Report

Aust. J. Plant Physiol., 1991, **18**, 435–7

Early Events in the Flowering Process: Key Issues from a Recent Workshop

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Significant advances in studies of flowering were discussed at a recent (February 1991) Australia–USA workshop. The 28 participants included geneticists, molecular and developmental biologists, morphologists and physiologists. Some of the contributions and some of the more important outcomes are summarised.

Gene Activity in Early Floral Organ Development

Genetic analysis in *Arabidopsis* combined with molecular techniques has revealed a fascinating picture of the control of floral organ type. 'Floral homeotic genes' with DNA-binding domains homologous to transcription factors have been identified and these express at various regions of the apex with a timing in flower development that 'educates' organ primordia as to which type of floral organ they are to become. Three major homeotic genes, *apetala 2* and *3* and *agamous*, plus a minor gene, *carpelloid*, are necessary to specify all flower organs. A model based on additive and interactive effects of these three major regulatory genes suffices for specification of all types of floral organs in *Arabidopsis*. The model also explains various genetic and environmentally induced fusions, deletions and duplications of floral organs seen in *Arabidopsis* (Bowman *et al.* 1991; Meyerowitz *et al.* 1991; Shannon and Meeks-Wagner 1992). Comparable genes operate in other species; for example the *'deficiens'* gene of *Antirrhinum* is homologous with *agamous*; it also codes for a regulatory protein and is likely to define floral organ differentiation (Carpenter and Coen 1990).

No doubt this floral model based on a dialogue between gene product and morphogenesis will be modified in the future. For example, the organ-forming epidermal cell layers of the apex do not behave independently of the deeper apical cell layers. Studies of periclinal chimeras of tomato by Szymkowiak (1990) have shown that carpel fasciation of a wild type epidermis can be induced by the *fasciated* mutant, when present chimerically as the subapical pith cell layer. Also, issues such as message and protein stability, and where and when these 'floral' genes are expressed, require further consideration. Furthermore, these 'floral' genes do not appear to define the arrangement and number of organs of each organ type. What, therefore, are the first events in the transition to flowering?

Geometry May Change First at the Apex

Floral organ determination (see above) occurs well after the fate of the meristem has been decided. A summary by Evans left no doubt that early events leading to floral determination at the shoot apex may be completed within a few hours of a suitable triggering event such as by photoperiodic treatment of plants (see McDaniel *et al.* 1991). Yet to date no changes in gene expression have been detected until after floral determination is morphologically evident. At this stage genes are switched on and other 'vegetative' genes may be switched off at the apex (Meeks-Wagner and Zagotta, unpublished). Floral-regulatory genes thus

0310-7841/91/040435$02.00

express late and, maybe, after transition from a vegetative to a floral pattern of arrangement of primordia. Is lack of early detection of expression of regulatory genes due to limitations of technique? Are early molecular changes merely quantitative, if occurring at all? Is it the geometry of organ positioning which must change before any shift in cellular and molecular specification of primordial type occurs? Early but non-specific activation of the apex could allow a shift in the number and position of primordia as a consequence of apical dome enlargement and the alteration of interprimordial biophysical forces.

The biophysical view was put cogently by Paul Green, from detailed observations over time of a single apex of *Anagallis* in its transition to flowering and in which they were able to follow surface cells in their division and enlargement (Hernandez *et al.* 1991). Simple rules could be applied to stresses and strains and these rules defined where and when primordia would form. Such a model of floral development involving early primordia prepatterning followed by later molecular determination of organ type would fit with the many instances in the literature of complete or partial reversion of early floral primordia initials. However, many issues remain unresolved and, in particular, issues of when first floral determination occurs. Singer and McDaniel's 1987 studies, for example, indicate that floral determination occurs before there is any visible shift in apical geometry and, sometimes, even before a meristem is apparent.

Genetics of Flowering

Central to the identification of component processes of flowering is the study of genetic differences. To understand the way in which gene products define organ type in *Arabidopsis* required such genetic information. In *Pisum* and *Lathyrus*, Murfet and coworkers in Hobart have isolated many flowering genes (Murfet 1989). A few may be homologues of the homeotic genes for floral organ specification found in *Arabidopsis*. Other genes have been identified which influence the response of flowering to environment (temperature and photoperiod), as well as genes for inhibitor and stimulus production and assimilate distribution and flowering. In some cases cause has had to be reasoned from effects, which is dangerous where pleiotropic changes are involved. The current use of specific mutants for gibberellin response or type of phytochrome offer a more precise and direct genetic approach.

Environmental Triggering of Flowering

Given the emphasis of the workshop on genes in development, classical questions of photoperiodism and of floral stimuli/inhibitors received scant attention. Effects of photosynthetic input have, however, been clarified recently. It is understandable that sugar supply to the shoot apex has large effects on flowering but what has now been established, at least for one species, is that there are separate and essential photoperiodic stimuli, the response to sugar supply being secondary (King and Evans 1991). Progress was also reported in studies of low temperature vernalisation response. Low temperatures have been shown to enhance gibberellin biosynthesis and, as a consequence, bolting. Also of considerable interest was the suggestion that vernalisation may act at the molecular level to reduce DNA methylation in the promotors of genes important for flowering, specifically at site(s) for the binding of regulatory proteins. Such a scenario fits with non graft-transmissibility of the vernalisation response and with the likely need for dividing cells for its expression. Furthermore, 8-azacytidine, which should reduce DNA methylation, could accelerate flowering in vernalisable lines of *Arabidopsis* (Bagnall, Burn and Dennis, unpublished).

Conclusions

The evidence presented at this workshop of control of floral organ differentiation by interacting regulatory gene products is a breakthrough in our understanding of flowering. Earlier events and the nature of native signal molecules remain less certain. However, the earliest apical events may involve shifts in physical stresses and strains leading to a

repositioning of primordia on the short apex. Such repatterning of the primordia on the apex provides the guides for subsequent floral organ formation. Earliest biochemical charges at the apex and the signals triggering these changes could, therefore, be both unspecific and complex.

Acknowledgments

My co-organisers of this conference, L. T. Evans and C. N. McDaniel, are thanked for their input to this workshop and their comments on this report.

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Manuscript received 8 May 1991, accepted 24 May 1991