxin. Each of these genes was expressed in transgenic petunia using strong plant promoters. Overproduction of the *iaaH* gene product had no phenotypic effect, confirming previous reports that IAM is not a natural intermediate in the plant auxin biosynthetic pathway. However, overexpression of the *iaaM* gene resulted in a 1000-fold overproduction of IAM and raised the endogenous IAA levels by 10-fold. This indicates that IAM is aspecifically converted to IAA in vivo. The transgenic plants exhibited many previously documented auxin responses. Enlargement of cells in all cell layers but the lower epidermis resulted in leaf curling. The plants appeared more woody due to increased xylem formation, and many roots formed in unusual places. Apical dominance was extreme in the transgenic plant even after removal of the shoot apex. Most surprisingly, these plants were fertile,

emphasizing the enormous flexibility of plant development.

In addition to the experiments described here, the overproduction of the *iaaH* and *iaaM* gene products in transgenic plants offers considerable future potential. The *iaaH* gene, which was not associated with an abnormal phenotype when overexpressed, may be useful as a bifunctional selectable marker. When cells from normal plants are cultured in vitro, auxin is required for growth but high auxin concentrations inhibit growth. Cells expressing the *iaaH* gene respond in a similar manner to naphthalene acetamide (NAM, a stable analog of IAM). By fusing this gene to an upstream regulatory region of interest, up-mutations can be selected for on low NAM media and down-mutations on high NAM media. At present, no negative selective marker exists for plants. The availability of such a marker will be particularly helpful in obtaining mutations in regulatory genes.

The most interesting prospects of Klee et al.'s in vivo approach lie in the area of studying hormonal effects on plant development. Their experiments involve overexpression of the *iaaM* gene from the 19S promoter which is assumed to be constitutive. Achieving temporal or tissue-specific overexpression of auxin is a logical next step. By using, for instance, a heat shock promoter, it will be possible to overproduce auxins at any time point during development. Similarly, tissue-specific promoters can be used to restrict auxin synthesis to certain cell types. It will be interesting to see the long-term effects of reversing the normal auxin gradients.

One can draw a parallel between Klee et al.'s experiments with auxin overproduction and the growth factor overproduction experiments in transgenic mice (Palmiter et al. 1983) because both manipulations affect growth. Yet this is only one parameter affected by auxin in plants. It is still unknown why auxin prompts some cell types to just enlarge or elongate while others form roots. Does auxin turn on different genes in different cells? A number of cDNAs have been isolated corresponding to genes that are differentially expressed in auxin-treated versus auxin-depleted tissue. The function of their protein products is unknown with the exception of a gene coding for β -1,3-glucanase, a cell wall-degrading enzyme, which could play a role in cell enlargement (Mohnen et al. 1985). These auxin-responsive genes may now be analyzed for their tissue-specific expression in the auxin-overproducing plants, giving a clue to their function. Such in vivo experiments will greatly advance our understanding of the pivotal role of auxins in plant development.

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