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Understanding the Genetic Basis of C_4 Kranz Anatomy with a View to Engineering C_3 Crops

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Abstract

One of the most remarkable examples of convergent evolution is the transition from C_3 to C_4 photosynthesis, an event that occurred on over 60 independent occasions. The evolution of C_4 is particularly noteworthy because of the complexity of the developmental and metabolic changes that took place. In most cases, compartmentalized metabolic reactions were facilitated by the development of a distinct leaf anatomy known as Kranz. C_4 Kranz anatomy differs from ancestral C_3 anatomy with respect to vein spacing patterns across the leaf, cell-type specification around veins, and cell-specific organelle function. Here we review our current understanding of how Kranz anatomy evolved and how it develops, with a focus on studies that are dissecting the underlying genetic mechanisms. This research field has gained prominence in recent years because understanding the genetic regulation of Kranz may enable the C_3-to-C_4 transition to be engineered, an endeavor that would significantly enhance crop productivity.

INTRODUCTION

Underpinning virtually all life on earth is the process of photosynthesis, whereby plants, algae, and photosynthetic bacteria use light energy to fix inorganic carbon dioxide (CO_2) into organic sugars. The most common form of photosynthesis is termed C_3 because CO_2 is fixed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) into a three-carbon compound (3). Despite its ubiquity, however, C_3 photosynthesis is hampered by the inability of RuBisCO to faithfully distinguish between CO_2 and O_2, leading to competing carboxylation and oxygenation reactions (reviewed in 87). Oxygenation, which is elevated in hot, dry environments, yields phosphoglycolate, which has to be recycled in an energy-consuming process termed photorespiration (reviewed in 4). Both temperature increases and drought stress thus increase photorespiration and decrease photosynthetic efficiency in C_3 plants.

A variety of strategies to concentrate CO_2 around RuBisCO and thus to reduce photorespiration can be found in nature. These include the carbon-concentrating mechanisms of many cyanobacteria and green algae, and both crassulacean acid metabolism and C_4 photosynthesis in flowering plants (reviewed in 108). Of these divergent strategies, C_4 photosynthesis is the most agronomically important, because productive crop species such as maize, sorghum, and sugarcane are C_4, as are many weeds. The most common form of the C_4 pathway (50) involves the spatial separation of metabolic reactions between two cell types organized within a characteristic leaf anatomy known as Kranz (named as such because Kranz is German for wreath and the two cell types form wreaths around the leaf veins) (8, 48). CO_2 is initially fixed by an O_2-insensitive carboxylase into a four-carbon compound (hence C_4 pathway) in the outer mesophyll cells of the leaf, and then the C_4 compound is transferred to inner vascular sheath cells, where it is decarboxylated to release CO_2 for refixation by RuBisCO (reviewed in 42, 71). This intercellular C_4 shuttle concentrates CO_2 at the site of RuBisCO, leading to low levels of photorespiration and enhanced photosynthetic efficiency relative to C_3 plants, particularly in hot and/or dry environments.

The more efficient C_4 pathway, which evolved from the C_3 pathway on multiple independent occasions (114), accounts for approximately 25% of primary productivity on the planet despite being used by only 3% of species (30, 132). The seeming ease with which the transition from C_3 to C_4 repeatedly occurred, combined with the superior productivity of the C_4 pathway, has prompted ambitious attempts to engineer C_3 crops such as rice to use the C_4 pathway (55, 140). This goal can be achieved, however, only with a more substantial understanding of the fundamental mechanisms that underpin both the metabolic pathway and the anatomical framework within which it operates. Although the genes encoding enzymes of the C_4 pathway were identified over 30 years ago (reviewed in 71), forward genetic screens over many years failed to provide any insight into the genes that regulate Kranz anatomy (e.g., 72, 109). More recently, however, genome-wide analyses have started to reveal the genetic complexity underpinning Kranz development (41, 145, 146, 148). In this review, with a focus on leaf anatomy, we cover both past successes and future challenges in the endeavor to genetically dissect the developmental innovations that have underpinned the evolution of C_4.

C_4 LEAF ANATOMY

Single Cell Versus Kranz

In C_3 leaves of monocots and eudicots, veins are generally encircled by one or more layers of vascular sheath cells (mestome sheath and/or bundle sheath) and are separated from adjacent veins by at least six mesophyll cells. RuBisCO and, hence, C_3 carbon fixation primarily occur in the mesophyll cells, whereas vascular sheath cells load metabolites into the vein, provide structural support,

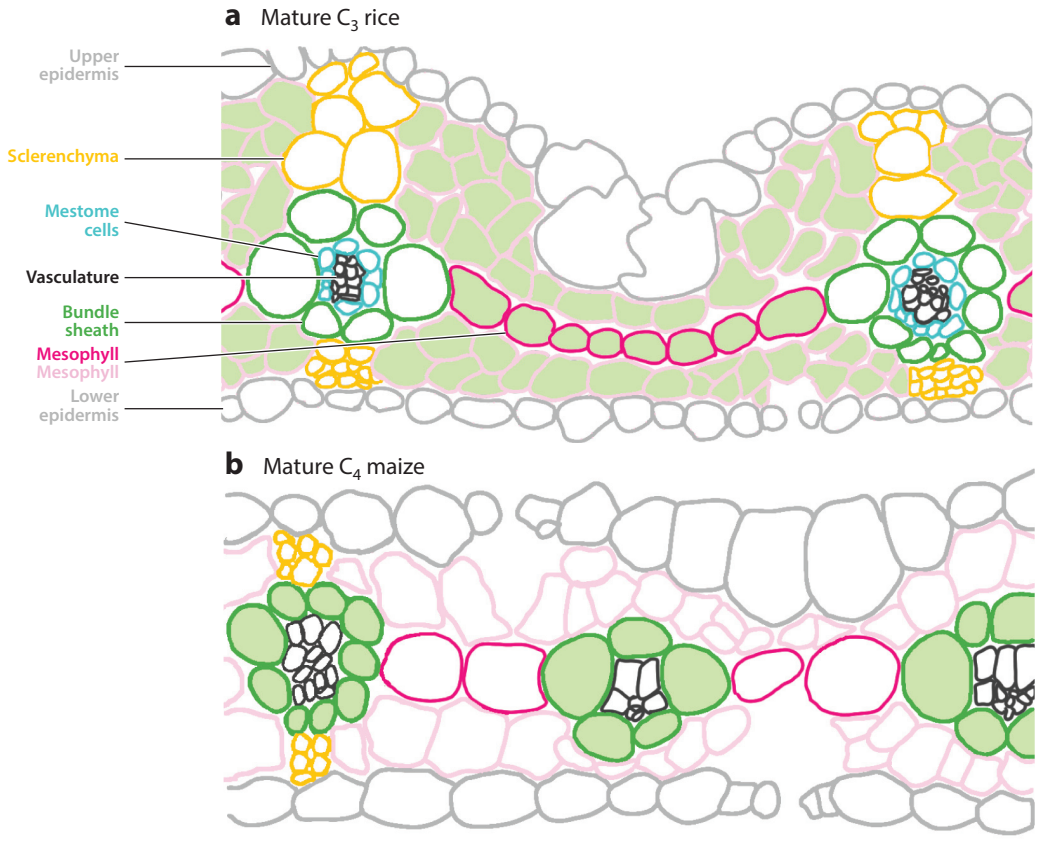

Figure 1

Comparison of C_3 and C_4 leaf anatomy. Diagrams showing transverse cross sections of mature C_3 rice (*a*) and C_4 maize (*b*) leaves. Cell outlines: upper and lower epidermis (*gray*), sclerenchyma (strengthening tissue comprised of cells with thickened cell walls) (*yellow*), vasculature (*black*), mestome sheath (*blue*), bundle sheath (*dark green*), and mesophyll (*light and dark pink*). The middle layer of mesophyll cells (*dark pink*) highlights the difference in cell number between veins in C_3 and C_4 species. The localization of RuBisCO enzyme accumulation (*light green*) also differs. Abbreviation: RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase.

and are proposed to facilitate cavitation repair during drought stress (reviewed in 47). Although the C_4 photosynthetic pathway can also operate in the context of single cells, with initial fixation and subsequent decarboxylation reactions split between distinct intracellular compartments (33, 141), the majority of C_4 species compartmentalize reactions between two distinct cell types in the leaf. In C_4 leaves with Kranz anatomy, concentric rings of photosynthetic vascular sheath and mesophyll cells surround each vein, and adjacent veins are often separated by just two mesophyll cells (8) (**Figure 1**).

Kranz Variations

The Kranz anatomy found in maize that is illustrated in **Figure 1** is representative of the classical NADP-ME subtype in which decarboxylation is carried out by NADP-dependent malic enzyme in a single layer of bundle sheath cells surrounding each vein. There is, however, a great deal

Table 1 Poaceae Kranz subtypes[a]

Subtype	Anatomical variations				
	Mestome sheath	Number of BS cell layers	BS chloroplasts	BS suberin layer	Other
Classical NADP-ME (includes maize and *Setaria*) (**Figure 1**)	Absent	1	Agranal and centrifugal	Present	NA
Classical NAD-ME	Present	1	Granal and centripetal	Absent	NA
Classical PEP-CK	Present	1	Granal and centrifugal or peripheral	Present	NA
Arundinelloid	Absent	1	Agranal and centrifugal	Present	Presence of distinctive cells that are similar to BS but not associated with a vascular bundle
Aristidoid	Absent	2	Agranal and centrifugal in the inner Kranz BS, granal and centripetal in the outer BS	Absent	NA
Stipagrostoid	Present	1	Reduced grana and centripetal	Absent	NA
Eriachneoid	Present	1	Granal and centrifugal	Absent	NA
Neurachneoid	Absent	2	Reduced grana and centrifugal in the inner Kranz BS, few chloroplasts in the outer BS	Present	There are examples of this subtype with either NADP-ME or PEP-CK biochemistry. These are associated with further subtle anatomical variations.
Triodioid	Present	1	Granal and centrifugal	Absent	BS form extensions toward chlorenchyma.

[a]Table adapted from Reference 34.

