Identification of cup-shaped cotyledon: New Ways to Think about Organ Initiation

It is easy to obtain lots of data with modern sequencing techniques, but often the informative sequences are those that connect to a mutant phenotype; of the 4000+ binding sites or 2000+ differentially expressed genes in an experiment, the genes that are important were most likely first defined by a mutant phenotype. A phenotype is proof that the gene is important regardless of what the transcriptome or proteome are doing. Early papers that described mutants carefully and determined the causal encoding gene made essential contributions to future investigations. One such example is the identification and characterization of the cup-shaped cotyledon (cuc) mutant by Aida et al. (1997). The careful, precise analysis and observations made in this stellar contribution to plant biology set the ground-work for future developmental studies. Later work uncovered that CUC genes function in establishing boundaries in many species. The phenotype, in combination with its expression pattern, provided new ways to think about organ initiation as well as organ elaboration and the connections between leaves, serrations, and ligules.

Aida and colleagues found the cuc mutant in a population of Arabidopsis that carried a transgenic Ac element and the TAG1 endogenous transposable element. They crossed it to Landsberg erecta and saw a segregation pattern that suggested two genes were responsible for the phenotype, which they named CUC1 and CUC2. The cuc1 cuc2 mutant had an interesting phenotype; the two normally separate cotyledons, which are embryonic leaves, had formed a cup and the shoot apical meristem stopped growing. From looking at the vasculature, they deduced the cup structure was formed of fused cotyledons. They carried out histological analysis and saw that the tissue organization in the cotyledons was normal with a clearly defined epidermis and mesophyll layers but that the small cells of the meristem were lacking. They looked earlier in development at embryos using plants homozygous for one mutant and segregating for the other. Their beautiful cleared images outlined normal development nicely, and showed that the defect occurred after the globular stage, when normal embryos take on a heart shape. Thus, CUC1 and CUC2 were needed during embryogenesis to distinguish the two cotyledons as well as to promote the formation of the meristem.

They then asked if they could bypass the embryonic lethality by culturing the embryos in media. This clever experiment allowed them to determine the role of CUC genes after embryogenesis. Each single mutation dampened the frequency of adventitious meristem formation and the double mutant had the strongest effect. However, they were able to regenerate plants that were homozygous for cuc1 and cuc2. Interestingly, although the leaves that formed were normal, they saw fusion defects in the flowers. Sepals were often fused into a ring of tissue, and stamens also showed fusions. This experiment told them that the defect found in the embryo was also present in other organs that initiate within the same whorl. Cloning CUC2 using the endogenous TAG1 transposon showed that it was homologous to the Petunia no apical meristem (nam) mutant that had a similar phenotype (Souter et al., 1996). The gene family was named NAC for NAM and CUC. CUC1 was later identified as a member of the same family.

The word “boundary” became synonymous with CUC function once its expression pattern was revealed. Using in situ hybridization, a subset of the same authors showed that CUC2 was expressed at the globular stage in between the future cotyledons (Aida et al., 1999). They compared SHOOTMERISTEMLESS (STM) expression at the same time and found that at this early stage, the expression of the two genes overlapped. As the cotyledons expanded, the CUC2 expression narrowed to wrap around the zone of STM expression. The pattern suggested that CUC genes function in making a boundary and that these boundaries are required for organogenesis. Not only is this boundary needed to separate the two cotyledons or separate the sepals in the whorl of the flower, but also needed to separate determinate organs, such as cotyledons, from the indeterminate meristem. The meristem failure of cuc1 cuc2 is likely due to the lack of the boundary function. Intriguingly, the adventitious meristems escaped this fate.

CUC genes were also discovered to function in leaf shape in a broad number of species. In Arabidopsis, the serrations on a leaf are due to regulation of CUC2 by the microRNA miR164 (Nikovics et al. 2006). Overexpression of the microRNA smoothed the margin while expression of a microRNA-resistant CUC2 produced deeper serrations. A collaborative group (Blein et al., 2008) explored the role of CUC genes in species with deeply lobed leaves: Aquilegia caerulea, Solanum lycopersicum, S. tuberosum, Cardamine hirsuta, and Pisum sativum. CUC genes were consistently expressed in a narrow strip of cells that defined the position of leaflets prior to the leaflet outgrowth. Using VIGS or other technologies, they found fewer lobes, fewer leaflets and leaflet fusions when the CUC genes were knocked down in each of these species (Blein et al. 2008). The work nicely supports ideas from Don Kaplan that serrations, lobes, and leaflets are an ontogenetic continuum (Kaplan, 2001).

In species where no cuc mutants have yet been identified, the expression pattern of meristem genes remains an interesting clue to the presence of a boundary. For example, in a more recent Plant Cell paper on maize leaf development (Johnston et al., 2014), genes were identified that showed increased expression at the position of the future ligule compared to distal blade or proximal sheath tissue; one of these was a CUC gene. In situ expression analysis showed CUC expression at the ligule as well as the boundary between incipient leaves and the
meristem, suggesting that the ligule forms at a boundary, similar to the formation of other lateral organs.

In summary, the original discovery of CUC2 by Aida et al. (1997) provided a valuable tool to investigate plant organogenesis as well as insight that led to a vital understanding of the importance of first establishing a boundary in order to make an organ.

ACKNOWLEDGMENTS

I would like to thank China Lunde and Annis Richardson for edits.

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REFERENCES


