Rubisco gene expression in C₄ plants

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Abstract

In leaves of most C₄ plants, ribulose 1,5 bisphosphate carboxylase (Rubisco) accumulates only in bundle sheath (bs) cells that surround the vascular centres, and not in mesophyll (mp) cells. It has been shown previously that in the C₄ dicots amaranth and Flaveria bidentis, post-transcriptional control of mRNA translation and stability mediate the C₄ expression patterns of genes encoding the large and small Rubisco subunits (chloroplast rbcL and nuclear RbcS, respectively). Translational control appears to regulate bs cell-specific Rubisco gene expression during early dicot leaf development, while control of mRNA stability appears to mediate bs-specific accumulation of RbcS and rbcL transcripts in mature leaves. Post-transcriptional control is also involved in the regulation of Rubisco gene expression by light, and in response to photosynthetic activity. Transgenic and transient expression studies in F. bidentis provide direct evidence for post-transcriptional control of bs cell-specific RbcS expression, which is mediated by the 5' and 3' untranslated regions (UTRs) of the mRNA. Comparisons of Rubisco gene expression in these dicots and in the monocot maize indicate possible commonalities in the regulation of RbcS and rbcL genes in these divergent C₄ species. Now that the role of post-transcriptional regulation in C₄ gene expression has been established, it is likely that future studies of mRNA–protein interactions will address long-standing questions about the establishment and maintenance of cell type-specificity in these plants. Some of these regulatory mechanisms may have ancestral origins in C₃ species, through modification of pre-existing factors, or by the acquisition of novel C₄ processes.

Rubisco gene expression and C₄ photosynthesis

The Kranz-type leaf anatomy typically associated with C₄ photosynthesis is comprised of two photosynthetic cell types, the bundle sheath (bs) and mesophyll (mp) cells, which differ in their CO₂ assimilation functions (Hatch and Slack, 1970; Hatch, 1987; Berry et al., 1997; Edwards et al., 2004; Ueno, 2001; von Caemmerer and Furbank, 2003). These two cell types provide structural compartmentalization for separate sets of carboxylation and decarboxylation reactions that are essential for the function of the C₄ pathway. Within the leaves of C₄ plants, bs and mp cells each accumulate a distinct assortment of enzymes, encoded by nuclear as well as organelar genes, to catalyse the cell type-specific biochemical reactions. The organelles of each cell type often possess functional and morphological dimorphisms, and it is clear that characteristic CO₂ fixation capabilities of these plants require regulatory processes and interactions occurring within and between the different cellular compartments (Gutierrez et al., 1974; Walbot, 1977; Berry et al., 1997; Sheen, 1999; Majerana et al., 2005).

Ribulose 1,5-bisphosphate carboxylase (Rubisco), and its corresponding mRNAs, are specific to the bs cells of mature C₄ leaves (Fig. 1). This enzyme provides an excellent model system for investigating nuclear, cytoplasmic, and organelar processes that regulate photosynthetic gene expression in these plants. The rbcL and RbcS genes that encode the two subunits that make up this enzyme (large subunit, LSU, and small subunit, SSU, respectively) occur in separate cellular compartments (Mizirokko and Lorimer, 1983; Spreitzer and Salvucci, 2002). The rbcL gene is localized to the chloroplasts, and these transcripts are translated on prokaryotic-like plastid ribosomes. The RbcS gene family occurs within the nucleus, and cytoplasmic ribosomes translate RbcS mRNAs as a precursor protein (Dean et al., 1989; Wanner and Gruissem, 1991; Spreitzer, 1993; Ewing et al., 1998; Corey et al., 1999; Sasanuma, 2001). The precursor is