Abbreviations: BS, bundle sheath; NA, not applicable; NADP-ME, nicotinamide adenine dinucleotide phosphate malic enzyme; NAD-ME, nicotinamide adenine dinucleotide malic enzyme; PEP-CK, phosphoenolpyruvate carboxykinase.

of variation in Kranz forms between species. **Table 1** summarizes the variations found within the Poaceae grasses (of which maize is a member), but note that similar variations are found in other C_4 lineages, both monocot and eudicot (C_4 species evolved only within the angiosperms). In the four subfamilies of the Poaceae that contain C_4 species, there are at least nine distinct Kranz forms (34). Between the nine forms, there are five key traits that differ: (*a*) the number of bundle sheath cell layers around veins, (*b*) presence or absence of a mestome sheath, (*c*) presence or absence of a suberin layer between bundle sheath and mesophyll cells, (*d*) chloroplast positioning in the photosynthetic vascular sheath cell layer (centripetal or centrifugal), and (*e*) chloroplast dimorphism (or not) between photosynthetic vascular sheath and mesophyll cells. One further anatomical feature of note is the presence of distinctive cells in the Arundinelloid subtype, which are similar to bundle sheath cells in terms of both ultrastructure and C_4 enzyme expression, despite not being associated with a vascular bundle (17, 23, 142). The observed variation in Kranz forms presumably reflects the multiple evolutionary origins of the C_4 pathway (114).

Given the range of different Kranz types, can anything be considered a fundamental Kranz trait? One feature that does not vary across two-cell C_4 lineages is the presence of concentric layers of outer mesophyll and inner vascular sheath cells. The outer mesophyll cell layer is ideally placed to fix CO_2 that diffuses into the leaf through stomata, whereas the inner sheath layer enables decarboxylation at the site of RuBisCO. The two layers are in close contact to allow the transfer of metabolites. However, both vascular sheath and mesophyll cell layers are also present in C_3 leaves, and thus the key Kranz trait is the functionalization of the vascular sheath for photosynthesis rather than the presence of the distinct cell types per se. A second unifying feature of all Kranz forms is a ratio of vascular sheath to mesophyll cell volume (per unit leaf area) that is higher than in C_3 species, but even this shared trait can be realized in a number of different ways. For example, increased vein density and/or vascular sheath cell size increase the proportion of vascular sheath tissue in the leaf relative to that seen in C_3 leaves. In light of the variation observed, any investigation into the genetic basis of Kranz development requires an understanding of both the phylogenetic and ontogenetic origins of C_4 leaf anatomy.

C_4 EVOLUTION

Ecological Drivers

Despite the variations exhibited in both biochemistry and anatomy, C_4 photosynthesis remains one of the most striking examples of convergent evolution. The latest estimates suggest that there are 66–68 angiosperm lineages containing C_4 species, with eudicots and monocots accounting for 36 and 30–32 of the lineages, respectively (46, 114, 116). Within the grasses, the so-called PACMAD clade accounts for all 18 of the C_4 lineages, with the sister BEP clade containing none (138). Given the advantage of C_4 in conditions where effective O_2:CO_2 ratios are high, it is not surprising that the first C_4 origins coincided with a drop in atmospheric CO_2 around 30 million years ago (12). However, many origins have been dated much later (13), and a number have been correlated with periods of reduced annual precipitation (31) or associated with drought-tolerant saline resistant lineages (61). These observations suggest that C_4 may have been selected for—in part—as a water-conserving method (105), but given that C_4 species are more prevalent at higher temperatures in many habitats, temperature was also likely to have been an important ecological driver (32). Analyses of evolutionary trajectories from C_3 to Kranz leaf anatomy should thus view CO_2 levels, drought conditions, and temperature as potential agents of selection and should consider likely anatomical transitions that might have occurred in the face of such environmental pressures.

The C_3-to-Kranz Trajectory

The independent origins of C_4 are not evenly distributed across the angiosperm phylogeny, and many lineages that have experienced seemingly conducive environmental conditions for C_4 evolution contain no C_4 species. Instead, C_4 origins are clustered in certain clades, suggesting that within those clades preadaptive or enabling traits were acquired prior to the evolution of C_4 itself. A number of recent studies have attempted to determine what those traits might be. One approach quantified anatomical traits in C_3 and C_4 species of the PACMAD and BEP grasses (which account for 18 and 0 origins of C_4, respectively) and concluded that C_3 PACMAD grasses had larger vascular sheath to mesophyll cell volumes by comparison to BEP grasses (14). On the basis of this study, it has been suggested that increased vascular sheath cell volume (either through increased cell size or increased vein density) in some C_3 species may have been an enabling anatomical trait

for the subsequent evolution of Kranz anatomy (14) and that this trait may have facilitated better cavitation repair and thus enhanced fitness in the face of drought stress (47).

Other approaches aimed at dissecting the evolutionary transition from C_3 to C_4 have exploited the existence of so-called C_2 intermediate species that can be found in families that also contain both C_3 and C_4 species (114). These intermediates, which are characterized by the presence of a glycine shuttle and restriction of glycine decarboxylase activity (and hence photorespiration) to vascular sheath cells, were proposed to represent a crucial metabolic stepping stone from C_3 to C_4 (96). This proposal was supported by a modeling approach that used photosynthesis parameters to predict the evolutionary trajectory from C_3 to C_4 metabolism (51). By randomly adding various metabolic traits to the C_3 state, and by using the criterion that traits could be fixed only if they increased overall fitness, the model mapped acquisition of the C_2 glycine shuttle to an early stage of the trajectory, with evolution of the compartmentalized C_4 pathway mapping to a much later point (51). A second model that mapped a phenotypic landscape based on both biochemical and anatomical traits in C_2 intermediates similarly positioned the localization of glycine decarboxylase activity in vascular sheath cells at an early stage in the trajectory and C_4 cycle activation much later (151). It has been proposed that the final metabolic transition to C_4 could have been triggered by a detrimental nitrogen imbalance between vascular sheath and mesophyll cells in C_2 plants (caused by localized glycine decarboxylase activity in the vascular sheath) (92). Crucially, if such an imbalance existed in ancestral C_2 species, it could have been ameliorated by the evolution of a shuttle that moved nitrogen back from vascular sheath to mesophyll cells, a role that some of the reactions in C_4 photosynthesis could fulfill (92).

Although there is general consensus around the likely metabolic transition from C_2 to C_4, and agreement that metabolic changes were preceded by at least some anatomical modifications (reviewed in 115), ordering the anatomical transitions along the evolutionary trajectory is not straightforward. This is in part because many Kranz traits exist to a greater or lesser degree in C_3 species and as such, the trajectory probably differed depending on the precise ancestral condition in each lineage (88, 151). That said, analyses of both eudicot and monocot lineages point toward two key intermediate steps (29, 65, 88, 97, 115, 117, 129). Studies in the eudicot genera *Flaveria* and *Heliotropium* have been particularly informative in determining this trajectory because both contain a number of C_2 intermediate species. In *Flaveria*, phylogenetic analysis resolved *F. pringlei* and *F. robusta* as the closest C_3 species to the C_2 intermediates (64, 95). Conspicuously, both species exhibit greater chloroplast and mitochondrial volumes in vascular sheath cells than do other C_3 species in the genus (117). This suggests that the phenotype, which has been termed proto-Kranz, was an enabler for the subsequent evolution of C_2. Proto-Kranz anatomy has also been identified in C_3 species of *Heliotropium* (97) and in C_3 grasses of the PACMAD clade, but not of the BEP clade (65). Whether proto-Kranz has any fitness benefit (or cost) is not known, but RuBisCO accumulates in chloroplasts and glycine decarboxylase accumulates in mitochondria, suggesting that the vascular sheath cells are functionalized for photosynthesis and photorespiration. Functionalization can occur in mestome and/or vascular sheath cells, can involve reorientation of organelles within the sheath cells, and can be accompanied by increased vein density and/or ratio of vascular sheath to mesophyll cell volume (14, 29, 65, 88, 97, 115, 117, 129). Notably, the single unifying feature of the transition from C_3 to proto-Kranz, i.e., activation of organelle function in vascular sheath cells, is also the single shared trait between all Kranz types (34) and is the only trait that can clearly distinguish C_3 and C_4 species (88). The combined evidence from qualitative, quantitative, and modeling approaches is thus consistent with a C_3-to-Kranz trajectory that proceeded via proto-Kranz and then C_2 intermediates; both steps require modification of organelle biogenesis and function, with or without concomitant changes to vein density or vascular sheath cell volume.

ONTOGENY OF KRANZ ANATOMY

Formation of Veins

The key features of Kranz anatomy are patterned early in leaf development, with the vascular network providing the scaffold around which cell-type differentiation occurs. In both monocots and eudicots, leaf primordia are formed on the flanks of the shoot apical meristem (SAM), with distinct cell layers of the meristem contributing to different tissues in the leaf (reviewed in 130). In eudicot SAMs there are three cell layers (L1, L2, and L3), whereas monocots comprise just L1 and L2. Procambial meristems that initiate vascular development in the leaf are normally formed from L3- or L2-derived tissue in eudicots and from the innermost layer of L2-derived tissue in monocots, although in all cases cell fate is dictated by cell position in the innermost layer rather than by lineage (see Reference 131, for example).

The ontogeny of vascular development in C_4 plants has been studied primarily in maize (35, 112, 125) but also in other monocots (24, 134) and in eudicots such as *Flaveria* (94) and *Cleome* (1, 69). In all cases, after a new leaf primordium is initiated, procambial tissue that will form the midvein (the highest-rank order vein) is laid down acropetally (from base to tip). Depending on species, the other major veins of the leaf then develop acropetally, basipetally (tip to base), or both, before the lower-rank veins are initiated and develop basipetally (reviewed in 101). In eudicots, vein ranks are referred to consecutively as primary (1°) (i.e., midvein), 2°, 3°, and so on. 2° veins initiate from the midvein toward the leaf margins as the leaf lamina is formed, and then as the leaf expands, 3° and lower-rank veins form the reticulate vascular network characteristic of eudicot leaves. In monocots, the lowest-ranked veins have been inconsistently referred to as either small or minor intermediate veins (6, 101, 112, 125) but are here termed rank-2 intermediate veins (**Figure 2**). Both C_3 and C_4 monocots form lateral veins and rank-1 intermediate veins, whereas rank-2 intermediate veins are found only in C_4 leaves. A similar situation is seen in eudicots, for example, C_4 species of *Cleome* form 1°–7°-ranked veins, whereas closely related C_3 species form only 1°–6° ranks (69). The relatively high vein density associated with Kranz anatomy in C_4 leaves is thus achieved through the development of more lower-rank veins than in comparable C_3 leaves. This observation suggests that ground meristem tissue in the innermost leaf layers of C_4 leaves remains competent to form procambium for longer than equivalent tissue in C_3 leaves, and, thus, that a suppressor of mesophyll cell differentiation in the innermost leaf layer may be a key Kranz regulator.

