How do individual plant cells attain, and maintain, their specific identities? What are the relative contributions of cell lineage and cell-cell communication to this process? What are the molecules involved? Unraveling the mechanisms by which plant cells gain an understanding of their developmental context constitutes one of the most intriguing problems facing plant developmental biologists today.

One process in which the interpretation of positional signaling information is likely to be of particular importance is in the elaboration of Kranz ("wreath" or "halo") anatomy in the leaves of \( C_4 \) plants such as maize. In \( C_4 \) plants, two morphologically and biochemically distinct photosynthetic cell types, bundle sheath (BS) and mesophyll (M) cells, are arranged in concentric rings around leaf veins. Each cell type carries out a subset of the reactions that lead to photosynthetic carbon fixation, the key feature being that the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) enzyme is shielded from atmospheric oxygen through compartmentalization into the internal BS cells. The \( C_4 \) adaptation is thought to be particularly advantageous to plants growing in conditions of high light intensity and high temperature, as it largely eliminates competition between \( O_2 \) and \( CO_2 \) at the RuBPCase active site, thus preventing energetically wasteful photorespiration (Hatch, 1978).

The biochemistry of this metabolic system is indeed elegant (see Furbank and Taylor, 1995, for a recent review). \( CO_2 \) (as bicarbonate) is initially fixed in the M cell cytoplasm by phosphoenolpyruvate carboxylase to form the \( C_4 \) compound oxaloacetate. Oxaloacetate enters the M cell chloroplasts, where malate dehydrogenase reduces it to malate. Malate is transported to the BS cells and decarboxylated by one of three enzymes—NADP-dependent malic enzyme (ME), NAD-dependent ME, or phosphoenolpyruvate carboxykinase—depending on the plant species. In maize, an NADP-dependent ME releases \( CO_2 \) and the \( C_3 \) compound pyruvate. The \( CO_2 \) is reixed by RuBPCase in the BS chloroplasts and enters the Calvin cycle. The pyruvate diffuses back into the M cells, where it is phosphorylated to regenerate the original carbon acceptor, phosphoenolpyruvate.

In the absence of light, or the appropriate positional information (for example, in M cells greater than two cells from a vein), both BS and M cells appear to accumulate a more \( C_3 \)-like complement of photosynthetic enzymes (Langdale et al., 1988). It has therefore been proposed (Nelson and Langdale, 1992) that a light-induced factor acts in concert with M and/or BS cell-specific signals to coordinate the regulation of nuclear, chloroplastic, and mitochondri al genomes in each cell type, which is necessary for the establishment of \( C_4 \) photosynthesis. How is this achieved in the mature plant? Moreover, how do M and BS cells attain their specific fates early in leaf development before the onset of \( C_4 \) photosynthetic maturity?

In maize, a genetic approach has been taken to address these questions. A large number of mutants containing aberrant chloroplasts have been identified by virtue of their pale green leaf or seedling lethality (reviewed by Miles, 1982, 1994; see also Barkan, 1993; Barkan et al., 1994; Langdale et al., 1995; Schultes et al., 1996). The majority of these mutations affect chloroplast and/or cell maturation in both photosynthetic cell types and are thus likely to define genes that function well after BS or M cell identity has been determined. However, a small number of mutations that exhibit BS cell-specific phenotypes have been identified in this collection (Langdale et al., 1995). These are of interest because they are likely to define either genes with a role in the determination of BS/M cell identity or genes that act subsequently in BS/M cell photosynthetic maturation.

As yet, no mutations have been identified in which BS or M cell determination is altered. However, on pages 915–927 of this issue, Roth and colleagues have taken an important step toward understanding how BS and M cells differentiate with their identification and characterization of a mutation, bundle sheath defective 2-mutable (bsd2-m1), that disrupts the coordinated differentiation of BS and M cells. What is particularly interesting about the bsd2-m1 mutation is that it affects the accumulation of BS-specific \( C_4 \) gene products from a very early stage in development—before BS and M cells can be distinguished morphologically (but after the Kranz architecture has been established in the seedling leaf). In wild-type maize leaves, the specific accumulation of mRNAs encoding the large subunit of RuBPCase (\( rbcL \)) in BS progenitor cells is the earliest known marker for BS cell differentiation (Langdale et al., 1988). Thereafter, chloroplast-encoded \( rbcL \) transcripts are restricted to BS cells of wild-type leaves.