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Rubisco serves as the primary CO₂ assimilation enzyme in all plants, and many aspects of Rubisco gene expression are shared by both C₄ and C₃ species (Ellis, 1979; Miziorik and Lorimer, 1983; Taylor, 1989; Spreitzer, 1993; Furbank and Taylor, 1995). In C₄ and C₃ plants, the expression of both rbcL and RbcS genes is restricted primarily to leaves and other photosynthetic tissues. This enzyme accumulates very abundantly in the chloroplasts, and can represent as much as 50% of the total soluble protein in some C₃ species. Rubisco levels in C₄ leaves tend to be significantly lower (about 50%), since the enzyme is only present in bs cells (Furbank and Taylor, 1995). Expression of the rbcL and RbcS genes is highly regulated, and can be influenced by a variety of intrinsic and external factors. Regulation of rbcL and RbcS genes by light is a phenomenon that is probably shared by all plant species (Berry et al., 1985; Gilmartin et al., 1990; Wang et al., 1993a; Shiina et al., 1998; Sheen, 1999; Zhou et al., 2001). Other factors that have been shown to affect Rubisco gene expression include cell and tissue development (Wanner and Gruissem, 1991; Nelson and Langdale, 1992; Kubicki et al., 1994; Furbank and Taylor, 1995; Berry et al., 1997; Sheen, 1999; Patel et al., 2004; Majerana et al., 2005), photosynthetic metabolism (Sheen, 1990; Jiang et al., 1993; Krapp et al., 1993; Wang et al., 1993b; McCormac et al., 1997; Urwin and Jenkins, 1997; Acevedo-Hernandez et al., 2005), nutrients and hormones (Furbank and Taylor, 1995; Sheen, 1999; Okawa et al., 2004; Imai et al., 2005), as well as age, senescence, and disease (Hensel et al., 1993; Jiang et al., 1993; Bergera et al., 2004).

While transcriptional control is clearly involved in many of these processes (Nelson et al., 1984; Mullet, 1988; Thompson and Meagher, 1990; Wanner and Gruissem, 1991; Bansal et al., 1992; Furbank and Taylor, 1995; Urwin and Jenkins, 1997; Cheng et al., 1998; Sheen, 1999; Bergera et al., 2004; Acevedo-Hernandez et al., 2005), it is also well established that regulation at one or more post-transcriptional levels mediates many aspects of rbcL and RbcS gene expression (Silverthorne and Tobin, 1990; Thompson and Meagher, 1990; Wanner and Gruissem, 1991; Boinski et al., 1993; Gallie, 1993; Gillham et al., 1994; Rodermel et al., 1996; Roth et al., 1996; Berry et al., 1997; Shiina et al., 1998; Brutnell et al., 1999; Schuster et al., 1999; McCormac et al., 2001; Sinha et al., 2002; Patel et al., 2004, 2006). Most of these phenomena have been demonstrated in both C₄ and C₃ species, and probably represent basic regulatory processes that were retained by rbcL and RbcS genes during the divergence and evolution of C₄ plants. Thus, in both C₃ and C₄ species, expression of Rubisco genes is confined mostly to leaves, occurs primarily in light, and is highly responsive to signals that affect growth and development of the plant. The primary difference in Rubisco gene expression between these two plant groups is that in C₄ leaves, rbcL and RbcS mRNAs and protein accumulation has become restricted to only one of the two photosynthetic cell types that occur within the leaves, the bs cells (Fig. 1).

Since Rubisco functions as a photosynthetic enzyme in all plants, only a few modifications may be required to achieve C₄ function for this enzyme. C₄ photosynthesis requires that rbcL and RbcS gene expression becomes fully down-regulated in mp cells, while these genes continue to be expressed at very high levels in bs cells. Additional modifications appear to be required for most of the other photosynthetic enzymes in C₄ leaves, such as the bs cell-specific malic enzymes (NAD-ME or NADP-ME), or mp-specific phosphoenolpyruvate carboxylase (PEPCase, shown in Fig. 1, bottom panel) and pyruvate orthophosphate dikinase (PPDK). These enzymes have originated from non-photosynthetic C₃ ancestors, and have acquired many alterations to their basic expression patterns. Some new aspects of their expression are shared...
with the Rubisco genes (Hatch, 1987; Long and Berry, 1996; Furbank and Taylor, 1995; Sheen, 1999; Monson, 1999; Westhoff and Gowik, 2004; Sage, 2004). For example, genes encoding the C₄ forms of these enzymes have greatly enhanced levels of expression, so that they can accumulate at the abundant levels required for their new photosynthetic roles. In addition, their expression has become strongly restricted to only one of the two leaf cell types. The C₄ photosynthesis genes recruited from other pathways in both bs and mp cells have also had their expression confined to leaves and other photosynthetic tissues, and are regulated by light and photosynthetic metabolism (Berry et al., 1997; Furbank and Taylor, 1995; Sheen, 1999). Since there appears to be many common attributes in the expression of Rubisco and other photosynthetic genes in C₄ plants, it might be expected that they would all be regulated by similar mechanisms and/or control factors. However, close examination reveals that the various C₄ genes actually have distinctive expression characteristics, and several lines of evidence indicate that they are in fact regulated independently of each other (Berry et al., 1997; Sheen, 1999; Gowik et al., 2004). For this reason, mechanisms responsible for controlling rbcL and RbcS gene expression in C₄ plants, as described in this article, must be considered as being mostly limited to these genes, and are not likely to be the same as those responsible for regulating the expression of the other C₄ enzymes.