The timing and sequence of vascular initiation events in C_4 leaves have also been most studied in maize (summarized in **Figure 2**) (reviewed in 101). As in all grasses, maize leaves are characterized by a series of longitudinal veins that run parallel to the proximo-distal leaf axis. Lateral veins are formed approximately one plastochron (the time interval between primordia initiation) later than midvein differentiation. Lateral vein initiation and extension are completed within the first four plastochrons, whereas the initiation of both rank-1 and rank-2 intermediate veins continues to plastochron 6 (125). The midvein, lateral veins, and rank-1 intermediates extend throughout the leaf blade and sheath, whereas rank-2 intermediate veins are generally restricted to the leaf blade, terminating near the blade–sheath boundary (112). As such, the leaf blade has classical Kranz anatomy, whereas the leaf sheath has a more C_3-like anatomy with wider spaced veins and 6–8 mesophyll cells between adjacent vein pairs. Transverse veins that connect the longitudinal veins across the mediolateral leaf axis are the last to be initiated and are formed in a basipetal direction. Crucially, as intermediate veins are initiated and differentiate, they do so in a fixed position relative to neighboring veins and appear to use existing veins as a positional landmark (101).

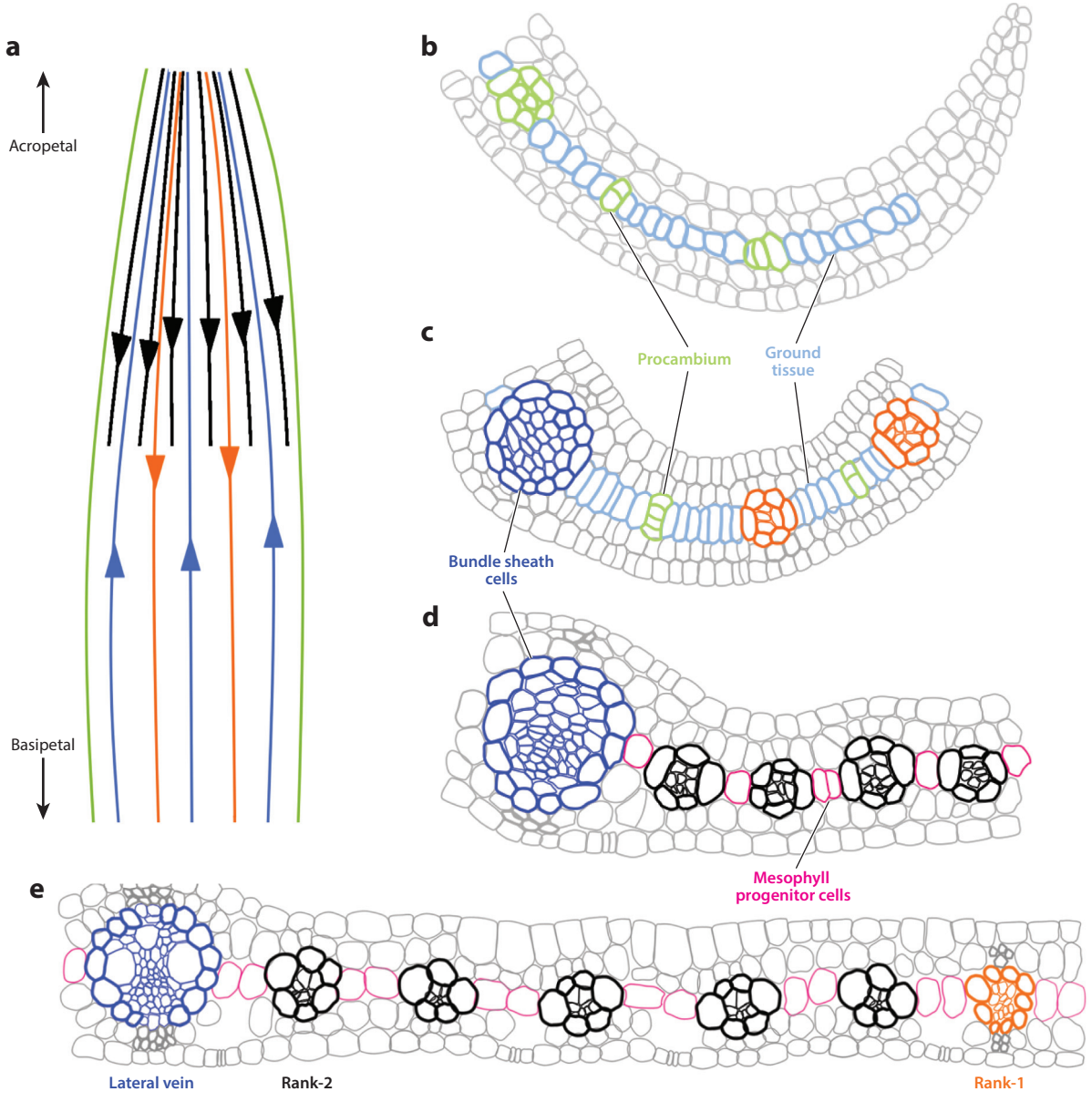

Figure 2

Leaf development in maize. (*a*) Direction of vein growth in the developing maize leaf blade: acropetal growth of midvein and lateral veins (*dark blue*) and basipetal growth of rank-1 (*orange*) and rank-2 (*black*) intermediate veins. (*b–d*) Diagrams of transverse leaf cross sections showing consecutive steps of tissue patterning. (*b*) Procambium initiation (*green*) occurs in the innermost layer of ground tissue (*light blue*). Procambium differentiates into various cell types to give vascular bundles, including bundle sheath (BS) cells. (*c*) Additional procambium (*green*) is initiated from ground tissue (*light blue*) whilst adjacent lateral (*dark blue*) and rank-1 intermediate (*orange*) veins are developing. (*d*) At the latter stages of leaf development, once veins have developed, e.g., lateral (*dark blue*) and rank-2 intermediates (*black*), mesophyll (M) progenitor cells (*middle layer*, *dark pink*) divide to give the BS-M-M-BS radial tissue patterning seen in maize. (*e*) Diagram of a transverse cross section of the mature maize leaf showing lateral vein (*dark blue*), rank-1 (*orange*) and rank-2 (*black*) intermediate veins, and the middle layer of mesophyll cells (*pink*). Images adapted from Reference 148 with permission.

Cell-Type Specification Around Veins

As veins develop, surrounding vascular sheath and mesophyll cells are specified from either procambial or ground meristem tissue. Interestingly, examination of cell division patterns across multiple C_3 and C_4 species demonstrated that the layer of vascular sheath cells closest to the vein is always derived from procambium, but in cases where two vascular sheath cell layers are present, the outer layer is derived from the surrounding ground meristem tissue (22). This distinction means that it is possible for bundle sheath cells to be derived from either procambial or ground meristem tissue, depending on whether an inner vascular sheath is present. For example, C_3 rice leaves have an inner mestome sheath and outer bundle sheath cell layer around veins, whereas C_4 maize leaves have a single bundle sheath layer. As such, both rice mestome sheath and maize bundle sheath cells are derived from procambium, and both rice bundle sheath cells and maize mesophyll cells are derived from ground meristem tissue (118). Given that procambial meristems themselves arise from ground meristem tissue in the leaf, however, this distinction is essentially one of timing.

Clonal analysis in maize revealed that mesophyll cells in the innermost leaf layer differentiate from the L2-derived tissue that also gives rise to procambium and bundle sheath cells (74), and a similar scenario was discovered in the C_4 grass *Stenotaphrum secundatum* (134). The fact that both bundle sheath and mesophyll cells originate from the same cell lineage indicates that the early specification of bundle sheath and mesophyll cell types in maize must be induced by positional signals, as opposed to lineage-based determinants. Later in development, however, when procambial centers have become committed to the vascular differentiation program, bundle sheath cell specification is restricted to that lineage. At this point, aberrant periclinal divisions within the bundle sheath cell layer result in two daughter bundle sheath cells, one of which is ectopically positioned in the mesophyll cell domain (57). Because bundle sheath and mesophyll cells differentiate in coordination with the vein they surround (i.e., cells around the midvein develop first and those around rank-2 intermediate veins develop last) (75, 77), it was proposed that positional signals emanating from developing veins induce the differentiation of C_4-like bundle sheath and mesophyll cells (76). This hypothesis was supported by the observation that in the husk leaves of maize, where veins are separated by at least ten mesophyll cells as opposed to the two seen in foliar leaves, cells more than a two-cell radius from a vein differentiated in a default manner as C_3 mesophyll (78). Despite being proposed over 25 years ago, a role for vein-derived positional signals in the patterning of bundle sheath and mesophyll cells of C_4 leaves has yet to be validated at a molecular level.

GENETIC REGULATION OF KRANZ DEVELOPMENT

Identification of Regulators

Traditional approaches to gene discovery such as forward genetic screens have offered little insight into the genetic regulators that underpin the development of Kranz anatomy. Mutagenesis screens with the C_4 grass *Panicum maximum* produced several lines with abnormal vein spacing and one wide vein spacing mutant (*large interveinal space 1*) with a CO_2 compensation value characteristic of C_3 photosynthesis (40). Unfortunately, however, these lines were lost before any molecular characterization could be carried out. More recent screens of 30,000 M2 lines of *Sorghum bicolor* led to the identification of two wide vein spacing mutants, both of which were underpinned by mutations in *CYTOCHROME P450–90D2* (*CYP90D2*), a gene that encodes an enzyme in the brassinosteroid biosynthesis pathway (109). In both mutants, altered vein spacing was caused by an increase in mesophyll cell size and number, but the leaf blades were also thicker than normal and

resembled leaf sheath tissue. It was thus concluded that perturbations in brassinosteroid synthesis and/or downstream signaling resulted in a shift from blade to sheath domain identity, as opposed to any specific disruptions to vascular patterning. More subtle phenotypes were reported to result from mutations in maize orthologs of the *Arabidopsis* transcription factors AtSHORTROOT (AtSHR) and AtSCARECROW (AtSCR) (126, 127). In *Arabidopsis*, AtSHR and AtSCR work together to specify cell types around vascular tissue in both the root and the shoot, with mutations in either gene leading to obvious defects in cell patterning and/or differentiation (5, 26, 53, 68, 123, 153). By contrast, only mild perturbations were observed in the maize mutants, with some vascular bundles exhibiting ectopic bundle sheath cells but many veins across the leaf developing normally. Notably, the failure to identify Kranz-specific defects in experiments designed to screen for mutations in single genes suggests either that mutations condition lethal phenotypes or that multiple genes and/or extensive regulatory redundancy are likely to be involved.

