Kranz Anatomy and the C_4 Pathway

James O Berry, *State University of New York, Buffalo, New York, USA*

C_4 photosynthesis incorporates novel leaf anatomy, metabolic specializations, and modified gene expression. The evolution of this pathway in some plants has facilitated their adaptation to high temperatures or arid conditions.

Secondary article

Article Contents

Introduction

Many plants that inhabit warm tropical or arid environments have evolved a specialized and efficient pathway for the dark reactions of photosynthesis. This pathway is termed C_4 photosynthesis, since atmospheric carbon dioxide is initially fixed into a four-carbon moleculeHatch, 1987). It is also referred to as the Hatch–Slack pathway.

The leaves of C_4 plant species possess a specialized leaf anatomy, termed Kranz anatomy, which consists of two morphologically and functionally distinct types of photosynthetic cells: mesophyll (mp) and bundle sheath (bs). The bs cells occur as a layer of cells forming a ring that surrounds each of the leaf veins, and these are surrounded in turn by one or more layers of mp cells. This wreath-like arrangement (**Figure 1**) (Kranz is the German word for wreath) serves to compartmentalize the two different sets of reactions that make up the C_4 pathway (Gutierrez *et al*., 1974; Hatch, 1987).

In C_4 plants, the initial fixation of atmospheric carbon dioxide (carboxylation phase) occurs in mp cells, and is accomplished by the mp-specific enzyme phosphoenolpyruvate carboxylase (PEPCase). The initial carboxylation of the three-carbon phosphoenolpyruvate (PEP) results in the formation of C_4 acids, which are then transported from the mp cells to the neighbouring bs cells. In bs cells, the C_4 acids are decarboxylated (decarboxylation phase), releasing carbon dioxide in these cells to levels several-fold higher than the concentration in air. In the subsequent reassimilation step, this carbon dioxide is incorporated into the Calvin cycle by the bs-specific enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The specialized biochemical reactions and cell types of the C_4 pathway thus work together as a 'carbon dioxide pump', to concentrate carbon dioxide in the vicinity of Rubisco and reduce metabolically wasteful photorespiration caused by the oxygenase activity of this enzyme (Hatch, 1987; Nelson and Langdale, 1992).

Kranz leaf anatomy and initial photosynthetic production of four-carbon acids distinguish C_4 plants from C_3 plants, which have only one photosynthetic cell type and use Rubisco to fix atmospheric carbon dioxide directly into the three-carbon molecule phosphoglyceric acid (3-PGA).

Evolution of the C_4 Pathway

C_4 photosynthesis is found in many plant species, mostly in monocots (such as maize, sugar cane and several grasses) but also in many dicots (such as amaranth and flaveria). Some plant genera contain C_3 as well as C_4 species, and some (such as *Flaveria* spp.) contain plants that can be classified as C_3–C_4 intermediates. Such a wide and diverse distribution in the occurrence of the C_4 pathway indicates that it has evolved independently and at many different times (Nelson and Langdale, 1992; Furbank and Taylor, 1995). Since all C_4 plants share a number of characteristics in terms of leaf anatomy, physiology and biochemistry, they stand as excellent examples of convergent evolution.

The C_4 pathway provides a means for maintaining photosynthetic efficiency under conditions of high temperature or water limitation by greatly reducing or eliminating photorespiration. Photorespiration is problematic in these situations because increasing temperatures enhance the oxygenase activity relative to the carboxylation activity of the Rubisco enzyme (Hatch, 1987; Ehleringer *et al*., 1997).

In addition, C_4 plants have a selective advantage under arid conditions. To minimize water loss through transpiration, plants must reduce the opening of their stomata, which leads to reduced carbon dioxide uptake and reduced release of oxygen. Since PEPCase can fix carbon dioxide from relatively low intracellular concentrations, C_4 plants show higher rates of photosynthesis than C_3 plants under conditions that promote high transpiration rates.

C_4 plants also show higher photosynthetic efficiencies under conditions of light saturation, such as occur in open plains or savannahs.