It is important to understand regulatory mechanisms that are responsible for the specialized cell-type specific Rubisco gene expression in C₄ plants, and thus ultimately underlie their enhanced photosynthetic capabilities. Research involving two C₄ dicots, Amaranthus and Flaveria bidentis (as described below), have provided evidence that regulation of the Rubisco rbcL and RbcS genes is complex, with regulation occurring at multiple levels. Most significantly, post-transcriptional control appears to predominate in the C₄ regulation of both Rubisco subunits.

Rubisco gene expression and early C₄ leaf development

In amaranth and Flaveria, Rubisco gene expression becomes established within the parameters of typical dicot leaf developmental. As these leaves expand from primordia through maturity, they are made up of a cellular mosaic with multiple lineages. While some polarity does occur from the base outward towards the edge, for the most part there is extensive variation and overlap in the size and developmental age of cells throughout the entire leaf (Steeves and Sussex, 1989). Within this somewhat uneven developmental framework, the amaranth RbcS and rbcL genes are expressed co-ordinately from the earliest stages of leaf formation, initially in the apical meristem and in the emerging leaf primordia. Expression continues during early vascular development in very young leaves, and is maintained through maturation and senescence (Wang et al., 1992, 1993b; Ramsperger et al., 1996). Although both Rubisco subunit genes are expressed continuously as leaf development progresses, in situ hybridization and immunolocalization analyses have revealed striking developmental-associated changes in the localization of rbcL and RbcS mRNAs and their corresponding proteins. These changes provide clues about regulatory processes involved in the establishment of C₄ gene expression.

Studies of very early meristem and leaf development in the C₄ dicot amaranth provided the initial evidence for post-transcriptional control of cell type-specific Rubisco gene expression (Ramsperger et al., 1996). It was found that the amaranth rbcL and RbcS mRNAs accumulate to very abundant levels within the apical meristem itself, and are evenly distributed throughout all cells of the leaf primordia. By contrast, the Rubisco LSU and SSU proteins do not show any accumulation within the meristem and are localized only to cells of the primordial ground meristem, with no accumulation anywhere else in the leaf primordium. The Rubisco mRNAs also show no cell-type specificity in leaves that have expanded to 2 mm in length. RbcS and rbcL transcripts accumulate abundantly within precursors to the bs cells that are located adjacent to the differentiating vascular centres, as well as in mp precursors that are located between the bs precursors and the epidermal cells. However, the Rubisco polypeptides do show strong specificity at the 2 mm stage; these accumulate only in bs precursor cells, but not at all within the mp precursors. At both of these early stages of development, the lack of correlation in the cellular accumulation patterns of the Rubisco transcripts and their encoded proteins is strongly suggestive of post-transcriptional control, with regulation occurring at the level of translation. These findings indicate that post-transcriptional control of Rubisco gene expression becomes established very early in leaf development, while the morphological differentiation of vascular centres and the two photosynthetic cell types is still in progress.

At 5 mm in length, amaranth leaves are fully developed morphologically, with differentiated vascular centres and clearly discernible Kranz anatomy. At this stage, the Rubisco mRNAs are still equally abundant in both bs and mp cells in a ‘C₄-like’ default pattern (Wang et al., 1992, 1993b). Unlike in the mp precursors of the earlier 2 mm leaves, some Rubisco peptides do accumulate in the mp cells of these older leaves, although at lower amounts than in the bs cells. It is likely that, by this stage, post-transcriptional repression (or lack of activation) of the Rubisco mRNAs present in mp cells is partially overcome, leading to ‘leaky’ translation and accumulation of
the Rubisco peptides in the mp as well as bs cells of the 5 mm long leaves.