Untangling the likely complex genetic networks underpinning Kranz development is a significant challenge, but over the past few years transcriptomics-based approaches have provided fresh insight. A study that was specifically designed to capture developmental regulators of Kranz anatomy took advantage of two distinct leaf types in maize: the foliar leaves, which act as the main photosynthetic tissue and exhibit conventional Kranz anatomy, and the husk leaves, which exhibit more C_3-like anatomy (78, 146). A similar developmental trajectory takes place in both leaf types between plastochrons (P) 1 and 3, when the midvein and lateral veins are initiated. After P3, however, the trajectory diverges in that rank-2 intermediate veins (and hence Kranz anatomy) are initiated in foliar but not husk leaves (146). On the basis of this distinction, transcriptomes were generated from both foliar and husk leaves at stages before (P1/2), during (P3/4), and after (P5) Kranz initiation (146). Given the developmental trajectory, a positive regulator of Kranz anatomy should be expressed at higher levels in foliar primordia than in husk primordia, prior to P5. In contrast, a negative regulator should be expressed at higher levels in husk primordia than in foliar primordia. Using these criteria, comparative expression analysis between the different developmental stages in foliar and husk leaves identified 283 candidate positive regulators and 142 candidate negative regulators of Kranz anatomy, with additional candidates identified through co-expression analysis (146). Prominent in the list of candidate regulators were many genes implicated in auxin signaling, and genes related to the SHR/SCR pathway.

Determining the function of potential Kranz regulators identified through bioinformatic analyses is not trivial when lists comprise more than a handful of candidate genes, not least because there are currently no C_4 species that are suitable for high-throughput transgenic analysis. As such, decisions about which (and how many) genes to work with are based at least in part on the species being used as an experimental chassis. Thus far, functional analyses of Kranz development have been carried out primarily in monocots—with maize and, more recently, green millet (*Setaria viridis*) (10, 15) being the most practical organisms of choice. Regardless of experimental system, however, candidate gene lists still need to be prioritized for functional analysis. A first step in this process is the correct assignment of gene orthology through accurate phylogenetic analysis. This is not straightforward, however, in the context of C_3 versus C_4 comparisons, because even with the 1,000 plants (1KP) transcriptome data set (93), genome-wide information for C_4 monocots is relatively underrepresented. As a consequence, cross-referencing candidate gene lists against other relevant transcriptome data sets is a necessity. For example, comparisons can be made with data sets that highlight genome-wide expression differences between closely related C_3 and C_4 species (1, 7, 45, 69, 92, 137, 143), between different developmental stages (79, 82, 83, 85, 106), and between isolated bundle sheath and mesophyll cell types (135). This approach allows the spatial and temporal expression dynamics of candidate genes to be mapped onto a range of phenotypic landscapes with or without Kranz anatomy; thus, it enabled prioritization of the 283 candidate

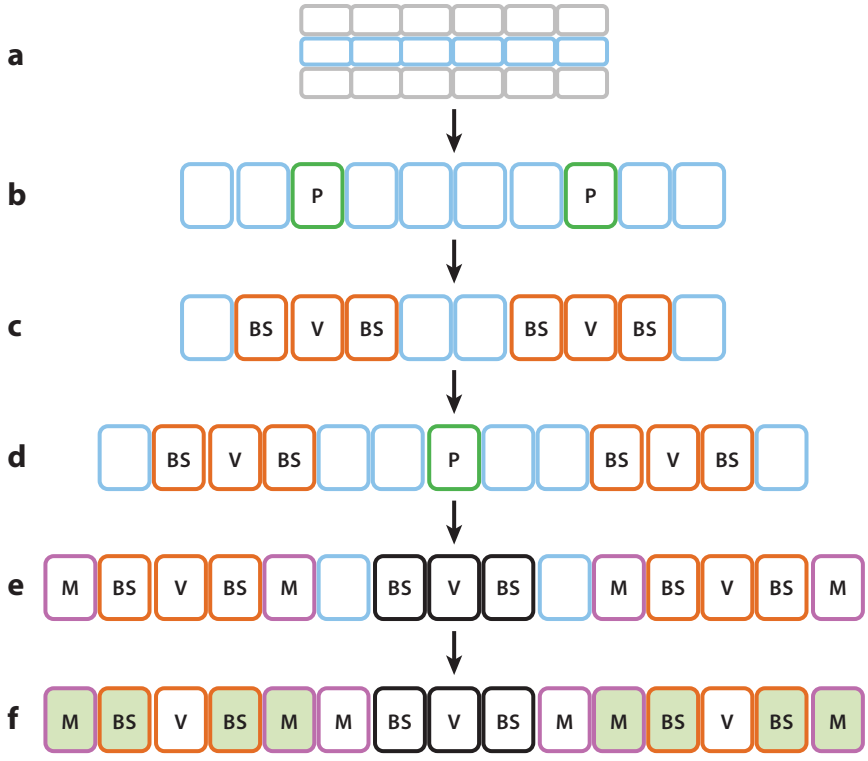

Figure 3

Steps of Kranz development. (*a*) Cells in the innermost leaf layer are competent (*pale blue*) to form procambium. (*b*) Procambium (P) (*green*) is initiated at intervals across the mediolateral leaf axis. (*c*) Procambial centers become committed to form vascular tissue (*orange*) [here rank-1 intermediate veins (V) and surrounding bundle sheath (BS) cells]. (*d*) As cell divisions increase the leaf width, further procambial centers are initiated between existing rank-1 intermediate veins. (*e*) Mesophyll (M) cells (*pink*) are specified around rank-1 intermediate veins at the same time as rank-2 intermediate procambial centers become specified (*black*). (*f*) BS and M cells around rank-1 intermediate veins become photosynthetic (*green fill*) at the same time as M cells are specified around rank-2 intermediates.

regulators identified in the maize foliar versus husk leaf analysis discussed above (41, 146). Ultimately, however, loss-of-function and gain-of-function analyses in planta are the only way to validate the necessity and sufficiency of gene function.

A Contextual Framework for Kranz Regulation

To provide a framework for interrogating the genetic network underpinning Kranz anatomy, it is useful to revisit the key steps in the developmental pathway. **Figure 3** summarizes those steps: (*a*) Cells of the innermost leaf layer become competent to form procambium, (*b*) procambium is initiated, (*c*) procambium is committed to form vascular centers, (*d*) cell types are radially patterned around veins, and (*e*) vascular sheath and mesophyll cells become photosynthetic. Importantly, two key factors distinguish Kranz from C_3 anatomy during this process: First, cells in the innermost leaf layer remain competent to form procambium for as long as cell divisions are increasing leaf width (as such, intermediate veins fill gaps between existing adjacent veins), and second, vascular

sheath (normally bundle sheath) cells become photosynthetic. While recognizing that the Kranz developmental pathway is likely to be a continuum, both spatially and temporally, dividing the process into the three steps of (*a*) procambium formation, (*b*) radial patterning of cell types around veins, and (*c*) photosynthetic functionalization is a helpful way to interrogate potential underlying genetic mechanisms. Each step will now be considered in turn.

Procambium Formation

For many years, auxin has been proposed as a regulator of vascular development. The canalization theory hypothesizes that auxin induces de novo specification of procambial initials from within a field of equivalent ground meristem cells and then guides the apical–basal trajectory of differentiating veins via polarized flow through dividing cells (113). There is now molecular evidence to support this theory, at least in the context of the *Arabidopsis* leaf where procambial centers are initiated at sites of auxin maxima (121). These auxin maxima are formed when (and where) neighboring cells with opposing polarity of the PIN-FORMED (PIN1) auxin efflux transporter converge. Auxin accumulation at the convergence point is perceived by the auxin responsive transcription factor MONOPTEROS/AUXIN RESPONSE FACTOR 5 (MP/ARF5) that then acts to elevate *PIN1* expression, the polar localization of which leads to canalization of auxin flow through a narrow strand of preprocambial cells (119, 120). Crucially, MP/ARF5 also activates expression of the HD-ZIP III transcription factor ARABIDOPSIS THALIANA HOMEOBOX 8 (AtHB8); this protein marks preprocambial cell identity, stabilizes PIN1 localization (27), and acts to maintain meristematic competence in the procambial center (62). The stabilization of PIN1 reinforces flow along the developing procambial strand and thus prevents auxin maxima (and hence new procambium) from forming in neighboring cells. Although the PIN1–MP/ARF5–AtHB8 feedback loop is sufficient to explain how auxin maxima induce vascular formation in *Arabidopsis*, it is how many maxima form that distinguishes C_3 anatomy from Kranz anatomy. Indeed, higher vein densities in the leaves of C_4 eudicots relative to C_3 eudicots have been associated with elevated levels of auxin biosynthesis and transport (56), but the mechanism that regulates precisely where procambium is initiated, and how far apart procambial initials are spaced, is not known.

In both eudicots and monocots, a correlation exists between the ability of ground meristem cells to proliferate and the extent to which procambium can be initiated. When cell proliferation is compromised, for example by overexpression of a negative cell-cycle regulator in *Arabidopsis* (63) or by mutation in the *ABNORMAL VASCULAR BUNDLES* (*AVB*) gene in rice (90), leaf size is reduced and fewer veins are initiated in the leaf. In contrast, when cell proliferation is increased by constitutive expression of the *AINTEGUMENTA* gene in *Arabidopsis*, leaves are bigger and more veins are initiated (63). This association between cell proliferation and vein density is seen most clearly in the uniform strap-shaped leaves of monocots, where in both C_3 and C_4 grasses there is a linear relationship between vein number and leaf blade width (21, 112). Given this relationship, the development of a higher vein density within a leaf of any given width may require a greater proportion of ground meristem cells to retain proliferation capacity through the specific developmental time window when inductive signals are transmitted. In this scenario, proliferation capacity could be a competence factor that distinguishes ground meristem cells in C_3 and C_4 leaf primordia, enabling more cells per unit area to respond to procambium-inducing auxin maxima in C_4 leaves than in C_3.

Auxin is unlikely to regulate procambium initiation in monocots through an identical gene network to that found in *Arabidopsis*, not least because monocots have more than one *PIN1* gene (103). However, auxin flow probably plays a role as evidenced by perturbed vascular development in rice and maize leaves grown on polar transport inhibitors (120, 136), and auxin maxima may

similarly arise through the formation of PIN1 convergence points (60), albeit with different *PIN1* gene family members having distinct roles in this process (102, 121). Specifically, SISTER OF PIN1 (SoPIN1) may act to establish convergence points in the epidermis, with PIN1a and PIN1b diverting auxin flow into internal tissues, thus establishing preprocambial strands (103). The question then becomes whether higher vein densities are established because auxin maxima are more closely spaced in C_4 leaves than in C_3 leaves or because more cells within a given field are competent to receive the signal. In the mutant and transgenic lines of C_3 and C_4 monocots that have been characterized so far, plants with closer vein spacing than normal have narrower leaves than wild type, and those with wider vein spacing have broader leaves (e.g., 37, 38, 109, 122, 128, 145). In the transition from C_3 to Kranz, however, the relationship between vein number and leaf width has to be perturbed. As such, genuine regulators of procambium positioning during Kranz development will be revealed only when manipulation of gene function, or maybe of auxin maxima, leads to an increased number of longitudinal veins in a C_3 leaf, or to a decreased number in a C_4 leaf, without any alteration to leaf width.