It is likely that the ancestors of most contemporary C_4 and C_3–C_4 intermediate plants evolved between 30 and 50 million years ago, in response to reduced carbon dioxide in the atmosphere, possibly in combination with elevated temperatures or limited water availability (Hatch, 1987; Ehleringer *et al*., 1997). The occurrence of C_3–C_4 intermediates indicates that there are multiple independent steps required for evolution of full C_4 capability. It is

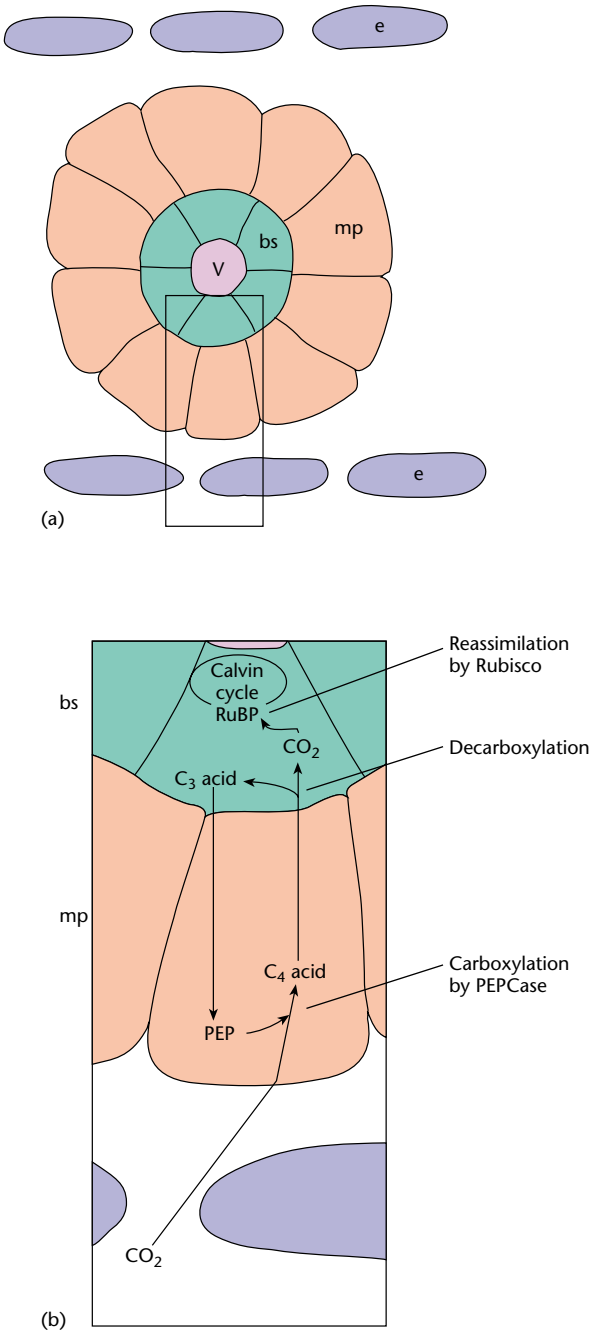

Figure 1 Diagramatic representation of Kranz anatomy and the C_4 pathway. (a) Typical Kranz leaf anatomy as observed within a leaf cross-section. (b) Simplified representation of the C_4 pathway superimposed on an enlarged diagram of the two cell types, indicating the cellular localization of carboxylation, decarboxylation and reassimilation reactions. v, vascular centre; bs, bundle sheath cells; mp, mesophyll cells; e, upper or lower leaf epidermal cells.

possible that in some intermediate species, C_4 evolution is still an ongoing process.

Developmental Biology of Cell and Tissue Specificity

In monocots, C_4 development occurs along a gradient within the leaf. The oldest cells in the outer portions of the leaf show fully differentiated Kranz anatomy, while younger, less differentiated regions near the leaf base show intermediate stages of C_4 development. In dicots, leaf development and differentiation of Kranz anatomy are less polarized (Nelson and Langdale, 1992; Berry, 1997).

In both monocots and dicots, the development of C_4 capacity is closely associated with the differentiation of Kranz anatomy. C_4 mp cells have an origin similar to chlorenchyma cells of C_3 plants. Bundle sheath cells differentiate from either ground meristem or procambium cells (depending on the species) adjacent to a developing leaf vein. The leaf veins, which are derived from procambium, develop prior to the bs cells and appear to be the primary positional determinant for bs cell differentiation. The production and cell-specific compartmentalization of C_4 enzymes (such as PEPCase only in mp cells) occurs after bs and mp differentiation is initiated, and for most of the C_4 enzymes correlates with the maturation of these cells.