The establishment of full bs cell specificity for the Rubisco proteins and mRNAs occurs as the amaranth leaves expand from 5 mm to 10 mm in length. During this time, the leaves undergo two highly co-ordinated developmental transitions that occur from the leaf apex to the leaf base (the basepetal direction) (Wang et al., 1992, 1993b). The first of these is a C$_3$–C$_4$ transition in Rubisco gene expression. During this transition, the Rubisco polypeptides and mRNAs completely disappear from the mp cells, thus becoming highly specific to bs cells. The C$_3$–C$_4$ transition in $rbc$L mRNA accumulation can be clearly observed in a section from a 7 mm long amaranth leaf in Fig. 2. At the same time, these leaves undergo a sink–source transition in carbon transport, a process in which they convert from a net importer to a net exporter of photoassimilate (Wang et al., 1993b). The simultaneous occurrence of these two transitions in the developing leaves suggests a link between the establishment of bs cell-specificity and the onset of photosynthetic activity. Both transitions are completed by the time the leaves have reached 10 mm in length (one-tenth final expansion). Once they have become source leaves, the C$_4$ localization pattern for Rubisco polypeptides and mRNAs, as shown in Fig. 1, is maintained throughout the rest of development (Wang et al., 1992, 1993b; Boinski et al., 1993).

True leaves do not develop in most dicot plants when these are grown in the absence of illumination (etiolation), and therefore it is not possible to determine the effects of light on C$_4$ leaf development and gene expression in these plants. However, cotyledons will develop on etiolated seedlings, and these have proven to be very useful for determining the effects of light on C$_4$ gene expression in dicots (Wang et al., 1993a). In light-grown amaranth seedlings, post-embryonic cotyledons possess characteristic Kranz type anatomy that is very similar to that of true leaves, and also undergo a C$_3$–C$_4$ transition in $RbcS$ and $rbc$L gene expression. At germination, the Rubisco mRNAs and proteins are present in both bs and mp cells of the cotyledons. These become progressively more localized to bs cells in the typical C$_4$-type pattern as the cotyledons expand, becoming fully bs specific by 7 d post-germination (Wang et al., 1993a). Unlike true leaves, cotyledons do not undergo a basipetal sink–source transition, and the C$_3$–C$_4$ transition occurs uniformly throughout the entire organ. Kranz anatomy also develops normally in etiolated amaranth cotyledons and, as in light-grown plants, the Rubisco mRNAs and proteins initially accumulate in the default ‘C$_3$-like’ pattern, although at reduced levels (Berry et al., 1985; Wang et al., 1993a). Even in the absence of any illumination, the Rubisco mRNAs and proteins still undergo a C$_3$–C$_4$ transition, becoming fully localized to the bs cells by 7 d after planting. These findings demonstrate that post-embryonic cotyledons of amaranth share a common developmental programme of C$_4$ Rubisco gene expression with true leaves, and that light is not necessary to establish full bs cell-specificity for $RbcS$ and $rbc$L gene expression in this C$_4$ dicot.

In common with leaves, the transition in Rubisco gene expression does not correlate with a developmental gradient; dicot cotyledons form from cell divisions that occur during embryogenesis and seed development (Scott and Possingham, 1982; Meinke, 1992). During early seedling germination, cotyledons serve primarily as a storage organ; with the transition to a photosynthetic function as the seedlings develop, between germination and complete photosynthetic development of the first true leaf. It is during this transition to photosynthetic function that the Rubisco mRNAs and proteins become specific to bs cells (Wang et al., 1993a). It is possible that the C$_3$–C$_4$ transition in cotyledons occurs because C$_4$ capacity is required during the short period of time when cotyledons are functioning as the only photosynthetic organs of the young seedlings.