Radial Patterning

It has previously been hypothesized that the patterning of vascular sheath and mesophyll cell types in Kranz anatomy is analogous to radial patterning of endodermal and cortical cell types around the root vasculature (35, 100). This suggestion arose because the vascular sheath layer in the shoot and the endodermal layer in the root have long been considered analogous, despite arising from different stem cell populations and at distinct developmental points (36). Comparative analyses of the SHR/SCR pathway have given traction to the idea of a shared patterning mechanism in shoots and roots, but other studies have suggested that in leaves the pathway regulates cell proliferation rather than cell patterning per se (25). Whether patterning or proliferation, however, there is currently no evidence to suggest that there is a distinct variant of the SHR/SCR pathway that operates around veins of C_3 as opposed to C_4 leaves.

In *Arabidopsis* roots, *AtSHR* is transcribed and translated in the vasculature, and the protein moves into the adjacent cell layer where it is bound and sequestered to the nucleus by AtSCR (20, 52). Together, this AtSHR/AtSCR complex regulates the expression of a suite of target genes that specify endodermal identity (18, 43, 81, 98). Furthermore, nuclear sequestration by AtSCR prevents AtSHR movement beyond the endodermis (20, 52). The absence of AtSCR and AtSHR in the cell layer adjacent to the endodermis leads to the formation of cortex. In shoots of *Atscr* and *Atshr* mutants, misspecification of cell types in the hypocotyl leads to aberrant gravitropic responses (68) and bundle sheath cell formation is impaired in the leaf (19). Interestingly, *AtSHR* is one of the genes that is coexpressed with *AtHB8* during procambium initiation in the *Arabidopsis* leaf (44), providing a direct link between vein formation and the patterning of surrounding cell types. Although discovered in *Arabidopsis*, three pieces of evidence point to AtSCR and AtSHR function being conserved in monocots: (*a*) The rice OsSHR protein is mobile in *Arabidopsis* (152) and interacts with AtSCR in vitro (20), (*b*) the maize *ZmSCR1* gene complements the *Atscr* mutant phenotype (84), and (*c*) both the OsSHR and AtSHR proteins induce supernumerary cortical cell layers when overexpressed in rice (54).

Transcriptome data sets have identified *SHR* and *SCR* orthologs as candidate regulators of Kranz anatomy (41, 145, 146). In maize, *ZmSHR1* is expressed in both the developing vasculature and bundle sheath cells (11, 82), whereas *ZmSCR1* is preferentially expressed in bundle sheath cells (11, 82). These expression profiles and the phenotypes of *Zmscr1* and *Zmshr1* mutants (126, 127) support a role for the SHR/SCR pathway in the regulation of radial patterning around leaf veins in maize and are consistent with the proposal that bundle sheath and mesophyll cell types

are specified by a positional signal emanating from the veins (76). However, a number of issues need to be resolved; for example, the phenotypes of both *Zmscr1* and *Zmshr1* mutants are subtle, with most of the vascular bundles in each leaf developing normally (126, 127). This observation suggests genetic redundancy, either in terms of individual genes or at the pathway level. Notably, there was a duplication of *SHR* at the base of the monocots, such that the majority of monocot genomes encode *SHR1* and *SHR2* genes orthologous to *AtSHR*. The situation in maize is further complicated by the recent maize whole genome duplication, which led to homeolog duplicates of both *ZmSCR1* and *ZmSHR2* (but not *ZmSHR1*).

In *Arabidopsis*, *INDETERMINATE DOMAIN* (*IDD*) genes such as *AtJACKDAW (AtJKD)* modulate activity of the SHR/SCR pathway (86, 104, 150). Three IDD genes have been identified as candidate Kranz regulators (41, 146). One is *NAKED ENDOSPERM1 (ZmNKD1)*, which acts redundantly with the duplicate *ZmNKD2* to regulate cell patterning in the aleurone (155), and the other two are *ZmRAVEN1* (*ZmRVN1*) (previously *ZmIDD2*) and *ZmIDD14*. Consistent with a role in modulating the activity of either *ZmSCR1* or *ZmSHR1* during Kranz development, *ZmNKD1* is preferentially expressed in mesophyll cells and *ZmRVN1* is preferentially expressed in bundle sheath cells of mature maize leaves (11, 82). Given the number of genes involved, however, and the presence of homeologs for many of them (at least in maize), functional analysis of this pathway is likely to take years, particularly because validation of a specific role in Kranz patterning will require elucidation of function in both C_3 and C_4 grasses.

Functionalization of Vascular Sheath Cells for Photosynthesis

Differences between developmental processes in bundle sheath and mesophyll cells result in cell types with distinct chloroplast morphology and metabolism in both C_3 and C_4 species (2, 66, 70, 80, 91). Insight into mechanisms operating in C_4 plants has been gained from forward mutagenesis experiments in maize, where screens for cell-type-specific perturbations in chloroplast development identified a class of *bundle sheath defective* mutants but failed to identify mutants with perturbed mesophyll chloroplast development (9, 72, 73, 111). Although these screens were by no means exhaustive, one interpretation of the failure to identify mesophyll-specific defects is that any such perturbations negatively impacted bundle sheath development, resulting in general rather than cell-specific chloroplast defects. In this scenario, chloroplast development in maize bundle sheath cells is dependent on signals from adjacent, functional mesophyll cells. Intriguingly, the opposite is inferred from mutagenesis in C_3 eudicots (mainly *Arabidopsis*), where a class of reticulated mutants has been identified in which bundle sheath cells differentiate normally but mesophyll cell development is abnormal (reviewed in 89). Most of the reticulated phenotypes are caused by mutations in genes encoding plastid-localized proteins that are involved in primary metabolism, but counterintuitively, at least some of the genes are expressed preferentially in vasculature-associated cells rather than mesophyll cells (67, 133, 139). On this basis, it has been proposed that mesophyll cell differentiation in C_3 eudicots is dependent on metabolic signals (and/or supplies) from adjacent veins and/or vascular sheath cells. Inevitably, comparisons between C_3 eudicots and C_4 monocots are complicated by the overlapping effects of C_3 versus C_4 and eudicot versus monocot traits, but the suggestion that metabolites function non-cell-autonomously to regulate chloroplast development in bundle sheath and mesophyll cells of both C_3 and C_4 plants (albeit with possibly opposing signal/receiver roles) is one that could focus future studies.

Although chloroplast biogenesis is regulated by many non-cell-autonomous signals, both environmental (e.g., light) and developmental, cell-autonomous processes also play a role, with nuclear-encoded transcription factors regulating the accumulation of many plastid-targeted products. Maize GOLDEN2 (ZmG2) was the first of these regulators to be identified, following the

isolation of a mutant with a bundle sheath defective phenotype (16, 49, 58, 73). Genome-wide expression profiles of isolated bundle sheath and mesophyll cells from a number of C_4 monocots and eudicots have also been used to identify potential transcriptional regulators of photosynthetic development in each cell type (1, 11, 28, 59, 82, 107, 135). To date, however, only ZmG2 has been functionally validated as a cell-specific regulator, with mutant plants exhibiting rudimentary chloroplasts in bundle sheath cells but normal mesophyll cell chloroplasts (16, 73). *ZmG2* encodes a myb-related transcription factor of the GARP family (49) that is expressed preferentially in bundle sheath cells, whereas the related gene *ZmG2-like1* (*ZmGLK1*) is expressed preferentially in mesophyll cells (110). This distinction contrasts with the situation in C_3 plants, where if more than one *GLK* gene is present in the genome, the genes act redundantly to promote photosynthetic development of mesophyll cells (39, 144, 154). Direct targets of GLK activity, at least in *Arabidopsis*, include a suite of genes encoding enzymes of the chlorophyll biosynthesis pathway and proteins required for light harvesting (149). On the basis of phylogenetic and expression analyses in a range of C_3 and C_4 species, it has been hypothesized that gene duplication and subsequent differential expression of *G2* and *GLK1* may have enabled C_4 evolution through neofunctionalization of GLK protein activity between the two cell types (144). Although this scenario is likely too simplistic, expression of either *ZmG2* or *ZmGLK1* from the constitutive maize ubiquitin promoter is sufficient to induce the sustained development of vascular sheath cell chloroplasts (both bundle sheath and mestome sheath) in rice (147), whereas constitutive activation of the endogenous *OsGLK1* gene does not have the same effect (99). This difference suggests either that the maize transgenes are not regulated in the same way as endogenous rice genes (transcriptionally and/or posttranscriptionally) or that orthologous transcription factors from C_3 and C_4 species may have inherently distinct effects on chloroplast development.

PROSPECTS FOR ENGINEERING KRANZ ANATOMY IN C_3 PLANTS

Attempts to engineer C_3 crops such as rice to use the C_4 pathway are currently held back by uncertainty over how Kranz anatomy is regulated in C_4 species. In an ideal world, elucidating the genetic mechanisms that underpin Kranz anatomy would be completed prior to engineering efforts. However, with the pressing need to increase rice yields, and the potential for C_4 rice to achieve that goal (55, 140), engineering attempts need to be made in parallel with discovery approaches. Thus far, constitutive expression of candidate Kranz regulators from maize has failed to induce C_4-like leaf anatomy in rice (145), a result that is perhaps not surprising given the likely complexity of regulatory mechanisms. Indeed, multiple genes may need to be modified simultaneously, with precise control of both spatial and temporal expression domains. Discovering the nature of those control mechanisms will take time, not least because gain-of-function experiments often bring about unexpected phenotypes. For example, expression of *ZmSHR1* in rice bundle sheath cells has no effect on vascular development or on bundle sheath cell specification but leads to the formation of supernumerary stomatal files in the overlying epidermis (124). This phenotype indicates that the SHR pathway may coordinate the positioning of veins and stomata in monocot leaves, a suggestion that is consistent with the fact that stomatal files are always positioned above (and/or below) the flanks of vascular bundles in the leaf. Although this hypothesis needs further testing, it is the first to propose a molecular link between the patterning of veins and stomata. As such, an engineering approach has provided novel insight into a fundamental developmental process. In a similar fashion, a possible mechanistic explanation for a key evolutionary event was provided by the recent finding that constitutive expression of either *ZmG2* or *ZmGLK1* in rice induces organelle development in bundle sheath cells to the same extent as seen in proto-Kranz species (147). The finding that modification of a single gene can recapitulate the transition from

C_3 to proto-Kranz suggests that the steps from proto-Kranz through C_2 to C_4 may be similarly straightforward once the key anatomical regulators are identified. Advances in transcriptomic approaches, genome editing technologies, and Kranz-related genetic resources mean that past hindrances to identification are quickly dispersing, and with collaborative efforts, the prospects for finding these regulators and engineering Kranz are brighter than ever before.

