LETTER TO THE EDITOR

The Role of Auxin in Plant Embryogenesis

In the June 1993 issue of THE PLANT CELL, the research paper by Liu et al. (1993) and the review article by Chasan (1993) addressed the important question of the role of auxin in plant embryogenesis. These articles are especially noteworthy because they discuss the literature on both zygotic and somatic embryos, each of which offers distinct advantages for studying the underlying mechanisms of plant embryogenesis. The intent of this letter is to place the work of Liu et al. (1993) in the context of what is already known about the transport, activity, and biosynthesis of auxin during plant embryogenesis.

Polar auxin transport has long been postulated to play a central role in plant embryogenesis. Microscale auxin transport assays have been used to show that the hypocotyls of mature embryos dissected from both gymnosperm and angiosperm seeds exhibit pronounced polar auxin transport toward the root end irrespective of their orientation (Greenwood and Goldsmith, 1970; Fry and Wangermann, 1976). This polar transport is completely blocked by 10 μM 2,3,5-triodobenzoic acid (TIBA) (Greenwood and Goldsmith, 1970), which suggests that the TIBA-mediated effects on cultured zygotic embryos observed by Liu et al. (1993) are directly attributable to the inhibition of polar auxin transport as opposed to being a secondary effect unrelated to auxin transport. In addition, Fry and Wangermann (1976) were the first to propose that the initiation of polarized auxin transport in the globular embryo might mediate the morphological polarity expressed in the later stages of plant embryogenesis.

The possible role of polar auxin transport in somatic embryogenesis was tested by Schiavone and Cooke (1967), who treated different stages of carrot somatic embryos with TIBA and a different auxin transport inhibitor, N-(1-naphthyl)phthalamic acid (NPA). Both auxin transport inhibitors at a concentration of 1 μM are able to block the ability of somatic embryos to undergo morphogenetic transitions to the subsequent stages: globular embryos undergo persistent spherical expansion, oblong embryos (an intermediate stage in somatic embryogenesis) continue axis elongation without any cotyledon initiation, and heart embryos develop additional growth axes on their hypocotyls. Further evidence that polar auxin transport is regulating somatic embryogenesis comes from microsurgical investigations, which have established that cut pieces of somatic embryos can often regenerate shoot structures from their apical ends and root structures from their basal ends (Schiavone and Racusen, 1990, 1991). Because the regeneration process is sensitive to exogenous auxin as well as to transport inhibitors (Schiavone, 1988), it is quite conceivable that the ability of developing somatic embryos to maintain structural polarity is also dependent on polar auxin transport.

In contrast to Schiavone and Cooke’s (1967) observations that carrot somatic embryos are profoundly affected by polar auxin transport inhibitors, Liu et al. (1993) reported that similarly treated zygotic embryos of Brassica juncea exhibit typical axis elongation and cotyledon positioning at the embryonic apex, but the cotyledons emerge as a fused collarlike structure around the apex rather than as two discrete lateral structures. Their interpretation that a reduction in polar auxin transport is responsible for the altered cotyledon morphology was strengthened by their finding that the pin1-1 mutant of Arabidopsis, which is known to have reduced auxin transport in its inflorescence (Okada et al., 1991), exhibits the same collarlike cotyledon structure in its embryo.

We believe that the difference in the responses of carrot somatic embryos and B. juncea zygotic embryos to auxin polar transport inhibitors suggests a provocative hypothesis about the role of auxin in embryonic pattern formation. If we assume that these differences cannot be ascribed to species differences or other trivial factors, then it seems reasonable to hypothesize that embryonic pattern formation is actually regulated by two overlapping mechanisms: (1) a positional mechanism that arises as a maternal effect from the ovular tissue surrounding the zygotic embryo or as a consequence of the polarized position of the egg cell and/or early embryo within the embryo sac, and (2) an auxin-mediated mechanism that is established by the initiation of polar auxin transport in the late globular embryo. Either mechanism is apparently sufficient by itself to regulate the initiation of the embryonic axis and the apical positioning of cotyledon structures, because transport inhibitors prevent these processes in somatic embryos, which would seem to lack an active positional mechanism, but have no effect on zygotic embryos, in which the positional mechanism appears to persist even in embryo culture. Finally, the emergence of two discrete cotyledons appears to depend solely on auxin transport because the inhibitors block this process in both somatic and zygotic embryos. This hypothesis of overlapping regulatory mechanisms has important experimental implications: the positional mechanism can be manipulated via genetic or physiological approaches in zygotic embryos only, whereas the auxin-mediated mechanism may be investigated more easily in somatic embryos, which lack the positional mechanism, than in zygotic embryos.

Liu et al. (1993) proposed two alternative models of localized auxin synthesis and subsequent transport to explain the origin of the cotyledons at the end of the globular stage. Although the available evidence on auxin biosynthesis during somatic embryogenesis lacks the spatial
resolution needed to discriminate between these two models, it does provide considerable insight into how auxin gradients may be established in developing embryos. Using gas chromatography/mass spectrometry, Michalczuk et al. (1992a) demonstrated that 2,4-D-treated embryogenic callus (whose proembryogenic masses may be considered the somatic equivalent of preglobular embryos) has extraordinary levels of endogenous auxin, i.e., approximately 60 ng/g f. wt. free and 600 ng/g f. wt. total IAA, which is synthesized via a tryptophan-mediated pathway (Michalczuk et al., 1992b). It is difficult to imagine how effective auxin gradients could be established in preglobular embryos in the presence of such supraoptimal concentrations.

Endogenous IAA levels in young somatic embryos decline precipitously following transfer to 2,4-D-free medium, with the result that postglobular embryos contain between 15 to 30 ng/g f. wt. of total IAA (Michalczuk et al., 1992a), which is low enough to establish internal gradients of auxin activity. More important, this IAA is synthesized via a different, nontryptophan pathway (Michalczuk et al., 1992b), which implies that the induction of this second pathway is required for the initiation of the polar auxin transport thought to occur in the apex of the late globular embryo (Schiavone and Cooke, 1987; Liu et al., 1993). Thus, isolating the genes for the nontryptophan pathway may provide the probes necessary to discriminate between the two potential sites of auxin biosynthesis predicted by the alternative models of Liu et al. (1993).

Todd J. Cooke
Richard H. Racusen
Department of Botany
University of Maryland
College Park, MD 20742

Jerry D. Cohen
Horticultural Crops Quality Laboratory
USDA Beltsville Agricultural Research Center
Beltsville, MD 20705

REFERENCES