In some cases, compartmentalization of Rubisco occurs after development of Kranz anatomy is completed. In dark-grown maize plants, Rubisco is found in both bs and mp cells, and becomes bs-specific when the plants are illuminated. In the dicot amaranth, bs-specific localization of Rubisco occurs independently of light, but is coordinated with the carbon sink–source transition, a developmental process in which a dicot leaf changes from a net importer to a net exporter of photosynthetically fixed carbon.

Types of C_4 Biochemistry and Metabolic Differentiations

There are three major types of C_4 biochemistry, which are categorized according to the enzyme utilized for decarboxylating C_4 acids in bs cells. The three groups of C_4 plants also differ in the nature of the four-carbon acids that are transported to bs cells (Gutierrez *et al.*, 1974; Hatch, 1987).

NADP-ME-type C_4 plants (such as maize, sorghum and sugar cane) utilize a phosphate nicotinamide–adenine dinucleotide (NADP)-dependent malic enzyme (ME) for the decarboxylation reaction. This enzyme is located in the bs chloroplast. Malate produced in mp cells is transported to the bs chloroplasts, where the molecules are decarboxylated by the NADP-ME. This reaction releases carbon dioxide and forms pyruvate. Pyruvate produced by decarboxylation is transported back to the mp cells, where it is converted to PEP (the three-carbon substrate for

PEPCase) by the enzyme pyruvate orthophosphate dikinase (PPdK), to complete the C_4 cycle.

NAD-ME-type C_4 plants (such as amaranth and millet) use a nicotinamide–adenine dinucleotide (NAD)-dependent malic enzyme for decarboxylation, which is located in the bs mitochondria. These plants transport aspartate to bs cells, where it is transaminated to oxaloacetate (OAA), and then reduced to form malate. In bs mitochondria, the malate is decarboxylated to carbon dioxide and pyruvate.

PCK-type C_4 plants (several species of grasses) use a phosphoenolpyruvate carboxykinase (PCK) as the major decarboxylating enzyme, and this is located in the bs cytoplasm. These plants also transport aspartate to bs cells, where it is transaminated to OAA, and then directly decarboxylated by PEP carboxykinase to produce carbon dioxide and PEP.

To complete the C_4 cycle in both NAD-ME- and PCK-type C_4 plants, alanine is produced from PEP in bs cells, and this is the C_3 acid which is shuttled back to mp cells. There it is ultimately converted by PPdK to PEP for use in carbon dioxide fixation.

Cytology

In each C_4 group, the chloroplasts of mp cells are similar to those of C_3 plants. Mesophyll plastids possess normal thylakoid development and photosystem II (PSII) activity, and are distributed throughout the cytoplasm. The three C_4 groups differ in the localization, morphology and biochemistry of their bs chloroplasts.

NADP-ME-type C_4 species have bs chloroplasts that are localized to the centrifugal portion of the cells (away from the vascular centre). Some of these plants (maize, sorghum) have bs plastids that lack granal stacks and many of the polypeptides associated with PSII, while others (*Flaveria*) have bs plastids with normal PSII activity.

NAD-ME-type species have bs chloroplasts that are clustered tightly together with the mitochondria in the centripetal position of the cell (in toward the vascular tissue). These are similar to mp chloroplasts, possessing well-developed grana and normal PSII activity.

PCK species have bs plastids that are located in the centrifugal position, with granal stacks and PSII activity.

Energetics of the Pathway in Comparison to C_3 Metabolism

The C_4 pathway has a higher energy cost associated with photosynthetic carbon fixation, as compared to the basic C_3 pathway (if photorespiration is not taken into account) (Hatch, 1987). For each molecule of carbon dioxide fixed, a molecule of PEP must be regenerated at the cost of two adenosine triphosphate (ATP) molecules. In C_4 plants, the overall energy requirements of carbon fixation are five ATPs and two NADPH per carbon dioxide, whereas in C_3 plants only three ATPs and two NADPH are needed. Transport of the three-carbon and four-carbon metabolites between the two cell types is believed to occur along gradients through specialized plasmodesmata. Thus, there appears to be no additional energy requirement for the transport steps of the C_4 process. Taking these factors into account, the extra energy requirements of the C_4 pathway are more than compensated for by the energy saved when photorespiration is reduced or eliminated.