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LITERATURE CITED

1. Aubry S, Kelly S, Kumpers BM, Smith-Unna RD, Hibberd JM. 2014. Deep evolutionary comparison of gene expression identifies parallel recruitment of *trans*-factors in two independent origins of C_4 photosynthesis. *PLOS Genet*. 10(6):e1004365
2. Aubry S, Smith-Unna RD, Boursnell CM, Kopriva S, Hibberd JM. 2014. Transcript residency on ribosomes reveals a key role for the *Arabidopsis thaliana* bundle sheath in sulfur and glucosinolate metabolism. *Plant J*. 78(4):659–73
3. Bassham JA, Benson AA, Kay LD, Harris AZ, Wilson AT, Calvin M. 1954. The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. *J. Am. Chem. Soc.* 76(7):1760–70
4. Bauwe H, Hagemann M, Fernie AR. 2010. Photorespiration: players, partners and origin. *Trends Plant Sci*. 15(6):330–36
5. Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Hauser M-T, Aeschbacher RA. 1993. Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. *Development* 119:57–70
6. Bosabalidis AM, Evert RF, Russin WA. 1994. Ontogeny of the vascular bundles and contiguous tissues in the maize leaf blade. *Am. J. Bot.* 81(6):745–52
7. Bräutigam A, Kajala K, Wullenweber J, Sommer M, Gagneul D, et al. 2011. An mRNA blueprint for C_4 photosynthesis derived from comparative transcriptomics of closely related C_3 and C_4 species. *Plant Physiol*. 155(1):142–56
8. Brown WV. 1975. Variations in anatomy, associations, and origins of Kranz tissue. *Am. J. Bot.* 62(4):395–402
9. Brutnell TP, Sawers RJH, Mant A, Langdale JA. 1999. BUNDLE SHEATH DEFECTIVE2, a novel protein required for post-translational regulation of the *rbcL* gene of maize. *Plant Cell* 11(5):849–64
10. Brutnell TP, Wang L, Swartwood K, Goldschmidt A, Jackson D, et al. 2010. *Setaria viridis*: a model for C_4 photosynthesis. *Plant Cell* 22(8):2537–44
11. Chang Y-M, Liu W-Y, Shih AC-C, Shen M-N, Lu C-H, et al. 2012. Characterizing regulatory and functional differentiation between maize mesophyll and bundle sheath cells by transcriptomic analysis. *Plant Physiol*. 160(1):165–77
12. Christin P-A, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, et al. 2008. Oligocene CO_2 decline promoted C_4 photosynthesis in grasses. *Curr. Biol.* 18(1):37–43
13. Christin P-A, Osborne CP. 2014. The evolutionary ecology of C_4 plants. *New Phytol*. 204(4):765–81

14. Christin P-A, Osborne CP, Chatelet DS, Columbus JT, Besnard G, et al. 2013. Anatomical enablers and the evolution of C_4 photosynthesis in grasses. *PNAS* 110(4):1381–86
15. Coelho C, Huang P, Brutnell TP. 2017. *Setaria viridis* as a model for C_4 photosynthesis. In *Genetics and Genomics of Setaria*, ed. A Doust, X Diao, pp. 291–300. Cham, Switz.: Springer
16. Cribb L, Hall LN, Langdale JA. 2001. Four mutant alleles elucidate the role of the G2 protein in the development of C_4 and C_3 photosynthesizing maize tissues. *Genetics* 159(2):787–97
17. Crookston RK, Moss DN. 1973. A variation of C_4 leaf anatomy in *Arundinella hirta* (Gramineae). *Plant Physiol.* 52(5):397–402
18. Cui H, Hao Y, Kovtun M, Stolc V, Deng XW, et al. 2011. Genome-wide direct target analysis reveals a role for SHORT-ROOT in root vascular patterning through cytokinin homeostasis. *Plant Physiol.* 157(3):1221–31
19. Cui H, Kong D, Liu X, Hao Y. 2014. SCARECROW, SCR-LIKE 23 and SHORT-ROOT control bundle sheath cell fate and function in *Arabidopsis thaliana*. *Plant J.* 78(2):319–27
20. Cui H, Levesque MP, Vernoux T, Jung JW, Paquette AJ, et al. 2007. An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* 316(5823):421–25
21. Dannenhoffer JM, Ebert W Jr., Evert RF. 1990. Leaf vasculature in barley, *Hordeum vulgare* (Poaceae). *Am. J. Bot.* 77(5):636–52
22. Dengler NG, Dengler RE, Hattersley PW. 1985. Differing ontogenetic origins of PCR ("Kranz") sheaths in leaf blades of C_4 grasses (Poaceae). *Am. J. Bot.* 72(2):284–302
23. Dengler NG, Donnelly PM, Dengler RE. 1996. Differentiation of bundle sheath, mesophyll, and distinctive cells in the C_4 grass *Arundinella hirta* (Poaceae). *Am. J. Bot.* 83:1391–405
24. Dengler NG, Woodvine MA, Donnelly PM, Dengler RE. 1997. Formation of vascular pattern in developing leaves of the C_4 grass *Arundinella hirta*. *Int. J. Plant Sci.* 158:1–12
25. Dhondt S, Coppens F, De Winter F, Swarup K, Merks RMH, et al. 2010. SHORT-ROOT and SCARECROW regulate leaf growth in Arabidopsis by stimulating S-phase progression of the cell cycle. *Plant Physiol.* 154(3):1183–95
26. Di Laurenzio L, Wysocka-Diller J, Malamy J, Pysh L, Helariutta Y, et al. 1996. The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. *Cell* 86(3):423–33
27. Donner TJ, Sherr I, Scarpella E. 2009. Regulation of preprocambial cell state acquisition by auxin signaling in *Arabidopsis* leaves. *Development* 136(19):3235–46
28. Döring F, Streubel M, Bräutigam A, Gowik U. 2016. Most photorespiratory genes are preferentially expressed in the bundle sheath cells of the C_4 grass *Sorghum bicolor*. *J. Exp. Bot.* 67(10):3053–64
29. Dunning LT, Lundgren MR, Moreno-Villena JJ, Namaganda M, Edwards EJ, et al. 2017. Introgression and repeated co-option facilitated the recurrent emergence of C_4 photosynthesis among close relatives. *Evolution* 71(6):1541–55
30. Edwards EJ, Osborne CP, Strömberg CAE, Smith SA. 2010. The origins of C_4 grasslands: integrating evolutionary and ecosystem science. *Science* 328(5978):587–90
31. Edwards EJ, Smith SA. 2010. Phylogenetic analyses reveal the shady history of C_4 grasses. *PNAS* 107(6):2532–37
32. Edwards EJ, Still CJ. 2008. Climate, phylogeny and the ecological distribution of C_4 grasses. *Ecol. Lett.* 11(3):266–76
33. Edwards GE, Franceschi VR, Voznesenskaya EV. 2004. Single-cell C_4 photosynthesis versus the dual-cell (Kranz) paradigm. *Annu. Rev. Plant Biol.* 55:173–96
34. Edwards GE, Voznesenskaya EV. 2011. C_4 photosynthesis: Kranz forms and single-cell C_4 in terrestrial plants. In *C_4 Photosynthesis and Related CO_2 Concentrating Mechanisms*, ed. AS Raghavendra, RF Sage, pp. 29–61. Dordrecht, Neth.: Springer
35. Esau K. 1943. Ontogeny of the vascular bundle in *Zea mays*. *Hilgardia* 15(3):327–68
36. Esau K. 1965. *Plant Anatomy*. New York: John Wiley & Sons, Inc.
37. Feldman AB, Leung H, Baraoidan M, Elmido-Mabilangan A, Canicosa I, et al. 2017. Increasing leaf vein density via mutagenesis in rice results in an enhanced rate of photosynthesis, smaller cell sizes and can reduce interveinal mesophyll cell number. *Front. Plant Sci.* 8:1883

38. Feldman AB, Murchie EH, Leung H, Baraoidan M, Coe R, et al. 2014. Increasing leaf vein density by mutagenesis: laying the foundations for C_4 rice. *PLOS ONE* 9(4):e94947
39. Fitter DW, Martin DJ, Copley MJ, Scotland RW, Langdale JA. 2002. *GLK* gene pairs regulate chloroplast development in diverse plant species. *Plant J.* 31(6):713–27
40. Fladung M. 1994. Genetic variants of *Panicum maximum* (Jacq.) in C_4 photosynthetic traits. *J. Plant Physiol.* 143(2):165–72
41. Fouracre JP, Ando S, Langdale JA. 2014. Cracking the Kranz enigma with systems biology. *J. Exp. Bot.* 65(13):3327–39
42. Furbank RT. 2016. Walking the C_4 pathway: past, present, and future. *J. Exp. Bot.* 67(14):4057–66
43. Gallagher KL, Benfey PN. 2009. Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement. *Plant J.* 57(5):785–97
44. Gardiner J, Donner TJ, Scarpella E. 2011. Simultaneous activation of *SHR* and *ATHB8* expression defines switch to preprocambial cell state in Arabidopsis leaf development. *Dev. Dyn.* 240(1):261–70
45. Gowik U, Bräutigam A, Weber KL, Weber APM, Westhoff P. 2011. Evolution of C_4 photosynthesis in the genus *Flaveria*: How many and which genes does it take to make C_4? *Plant Cell* 23(6):2087–105
46. Grass Phylogeny Working Group II. 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C_4 origins. *New Phytol.* 193(2):304–12
47. Griffiths H, Weller G, Toy LFM, Dennis RJ. 2013. You're so vein: bundle sheath physiology, phylogeny and evolution in C_3 and C_4 plants. *Plant. Cell Environ.* 36(2):249–61
48. Haberlandt G. 1896. *Physiologische Pflanzenanatomie*. Leipzig, Ger.: Wilhelm Engelman
49. Hall LN, Rossini L, Cribb L, Langdale JA. 1998. GOLDEN 2: a novel transcriptional regulator of cellular differentiation in the maize leaf. *Plant Cell* 10(6):925–36
50. Hatch M, Slack C. 1966. Photosynthesis by sugar-cane leaves: a new carboxylation reaction and the pathway of sugar formation. *Biochem. J.* 101(1):103–11
51. Heckmann D, Schulze S, Denton A, Gowik U, Westhoff P, et al. 2013. Predicting C_4 photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. *Cell* 153(7):1579–88
52. Heidstra R, Welch D, Scheres B. 2004. Mosaic analyses using marked activation and deletion clones dissect *Arabidopsis* SCARECROW action in asymmetric cell division. *Genes Dev.* 18(16):1964–69
53. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, et al. 2000. The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* 101(5):555–67
54. Henry S, Dievart A, Divol F, Pauluzzi G, Meynard D, et al. 2017. *SHR* overexpression induces the formation of supernumerary cell layers with cortex cell identity in rice. *Dev. Biol.* 425(1):1–7
55. Hibberd JM, Sheehy JE, Langdale JA. 2008. Using C_4 photosynthesis to increase the yield of rice—rationale and feasibility. *Curr. Opin. Plant Biol.* 11(2):228–31
56. Huang C-F, Yu C-P, Wu Y-H, Lu M-YJ, Tu S-L, et al. 2017. Elevated auxin biosynthesis and transport underlie high vein density in C_4 leaves. *PNAS* 114:E6884–91
57. Jankovsky JP, Smith LG, Nelson T. 2001. Specification of bundle sheath cell fates during maize leaf development: roles of lineage and positional information evaluated through analysis of the *tangled1* mutant. *Development* 128(14):2747–53
58. Jenkins MT. 1927. A second gene producing golden plant color in maize. *Am. Nat.* 60:484–88
59. John CR, Smith-Unna RD, Woodfield H, Covshoff S, Hibberd JM. 2014. Evolutionary convergence of cell-specific gene expression in independent lineages of C_4 grasses. *Plant Physiol.* 165(1):62–75
60. Johnston R, Leiboff S, Scanlon MJ. 2015. Ontogeny of the sheathing leaf base in maize (*Zea mays*). *New Phytol.* 205(1):306–15
61. Kadereit G, Ackerly D, Pirie MD. 2012. A broader model for C_4 photosynthesis evolution in plants inferred from the goosefoot family (Chenopodiaceae s.s.). *Proc. R. Soc. B* 279(1741):3304–11
62. Kang J, Dengler N. 2002. Cell cycling frequency and expression of the homeobox gene *ATHB-8* during leaf vein development in *Arabidopsis*. *Planta* 216(2):212–19
63. Kang J, Mizukami Y, Wang H, Fowke L, Dengler NG. 2007. Modification of cell proliferation patterns alters leaf vein architecture in *Arabidopsis thaliana*. *Planta* 226(5):1207–18
64. Kapralov MV, Kubien DS, Andersson I, Filatov DA. 2011. Changes in Rubisco kinetics during the evolution of C_4 photosynthesis in *Flaveria* (Asteraceae) are associated with positive selection on genes encoding the enzyme. *Mol. Biol. Evol.* 28(4):1491–503