One significant effect of the bsd2-m1 mutation is to disrupt this pattern of \( rbcL \) transcript accumulation. Specifically, \( rbcL \) transcripts are detected in M progenitor cells at the same time as they appear in neighboring BS progenitor cells and continue to accumulate ectopically in M cells throughout leaf development. Furthermore, despite the presence of high levels of \( rbcL \) transcripts in both BS and M progenitor cells, neither subunit of RuBPCase is detectable in immature mutant leaf tissue. Defects in \( rbcL \) expression in the bsd2-m1 mutant are not correlated with
altered expression of nuclear C4 genes, which continue to be expressed in the correct cell types in this mutant. These data suggest that the Bsd2 gene product may play complementary roles in the regulation of rbcL (and possibly RbcS) expression in wild-type BS and M cells. In M cells, the Bsd2 gene product is hypothesized to repress the accumulation of rbcL transcripts, whereas in BS cells, it may act to increase the translational efficiency of the rbcL mRNAs and/or the stability of the RuBPCase large subunit protein.

Deficiencies in the accumulation of photosynthetic enzymes, or the corresponding mRNAs, are a common feature in mutants affected in photosynthetic function, but the mechanisms by which these effects are mediated have been difficult to define (see Mayfield et al., 1995). For example, the maize mutants hcf7, cps1-1, cps2-1, and cps2 also show a reduction in the level of rbcL transcripts, most likely due to an effect of the mutations on the stability of the rbcL message (Barkan, 1993). The low levels of rbcL transcripts in these mutants are correlated with a decrease in ribosome loading on the messages, but it is not yet known whether the effect is BS or M cell specific, or how early in leaf development the genes may act.

The fact that the bsd2-m1 mutation affects rbcL expression in both BS and M cells suggests that the Bsd2 gene product functions after BS and M cell identities have been defined. Nevertheless, the early manifestation of aberrant rbcL gene expression is consistent with a role for the Bsd2 protein in maintaining the functional distinction between BS and M cells, perhaps through a direct effect on rbcL gene expression. A second bundle sheath defective mutation, bsd1-m1, appears to act earlier in the process leading to the functional differentiation of BS and M cells in maize (Langdale and Kidner, 1994). Unlike bsd2-m1, the effects of the bsd1-m1 mutation are restricted to the BS cells of light-grown plants, where the mutation leads to a decrease in the expression of BS cell-specific C4 enzymes (RuBPCase and ME) and the aberrant development of BS chloroplasts. In the dark, however, the bsd1-m1 mutation also affects C4 photosynthetic gene expression in M cells without affecting M cell etioplast structure (Langdale and Kidner, 1994). In light of these data, it is possible that the Bsd1 gene product may participate in the integration of positional and light-induced cues, which is thought to establish the appropriate regimen of C4 gene expression in BS cells (Nelson and Langdale, 1992).

How should we proceed from here? The cloning of the Bsd1 gene has been reported (Langdale et al., 1995) and the cloning of Bsd2 isn't far behind (T.P. Brutnell and J.A. Langdale, personal communication). The sequences of the genes may (or may not) provide some insight into the roles of the corresponding proteins during the differentiation of BS and M cells. In any case, Bsd gene probes can be used to address some of the more pressing issues with respect to Bsd1 and Bsd2 function. At which developmental stage can Bsd1 and Bsd2 transcripts first be detected in maize leaf primordia? Is the expression of these genes restricted to specific cell types? In the meantime, double mutant analyses should establish epistatic relationships between bsd1 and bsd2, as well as between the bsd mutations and others with defects in chloroplast structure or function. Clearly, the functional characterization of the Bsd gene products will considerably advance our understanding of the processes through which BS and M cell identities are determined during maize leaf development.

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REFERENCES


C3 or C4? Maize Mutations and the Elaboration of Kranz Anatomy
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