Additional evidence for the photosynthetic control of Rubisco gene expression comes from studies of *Amaranthus tricolor* leaves (McCormac et al., 1997). Just prior to flowering, this plant produces three-coloured leaves that possess both photosynthetic (green) and non-photosynthetic (red and yellow) regions. The green regions, showing normal photosynthetic activity, also have normal C$_4$ expression patterns for both Rubisco genes. However, in the yellow and red sectors, a lack of photosystem II activity and reduced chlorophyll is associated with a loss of cell-specificity for the $rbcL$ and $RbcS$ mRNAs, which accumulate in both bs and mp cells of those regions. However, the lack of photosynthetic activity does not affect normal C$_4$ localization patterns of the Rubisco proteins, which remain specific to bs cells. This breakdown of correlation, in which the Rubisco mRNAs accumulate in both cell types while the proteins they encode accumulate only in the bs cells, is similar to

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**Fig. 2.** C$_3$–C$_4$ transition for $rbc$L mRNA accumulation in a young 7 mm-long amaranth leaf. Note that in this composite micrograph of an *in situ* hybridization, the young leaf is approximately two-thirds through the transition in Rubisco gene expression. C$_3$-type bs cell specificity for $rbc$L mRNA occurs near the leaf apex (A, on right), and C$_4$-like accumulation in both bs and mp cells occurs near the leaf base (B, on left). The C$_3$–C$_4$ transition zone (TZ) is observed approximately one-third of the way up the leaf from the base. This image is modified from Wang J-L, Turgeon R, Carr JP, Berry JG, (1993b) Carbon sink-to-source transition is coordinated with establishment of cell-specific gene expression in a C$_4$ plant. *The Plant Cell* 5, 289–296 (www.plantcell.org) Copyright American Society of Plant Biologists.
that observed in the very early 2 mm long amaranth leaves, and is indicative of translational control. Such observations support a model in which there are at least two separate levels of control that determine bs-specific Rubisco gene expression: developmental and photosynthesis-associated translational control (as occurs in very young 2 mm leaves and non-photosynthetic regions of A. tricolor leaves), as well as developmentally determined control of mRNA accumulation (as occurs in mature leaves).

Other studies have provided more direct evidence that in some C₄ plants control of transcript stability plays a major role in determining bs-specific accumulation of both Rubisco transcripts. Initial evidence for this control mechanism came from earlier studies which demonstrated that, as in intact leaves, the rbcL transcripts accumulate only in bs chloroplasts, and not in mp chloroplasts, that have been isolated and separated from fully developed, mature amaranth leaves. However, run-on transcription analysis using these same isolated plastids showed that rbcL mRNAs are transcribed at similar levels in chloroplasts isolated from both leaf cell types (Boinski et al., 1993). A recent study has demonstrated that 5’ and 3’ UTRs derived from a heterologous C₄ amaranth AhRbcS1 mRNA, in themselves, could confer at least partial bs-specific expression to a gusA reporter gene at the level of post-transcriptional mRNA accumulation, when these were constitutively expressed from a CaMV promoter in stable transgenic lines of F. bidentis (Patel et al., 2004). It is interesting that the heterologous amaranth UTRs were able to mediate normal, full tissue-specific mRNA accumulation (such as leaf versus root), suggesting that post-transcriptional regulatory mechanisms that determine organ- and tissue-specific mRNA accumulation may be more highly conserved in these two dicot species than those determining bs cell-specificity. Most significantly, a recent study has shown that the 5’ and 3’ UTRs of an endogenous F. bidentis RbcS mRNA (FbRbcS1), in themselves, were able to confer strong bs-specific expression to gfpA reporter gene at the level of mRNA and protein accumulation, when transcribed from a constitutive CaMV promoter (Patel et al., 2006). This phenomenon can be clearly observed in Fig. 3, where GFP expression from a constitutively expressed chimeric FbRbcS1-gfpA construct produced bright green fluorescence only in bs cells in an image taken from the leaf surface. This work has clearly demonstrated that bs-specific RbcS expression patterns, a defining characteristic of C₄ plant species, can be conferred to chimeric RbcS-reporter mRNAs even when these are constitutively transcribed in all cell types. This post-transcriptional control of cell type specificity is mediated by the 5’ and 3’ UTRs of the FbRbcS1 mRNAs. Taken together, the findings discussed here provide strong evidence that control of cell type-specific Rubisco mRNA accumulation in the mature C₄ leaves is mediated post-transcriptionally by cell-type specific degradation of these transcripts in mp cells, or possibly by selective stabilization of these transcripts in bs cells.