65. Khoshravesh R, Stinson CR, Stata M, Busch FA, Sage RF, et al. 2016. C_3–C_4 intermediacy in grasses: organelle enrichment and distribution, glycine decarboxylase expression, and the rise of C_2 photosynthesis. *J. Exp. Bot.* 67(10):3065–78
66. Kinsman EA, Pyke KA. 1998. Bundle sheath cells and cell-specific plastid development in *Arabidopsis* leaves. *Development* 125(10):1815–22
67. Knappe S, Löttgert T, Schneider A, Voll L, Flügge U-I, Fischer K. 2003. Characterization of two functional *phosphoenolpyruvate/phosphate translocator* (*PPT*) genes in *Arabidopsis-AtPPT1* may be involved in the provision of signals for correct mesophyll development. *Plant J.* 36(3):411–20
68. Kukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey PN, et al. 1998. Genetic evidence that the endodermis is essential for shoot gravitropism in *Arabidopsis thaliana. Plant J.* 14(4):425–30
69. Külahoglu C, Denton AK, Sommer M, Maß J, Schliesky S, et al. 2014. Comparative transcriptome atlases reveal altered gene expression modules between two Cleomaceae C_3 and C_4 plant species. *Plant Cell* 26(8):3243–60
70. Laetsch WM. 1974. The C_4 syndrome: a structural analysis. *Annu. Rev. Plant Physiol.* 25:27–52
71. Langdale JA. 2011. C_4 cycles: past, present, and future research on C_4 photosynthesis. *Plant Cell* 23(11):3879–92
72. Langdale JA, Hall LN, Roth R. 1995. Control of cellular differentiation in maize leaves. *Philos. Trans. R. Soc. B* 350:53–57
73. Langdale JA, Kidner CA. 1994. *bundle sheath defective*, a mutation that disrupts cellular differentiation in maize leaves. *Development* 120:673–81
74. Langdale JA, Lane B, Freeling M, Nelson T. 1989. Cell lineage analysis of maize bundle sheath and mesophyll cells. *Dev. Biol.* 133(1):128–39
75. Langdale JA, Metzler MC, Nelson T. 1987. The *argentia* mutation delays normal development of photosynthetic cell-types in *Zea mays*. *Dev. Biol*. 122(1):243–55
76. Langdale JA, Nelson T. 1991. Spatial regulation of photosynthetic development in C_4 plants. *Trends Genet*. 7(6):191–96
77. Langdale JA, Rothermel BA, Nelson T. 1988. Cellular patterns of photosynthetic gene expression in developing maize leaves. *Genes Dev*. 2:106–15
78. Langdale JA, Zelitch I, Miller E, Nelson T. 1988. Cell position and light influence C_4 versus C_3 patterns of photosynthetic gene expression in maize. *EMBO J.* 7:3643–51
79. Lauterbach M, Billakurthi K, Kadereit G, Ludwig M, Westhoff P, Gowik U. 2017. C_3 cotyledons are followed by C_4 leaves: intra-individual transcriptome analysis of *Salsola soda* (Chenopodiaceae). *J. Exp. Bot.* 68(2):161–76
80. Leegood RC. 2008. Roles of the bundle sheath cells in leaves of C_3 plants. *J. Exp. Bot.* 59(7):1663–73
81. Levesque MP, Vernoux T, Busch W, Cui H, Wang JY, et al. 2006. Whole-genome analysis of the SHORT-ROOT developmental pathway in *Arabidopsis. PLOS Biol.* 4(5):e143
82. Li P, Ponnala L, Gandotra N, Wang L, Si Y, et al. 2010. The developmental dynamics of the maize leaf transcriptome. *Nat. Genet.* 42(12):1060–67
83. Li Y, Ma X, Zhao J, Xu J, Shi J, et al. 2015. Developmental genetic mechanisms of C_4 syndrome based on transcriptome analysis of C_3 cotyledons and C_4 assimilating shoots in *Haloxylon ammodendron. PLOS ONE* 10(2):e0117175
84. Lim J, Jung JW, Lim CE, Lee M-HH, Kim BJ, et al. 2005. Conservation and diversification of *SCARECROW* in maize. *Plant Mol. Biol.* 59(4):619–30
85. Liu W-Y, Chang Y-M, Chen SC-C, Lu C-H, Wu Y-H, et al. 2013. Anatomical and transcriptional dynamics of maize embryonic leaves during seed germination. *PNAS* 110(10):3979–84
86. Long Y, Smet W, Cruz-Ramírez A, Castelijns B, de Jonge W, et al. 2015. Arabidopsis BIRD zinc finger proteins jointly stabilize tissue boundaries by confining the cell fate regulator SHORT-ROOT and contributing to fate specification. *Plant Cell* 27(4):1185–99
87. Lorimer GH. 1981. The carboxylation and oxygenation of ribulose 1,5-bisphosphate: the primary events in photosynthesis and photorespiration. *Annu. Rev. Plant Physiol.* 32(1):349–82
88. Lundgren MR, Osborne CP, Christin P-A. 2014. Deconstructing Kranz anatomy to understand C_4 evolution. *J. Exp. Bot.* 65(13):3357–69

89. Lundquist PK, Rosar C, Bräutigam A, Weber APM. 2014. Plastid signals and the bundle sheath: mesophyll development in reticulate mutants. *Mol. Plant* 7(1):14–29
90. Ma L, Sang X, Zhang T, Yu Z, Li Y, et al. 2017. *ABNORMAL VASCULAR BUNDLES* regulates cell proliferation and procambium cell establishment during aerial organ development in rice. *New Phytol*. 213(1):275–86
91. Majeran W, Cai Y, Sun Q, van Wijk KJ. 2005. Functional differentiation of bundle sheath and mesophyll maize chloroplasts determined by comparative proteomics. *Plant Cell* 17(11):3111–40
92. Mallmann J, Heckmann D, Bräutigam A, Lercher MJ, Weber APM, et al. 2014. The role of photorespiration during the evolution of C_4 photosynthesis in the genus *Flaveria*. *eLife* 3:e02478
93. Matasci N, Hung L-H, Yan Z, Carpenter EJ, Wickett NJ, et al. 2014. Data access for the 1,000 Plants (1KP) project. *Gigascience* 3(1):17
94. McKown AD, Dengler NG. 2009. Shifts in leaf vein density through accelerated vein formation in C_4 *Flaveria* (Asteraceae). *Ann. Bot.* 104:1085–98
95. McKown AD, Moncalvo J-M, Dengler NG. 2005. Phylogeny of *Flaveria* (Asteraceae) and inference of C_4 photosynthesis evolution. *Am. J. Bot.* 92(11):1911–28
96. Monson RK, Rawsthorne S. 2000. CO_2 assimilation in C_3–C_4 intermediate plants. In *Photosynthesis: Physiology and Metabolism*, ed. RC Leegood, TD Sharkey, S von Caemmerer, pp. 533–50. Dordrecht, Neth.: Kluwer Acad. Publ.
97. Muhaidat R, Sage TL, Frohlich MW, Dengler NG, Sage RF. 2011. Characterization of C_3–C_4 intermediate species in the genus Heliotropium L. (Boraginaceae): anatomy, ultrastructure and enzyme activity. *Plant Cell Environ*. 34(10):1723–36
98. Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* 413(6853):307–11
99. Nakamura H, Muramatsu M, Hakata M, Ueno O, Nagamura Y, et al. 2009. Ectopic overexpression of the transcription factor *OsGLK1* induces chloroplast development in non-green rice cells. *Plant Cell Physiol*. 50(11):1933–49
100. Nelson T. 2011. The grass leaf developmental gradient as a platform for a systems understanding of the anatomical specialization of C_4 leaves. *J. Exp. Bot.* 62(9):3039–48
101. Nelson T, Dengler N. 1997. Leaf vascular pattern formation. *Plant Cell* 9:1121–35
102. O'Connor DL, Elton S, Ticchiarelli F, Hsia MM, Vogel JP, Leyser O. 2017. Cross-species functional diversity within the PIN auxin efflux protein family. *eLife* 6:e31804
103. O'Connor DL, Runions A, Sluis A, Bragg J, Vogel JP, et al. 2014. A division in PIN-mediated auxin patterning during organ initiation in grasses. *PLOS Comput. Biol.* 10(1):e1003447
104. Ogasawara H, Kaimi R, Colasanti J, Kozaki A. 2011. Activity of transcription factor JACKDAW is essential for SHR/SCR-dependent activation of *SCARECROW* and *MAGPIE* and is modulated by reciprocal interactions with MAGPIE, SCARECROW and SHORT ROOT. *Plant Mol. Biol.* 77(4–5):489–99
105. Osborne CP, Sack L. 2012. Evolution of C_4 plants: a new hypothesis for an interaction of CO_2 and water relations mediated by plant hydraulics. *Philos. Trans. R. Soc. B* 367(1588):583–600
106. Pick TR, Bräutigam A, Schlüter U, Denton AK, Colmsee C, et al. 2011. Systems analysis of a maize leaf developmental gradient redefines the current C_4 model and provides candidates for regulation. *Plant Cell* 23(12):4208–20
107. Rao X, Lu N, Li G, Nakashima J, Tang Y, Dixon RA. 2016. Comparative cell-specific transcriptomics reveals differentiation of C_4 photosynthesis pathways in switchgrass and other C_4 lineages. *J. Exp. Bot.* 67(6):1649–62
108. Raven JA, Cockell CS, De La Rocha CL. 2008. The evolution of inorganic carbon concentrating mechanisms in photosynthesis. *Philos. Trans. R. Soc. B* 363(1504):2641–50
109. Rizal G, Thakur V, Dionora J, Karki S, Wanchana S, et al. 2015. Two forward genetic screens for vein density mutants in sorghum converge on a cytochrome P450 gene in the brassinosteroid pathway. *Plant J.* 84(2):257–66
110. Rossini L, Cribb L, Martin DJ, Langdale JA. 2001. The maize *Golden2* gene defines a novel class of transcriptional regulators in plants. *Plant Cell* 13(5):1231–44
111. Roth R, Hall LN, Brutnell TP, Langdale JA. 1996. *bundle sheath defective2*, a mutation that disrupts the coordinated development of bundle sheath and mesophyll cells in the maize leaf. *Plant Cell* 8(5):915–27