Regulation of Enzyme Activity and Synthesis

As part of a complex biochemical pathway, the various C_4 enzymes show multiple independent and interactive regulatory mechanisms (Furbank and Taylor, 1995; Berry *et al*., 1997). Activities of the various enzymes are modulated by feedback regulation, light, carbon metabolism, energy levels and photosynthetic activity. For some C_4 enzymes, regulation of activity has been carried over from the basic C_3 metabolic form of the enzyme, such as activation of PEPCase and PPdK by phosphorylation.

At the molecular level, development of C_4 capacity appears to involve many independent modifications to already existing C_3-type expression patterns for numerous metabolic, photosynthetic and developmental genes.

Genes encoding Rubisco, an abundant enzyme in all photosynthetic cells of C_3 plants, must be selectively downregulated in C_4 mp cells but not in bs cells, so that the enzyme accumulates only in bs cells. On the other hand, genes that encode enzymes with no photosynthetic function in C_3 plants, but which have acquired photosynthetic function in C_4 plants (i.e. PEPCase, PPdK, NAD- or NADP-malic enzymes), have had their expression modified in two ways. First, expression has been greatly enhanced, so these enzymes can be present at the very abundant levels required for photosynthesis. Second, expression has become selectively restricted, so that these new photosynthetic enzymes accumulate in only one cell type.

For many C_4 genes, control of cell type-specific expression appears to involve regulation of transcription, as well as posttranscriptional regulation (mRNA stability or translation). In addition, many of the C_4 genes are light-regulated, so that their expression is activated in light and inactivated in darkness.

References

Berry JO, McCormac DJ, Long JJ, Boinski JJ and Corey A (1997) Photosynthetic gene expression in amaranth, an NAD-ME type C_4 dicot. *Australian Journal of Plant Physiology* **24**: 423–428.

Ehleringer JR, Cerling TE and Helliker BR (1997) C4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* **112**: 285–299.

Furbank RT and Taylor WC (1995) Regulation of photosynthesis in C_3 and C_4 plants: a molecular approach. *The Plant Cell* **7**: 797–807.

Gutierrez M, Gracen VE and Edwards GE (1974) Biochemical and cytological relationships in C4 plants. *Planta* **119**: 279–300.

Hatch MD (1987) C_4 photosynthesis: a unique blend of modified biochemistry, anatomy, and ultrastructure. *Biochemica et Biophysica Acta* **895**: 81–106.

Nelson T and Langdale JA (1992) Developmental genetics of C_4 photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 25–47.

Further Reading

Hatch MD (1992) C_4 photosynthesis: an unlikely process full of surprises. *Plant and Cell Physiology* **33**: 333–342.

Hattersley PW and Watson L (1992) Diversification of photosynthesis. In: Chapman GP (ed.) *Grass Evolution and Domestication*. Cambridge: Cambridge University Press.

Langdale JA and Nelson T (1991) Spatial regulation of photosynthetic development in C_4 plants. *Trends in Genetics* **7**: 191–196.

Long JJ and Berry JO (1996) Tissue-specific and light-mediated expression of the C_4 photosynthetic NAD-dependent malic enzyme of amaranth mitochondria. *Plant Physiology* **112**: 473–482.

Sheen J (1991) Molecular mechanisms underlying the differential expression of maize pyruvate, orthophosphate dikinase genes. *The Plant Cell* **3**: 225–245.

von Caemmerer S and Furbank RT (eds) (1997) C_4 photosynthesis: 30 (or 40) years on. *Australian Journal of Plant Physiology* (Special Issue) **24** (4).

Wang J-L, Turgeon R, Carr JP and Berry JO (1993) Carbon sink-to-source transition is co-ordinated with establishment of cell-specific gene expression in a C_4 plant. *The Plant Cell* **5**: 289–296.