### Post-transcriptional regulation of light-mediated Rubisco gene expression

Rubisco gene expression is strongly controlled by light at the level of translation in the C₄ dicots amaranth and F. bidentis. In seedlings of both species, RbcS and rbcL mRNAs accumulate in the present or absence of light, but polypeptide synthesis occurs only when these plants are illuminated (Berry et al., 1985, 1986, 1988, 1990; M Patel and JO Berry, unpublished data). When dark-grown seedlings are transferred to light (light-shift), synthesis of the SSU and LSU polypeptides is induced very rapidly, without corresponding increases in levels of their transcripts. In amaranth, RbcS and rbcL mRNAs are associated with polysomes only in light-grown and light-shifted seedlings (when Rubisco synthesis occurs), not in dark-grown seedlings (when synthesis does not occur), providing evidence for regulation of translational initiation. In another set of experiments, it was found that when light-grown plants were transferred to darkness (dark-shift), synthesis of both Rubisco subunits was rapidly repressed. Although Rubisco synthesis was reduced in dark-shifted amaranth seedlings, RbcS and rbcL transcripts remain in...
association with polysomes, indicating that regulation of translation elongation can occur as well.

In an effort to isolate regulatory factors that post-transcriptionally regulate C₄ Rubisco gene expression, biochemical analysis has been used to identify and characterize RNA binding proteins that interact specifically with 5’ regions of rbcL mRNA (McCormac et al., 2001; JO Berry and M Patel, unpublished results). In vivo mapping of protein binding sites in the 5’ UTR of rbcL mRNA using RNA heelprinting analysis has identified only three major sites of mRNA–protein interaction that correlate with light-induced polysome association and translation of rbcL mRNA. A current model is that protein binding at these sites is required for light-mediated activation of rbcL translation, and that function of these binding proteins might overlap with post-transcriptional activation of rbcL expression in bs chloroplasts. Gel-shift and UV crosslinking studies identified an rbcL-specific mRNA binding protein with specificity for the 5’ portion of the transcript, with an approximate MW of 47 kDa (designated p47). Taken together, the data suggest three possible functions for this protein (McCormac et al., 2001). First, p47 could be a regulatory protein that specifically binds to rbcL mRNAs and mobilizes them to polysomes for translation. Second, it could be responsible for translation-associated processing of the initially transcribed rbcL precursor RNA, producing the shorter mature transcripts with a 5’ end terminating at the –66 position. Third, p47 could be responsible for protecting polysome-bound rbcL mRNAs from degradation following processing at the –66 site. These functions are not exclusive, and this protein could have multiple roles in the translation/ stabilization of rbcL mRNA. It is also possible that cytoplasmic- and plastid-localized mRNA binding proteins such as p47, which are involved with light-mediated Rubisco gene expression, might be associated with more than one aspect of Rubisco gene expression, such as the post-transcriptional regulation of bs cell-specific expression.

Comparisons of Rubisco gene expression in C₄ dicots and monocots

Some aspects of Rubisco gene expression described above may be unique to C₄ dicots such as amaranth and Flaveria. However, comparisons of Rubisco gene expression in dicot and monocot species indicate that at least some regulatory processes may be shared by both C₄ plant groups. Expression patterns for genes encoding Rubisco and other photosynthetic enzymes in maize have been covered in several reviews (Nelson and Langdale, 1989, 1992; Langdale and Nelson, 1991; Dengler and Nelson, 1999). Maize leaf development is typical of most grasses, consisting of cells that originate mostly from an intercalary meristem located at the base of the leaf (Steeves and Sussex, 1989; Sylvester et al., 1990; Nelson and Langdale, 1992). This leads to the development of a leaf that is comprised primarily of a linear gradient of cells, with younger cells occurring at the base and older cells at the tip, with much more polarization and uniformity than a C₄ dicot leaf. As in amaranth leaves, rbcL and RbcS mRNAs accumulate initially very early in development, before the morphological differentiation of bs and mp cells. Unlike dicots, the initial Rubisco transcripts show cell specificity from their earliest detection, accumulating only in a ring of bs precursor cells immediately surrounding the differentiating vascular centres. The accumulation of the Rubisco transcripts in the bs precursor cells of the leaf primordia, and in new cells near the basal meristem precedes the appearance of their corresponding proteins in the leaf bs cells. Thus, the transcripts are not used immediately for Rubisco synthesis (Nelson et al., 1984; Nelson and Langdale, 1992). As leaf development progresses in illuminated maize plants, localization of the Rubisco mRNAs and subunit proteins remains highly specific to bs cells.