112. Russell SH, Evert RF. 1985. Leaf vasculature in *Zea mays* L. *Planta* 164:448–58
113. Sachs T. 1981. The control of the pattern differentiation of vascular tissue. *Adv. Bot. Res.* 9:151–62
114. Sage RF, Christin P-A, Edwards EJ. 2011. The C_4 plant lineages of planet Earth. *J. Exp. Bot.* 62(9):3155–69
115. Sage RF, Khoshravesh R, Sage TL. 2014. From proto-Kranz to C_4 Kranz: building the bridge to C_4 photosynthesis. *J. Exp. Bot.* 65(13):3341–56
116. Sage RF, Sage TL, Kocacinar F. 2012. Photorespiration and the evolution of C_4 photosynthesis. *Annu. Rev. Plant Biol.* 63:19–47
117. Sage TL, Busch FA, Johnson DC, Friesen PC, Stinson CR, et al. 2013. Initial events during the evolution of C_4 photosynthesis in C_3 species of *Flaveria*. *Plant Physiol.* 163(3):1266–76
118. Sakaguchi J, Fukuda H. 2008. Cell differentiation in the longitudinal veins and formation of commissural veins in rice (*Oryza sativa*) and maize (*Zea mays*). *J. Plant Res.* 121(6):593–602
119. Sauer M, Balla J, Luschnig C, Wiśniewska J, Reinöhl V, et al. 2006. Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes Dev.* 20(20):2902–11
120. Scarpella E, Boot KJM, Rueb S, Meijer AH. 2002. The procambium specification gene *Oshox1* promotes polar auxin transport capacity and reduces its sensitivity toward inhibition. *Plant Physiol.* 130(3):1349–60
121. Scarpella E, Marcos D, Friml JJ, Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes Dev.* 20(8):1015–27
122. Scarpella E, Rueb S, Meijer AH. 2003. The *RADICLELESS1* gene is required for vascular pattern formation in rice. *Development* 130(4):645–58
123. Scheres B, Di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, et al. 1995. Mutations affecting the radial organization of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121:53–62
124. Schuler ML, Sedelnikova OV, Walker BJ, Westhoff P, Langdale JA. 2018. SHORTROOT-mediated increase in stomatal density has no impact on photosynthetic efficiency. *Plant Physiol.* 176:757–72
125. Sharman B. 1942. Developmental anatomy of the shoot of *Zea mays* L. *Ann. Bot.* 6:245–84
126. Slewinski TL, Anderson AA, Price S, Withee JR, Gallagher K, Turgeon R. 2014. Short-Root1 plays a role in the development of vascular tissue and Kranz anatomy in maize leaves. *Mol. Plant* 7(8):1388–92
127. Slewinski TL, Anderson AA, Zhang C, Turgeon R. 2012. *Scarecrow* plays a role in establishing Kranz anatomy in maize leaves. *Plant Cell Physiol.* 53(12):2030–37
128. Smillie IRA, Pyke KA, Murchie EH. 2012. Variation in vein density and mesophyll cell architecture in a rice deletion mutant population. *J. Exp. Bot.* 63(12):4563–70
129. Stata M, Sage TL, Hoffmann N, Covshoff S, Ka-Shu Wong G, Sage RF. 2016. Mesophyll chloroplast investment in C_3, C_4 and C_2 species of the genus *Flaveria*. *Plant Cell Physiol.* 57(5):904–18
130. Steeves TA, Sussex IM. 1989. *Patterns in Plant Development*. Cambridge, UK: Cambridge Univ. Press
131. Stewart RN, Derman H. 1975. Flexibility in ontogeny as shown by the contribution of shoot apical layers to leaves of periclinal chimeras. *Am. J. Bot.* 62:935–47
132. Still CJ, Berry JA, Collatz GJ, DeFries RS. 2003. Global distribution of C_3 and C_4 vegetation: carbon cycle implications. *Global Biogeochem. Cycles* 17(1):1006
133. Streatfield SJ, Weber A, Kinsman EA, Hausler RE, Li J, et al. 1999. The phosphoenolpyruvate/phosphate translocator is required for phenolic metabolism, palisade cell development, and plastid-dependent nuclear gene expression. *Plant Cell* 11(9):1609–22
134. Sud R, Dengler NG. 2000. Cell lineage of vein formation in variegated leaves of the C_4 grass *Stenotaphrum secundatum*. *Ann. Bot.* 86(1):99–112
135. Tausta SL, Li P, Si Y, Gandotra N, Liu P, et al. 2014. Developmental dynamics of Kranz cell transcriptional specificity in maize leaf reveals early onset of C_4-related processes. *J. Exp. Bot.* 65(13):3543–55
136. Tsiantis M, Brown MI, Skibinski G, Langdale JA. 1999. Disruption of auxin transport is associated with aberrant leaf development in maize. *Plant Physiol.* 121(4):1163–68
137. van den Bergh E, Külahoglu C, Bräutigam A, Hibberd JM, Weber APM, et al. 2014. Gene and genome duplications and the origin of C_4 photosynthesis: birth of a trait in the Cleomaceae. *Curr. Plant Biol.* 1:2–9
138. Vicentini A, Barber JC, Aliscioni SS, Giussani LM, Kellogg EA. 2008. The age of the grasses and clusters of origins of C_4 photosynthesis. *Glob. Chang. Biol.* 14(12):2963–77

139. Voll L, Häusler RE, Hecker R, Weber A, Weissenböck G, et al. 2003. The phenotype of the *Arabidopsis cue1* mutant is not simply caused by a general restriction of the shikimate pathway. *Plant J.* 36(3):301–17
140. von Caemmerer S, Quick WP, Furbank RT. 2012. The development of C_4 rice: current progress and future challenges. *Science* 336(6089):1671–72
141. Voznesenskaya EV, Franceschi VR, Kiirats O, Freitag H, Edwards GE. 2001. Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. *Nature* 414(6863):543–46
142. Wakayama M, Ohnishi J, Ueno O. 2006. Structure and enzyme expression in photosynthetic organs of the atypical C_4 grass *Arundinella hirta*. *Planta* 223(6):1243–55
143. Wang L, Czedik-Eysenberg A, Mertz RA, Si Y, Tohge T, et al. 2014. Comparative analyses of C_4 and C_3 photosynthesis in developing leaves of maize and rice. *Nat. Biotechnol.* 32(11):1158–65
144. Wang P, Fouracre J, Kelly S, Karki S, Gowik U, et al. 2013. Evolution of *GOLDEN2-LIKE* gene function in C_3 and C_4 plants. *Planta* 237(2):481–95
145. Wang P, Karki S, Biswal AK, Lin H-C, Dionora MJ, et al. 2017. Candidate regulators of early leaf development in maize perturb hormone signalling and secondary cell wall formation when constitutively expressed in rice. *Sci. Rep.* 7(1):4535
146. Wang P, Kelly S, Fouracre JP, Langdale JA. 2013. Genome-wide transcript analysis of early maize leaf development reveals gene cohorts associated with the differentiation of C_4 Kranz anatomy. *Plant J.* 75(4):656–70
147. Wang P, Khoshravesh R, Karki S, Tapia R, Balahadia CP, et al. 2017. Re-creation of a key step in the evolutionary switch from C_3 to C_4 leaf anatomy. *Curr. Biol.* 27:3278–87
148. Wang P, Vlad D, Langdale JA. 2016. Finding the genes to build C_4 rice. *Curr. Opin. Plant Biol.* 31:44–50
149. Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA. 2009. GLK transcription factors coordinate expression of the photosynthetic apparatus in *Arabidopsis*. *Plant Cell* 21(4):1109–28
150. Welch D, Hassan H, Blilou I, Immink R, Heidstra R, Scheres B. 2007. *Arabidopsis* JACKDAW and MAGPIE zinc finger proteins delimit asymmetric cell division and stabilize tissue boundaries by restricting SHORT-ROOT action. *Genes Dev.* 21(17):2196–204
151. Williams BP, Johnston IG, Covshoff S, Hibberd JM. 2013. Phenotypic landscape inference reveals multiple evolutionary paths to C_4 photosynthesis. *eLife* 2:e00961
152. Wu S, Lee C-M, Hayashi T, Price S, Divol F, et al. 2014. A plausible mechanism, based upon SHORT-ROOT movement, for regulating the number of cortex cell layers in roots. *PNAS* 111(45):16184–89
153. Wysocka-Diller JW, Helariutta Y, Fukaki H, Malamy JE, Benfey PN. 2000. Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. *Development* 127(3):595–603
154. Yasumura Y, Moylan EC, Langdale JA. 2005. A conserved transcription factor mediates nuclear control of organelle biogenesis in anciently diverged land plants. *Plant Cell* 17(7):1894–907
155. Yi G, Neelakandan AK, Gontarek BC, Vollbrecht E, Becraft PW. 2015. The *naked endosperm* genes encode duplicate INDETERMINATE domain transcription factors required for maize endosperm cell patterning and differentiation. *Plant Physiol.* 167(2):443–56