Maize leaves can still undergo a C₃–C₄ transition in Rubisco gene expression. However, although monocot leaves do undergo a sink–source transition, it does not progress basepetally as in dicots (Qiu et al., 2007), and the two transitions do not correlate in this plant. Leaves will develop on etiolated maize seedlings and Rubisco mRNAs and proteins accumulate, but at greatly reduced levels relative to light-grown plants (Sheen and Bogorad, 1985, 1986). The rbcL and RbcS mRNAs and proteins within the etiolated leaves are not cell type-specific, and accumulate in both bs and mp cells (Sheen and Bogorad, 1985, 1986, 1987a, b; Langdale et al., 1988; Nelson and Langdale, 1992). When the etiolated plants are illuminated, RbcS and rbcL gene expression increases in bs cells and becomes repressed in mp cells, with full bs cell-specificity becoming established within 12 h of exposure to light. Thus for maize leaves, light is a primary determining factor in the transition from the initial ‘C₃-like’ default state to the more specialized ‘C₄ type’ expression pattern.

In both amaranth and maize, C₄ leaf development is associated with two states of Rubisco gene expression. There is an initial ‘C₃-like’ default pattern that is likely the ancestral programme, and more specialize ‘C₄-type’ that occurs following a specific developmental transition. In amaranth, this signal is associated with changes in carbon transport status that work independently of light. In maize, this transition only occurs when etiolated plants are used as the starting material, with light as the primary determinant for bs specificity. This would suggest that entirely different signals influence C₄ patterns of gene expression in these two plants. In support of this, transient expression analysis using a maize RbcS–m3 reporter gene construct has identified two specific photoreceptors, phytochrome and a blue-light receptor, that promote bs cell-specificity for this maize gene (Purcell et al., 1995). However, there may also be some commonality in events...
leading to the C₃–C₄ transition in the leaves of both species. Changes in photosynthetic production, accumulation, or transport brought about by the sink–source transition (in amaranth) or the initiation of photosynthesis in the light (in maize) could result in the movement of similar metabolic signals that directly or indirectly affect \( rbcL \) and \( RbcS \) gene expression patterns within the C₄ leaves (Berry et al., 1997; Nelson and Langdale, 1992; Sheen, 1999).

As in the C₄ dicots, post-transcriptional control appears to be involved in regulating the maize \( RbcS \) and \( rbcL \) genes. Transcription studies using isolated maize bs and mp plastids from the C₄ monocots maize and sorghum showed post-transcriptional control of \( rbcL \) mRNA accumulation, similar to those reported for amaranth plastids (Boinski et al., 1993; Kubicki et al., 1994). In addition, run-on transcription analysis using nuclei from separated bs and mp cells have demonstrated that specific accumulation of \( RbcS \) transcripts in C₄ maize leaves also involves differences in mRNA stability in the two cell types (Schaffner and Sheen, 1992; Sheen, 1999). Post-transcriptional control of light-induced Rubisco gene expression has also been reported for maize (Nelson et al., 1984; Markelz et al., 2003). When these studies are considered together with the amaranth and \( F. \) bidentis findings discussed here, it becomes apparent that in spite of underlying differences in developmental timing and leaf structure, at least some of the underlying processes responsible for the establishment and maintenance of bs cell-specific Rubisco gene expression may be conserved between monocot and dicot C₄ species.

**Future directions**

Rubisco gene expression in C₄ plants provides a unique, clearly defined system to investigate mechanisms responsible for cell-type specific gene expression of both organelle- and nuclear-encoded genes. However, molecular mechanisms involved in the specialized C₄ regulation of \( RbcS \) and \( rbcL \) genes are still not well understood. In part, this may be due to the complexities of C₄ systems. The evidence to date indicates that C₄ gene expression is complex, incorporating multiple levels of regulation that function interactively to achieve full bs or mp cell specificity. It appears that there is no 'global regulator' of C₄ expression; genes encoding different photosynthetic enzymes show unique patterns of expression indicative of independent regulation. By comparison, plant gene regulation studies that have focused on more amenable C₃ and algal model systems have progressed more rapidly. By using the knowledge derived from such model systems as a basis for studies of gene expression in C₄ species, it should be possible to expedite understanding of the specialized gene expression patterns that define photosynthetic capabilities of plants that utilize this intriguing pathway of CO₂ assimilation.

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