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Cell position and light influence C4 versus C3 patterns of photosynthetic gene expression in maize

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C4 plants such as maize partition photosynthetic activities in two morphologically distinct cell types, bundle sheath (BS) and mesophyll (M), which lie as concentric layers around veins. We show that both light and cell position relative to veins influence C4 photosynthetic gene expression. A pattern of gene expression characteristic of C3 plants [ribulose bisphosphate carboxylase (RuBPCase) and light-harvesting chlorophyll a/b binding protein in all photosynthetic cells] is observed in leaf-like organs such as husk leaves, which are sparsely vascularized. This pattern of gene expression reflects direct fixation of CO_2 in the C3 photosynthetic pathway, as determined by O_2 inhibition assays. Light induces a switch from C3-type to C4-type gene expression patterns in all leaves, primarily in cells that are close to a vein. We propose that light causes repression of RuBPCase expression in M cells, by a mechanism associated with the vascular system, and that this is an essential step in the induction of C4 photosynthesis.

Key words: maize/C4-C3 photosynthesis/light regulation/spatial regulation

Introduction

Non-succulent plants are categorized as C3 or C4 on the basis of the photosynthetic pathway they employ. In C3 plants, CO_2 is fixed directly in the Calvin cycle by ribulose bisphosphate carboxylase (RuBPCase). O_2 inhibits this reaction by competing with CO_2, and fixed O_2 causes energy loss through photorespiration (for a review see Zelitch, 1975). C4 plants circumvent this problem by concentrating CO_2 at the active site of RuBPCase. The C4 pathway separates photosynthetic functions into two morphologically distinct cell-types, bundle sheath (BS) and mesophyll (M) (for a review see Edwards and Huber, 1981). CO_2 is first fixed into C4 acids in the M cells and then transferred to the BS. Subsequent decarboxylation in the BS releases CO_2 to be fixed in the Calvin cycle, as in C3 plants.

In all C4 plants, each of the two photosynthetic cell-types has a diagnostic complement of C4 photosynthetic enzymes (Edwards and Huber, 1979). In maize, the developmental accumulation of these enzymes and their corresponding mRNAs is well documented (Williams and Kennedy, 1978; Miranda *et al.*, 1981; Mayfield and Taylor, 1984; Martineau and Taylor, 1985; Aoyagi and Bassham, 1986; Langdale *et al.*, 1987, 1988). C4 proteins accumulate as BS and M cells become morphologically differentiated, whereas C4 mRNAs, in particular BS cell-specific messages, can accumulate prior to the morphological distinction of the two cell-types (Langdale *et al.*, 1988). Around individual veins, RuBPCase mRNAs accumulate in BS cells before M cell-specific mRNAs accumulate. Although all of these enzymes appear to be developmentally regulated, the expression of each is greatly enhanced by light (Nelson *et al.*, 1984; Sheen and Bogorad, 1987). Light plays additional roles, however, because RuBPCase is not correctly compartmentalized in etiolated leaves (Sheen and Bogorad, 1985).

Our previous work has provided two indications that the accumulation of photosynthetic gene products in BS and M cells is associated with vascular development (Langdale *et al.*, 1987, 1988). First, the overall pattern of BS and M development throughout the leaf reflects the earlier manifest pattern of vein formation in the same leaf. This suggests that the veins limit transport of factors essential for the development of these cell-types. Second, C4 enzyme accumulation appears to be regulated locally around individual veins. In the maize mutant *argentia*, in which C4 enzyme accumulation is delayed, cells become photosynthetically competent in clusters around individual veins rather than uniformly across the leaf. M cells can accumulate C4 enzymes in the absence of adjacent, activated BS cells, indicating that the necessary 'activator' is not a photosynthetic metabolite.

If the regulation of C4 photosynthetic gene expression is associated with vascular development, it can be predicted that in leaves with altered patterns of vein spacing, photosynthetic gene expression differs from normal. In foliar leaves, BS and M cells form a typical Kranz-type leaf anatomy in which they lie as concentric layers around each vein (Brown, 1975). The BS cells comprise the layer closest to the vein. Vascular differentiation positions approximately four photosynthetic cells (two BS and two M) between adjacent veins. In a number of leaf-like organs, for example husk leaves and prophylls (which surround the ear), this pattern is altered such that two BS and as many as 20 M cells separate adjacent veins. We have examined the cellular pattern of C4 enzyme accumulation in leaf-like organs. In this paper we present evidence showing that C3-type gene expression occurs in some of these organs under normal conditions and that this pattern of gene expression reflects exclusive use of the C3 photosynthetic pathway. We show that light induces the cell-specific expression of C4 genes in both foliar leaves and husk leaves, primarily in cells that are close to a vein. More distant cells retain a C3-type pattern. The morphological differentiation of BS cell chloroplasts, associated with a switch from C3 to C4 photosynthesis, is also induced by light.

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Table I. Summary of photosynthetic enzyme accumulation patterns in etiolated, light-shifted and light-grown seedling leaves

	Etiolated leaves		Light-shifted leaves		Light-grown leaves	
	Base	Tip	Sheath	Blade	Sheath	Blade[c]
RuBPCase						
Protein	–	BS/M	–	BS	–	BS
LSu mRNA	BS/M	BS/M	BS/M	BS	BS	BS
SSu mRNA	BS/M	BS/M	BS/M	BS	BS	BS
ME						
Protein	–	–	–	BS[a]	–	BS
mRNA	–	–	BS[a]	BS	BS[a]	BS
PEPCase						
mRNA	–	M[a]	–	M	M[a]	M
PPdK						
Protein	–	–	–	M[a]/BS[a]	–	M/BS
MDH						
Protein	–	–	–	M[a]/BS[a,b]	–	M/BS[b]
LHCP-II						
Protein	–	–	M	M/BS	M/BS[a]	M/BS

[a]Low levels of protein or mRNA detected.
[b]Possible artefact [the detection of MDH in BS cells has been extensively discussed previously (Langdale *et al.*, 1987)].
[c]The accumulation patterns observed in blades 1–3 were identical. However, levels of all enzymes were highest in leaf 3>2>1.

Results

Light induces compartmentalization of RuBPCase gene products and cell-specific expression of C4 genes

The enzymes of the C4 pathway are compartmentalized such that RuBPCase and malic enzyme (ME) accumulate in the BS cells, whereas malate dehydrogenase (MDH) and phosphoenolpyruvate carboxylase (PEPCase) accumulate in the M cells (Edwards and Huber, 1979; Broglie *et al.*, 1984). Pyruvate phosphate dikinase (PPdK) accumulates in both cell-types, but its function in BS cells is unknown (Aoyagi and Nakamoto, 1985). The regulation of this compartmentalization, although a major step in the establishment of the C4 pathway, is poorly understood. Previous reports suggested that RuBPCase mRNAs accumulate in both BS and M cells in etiolated tissue (Sheen and Bogorad, 1985) whereas C4 enzymes accumulate cell-specifically (Sheen and Bogorad, 1987). These experiments, which were carried out using RNA from separated BS and M cells, indicated that light regulates the cell-specific compartmentalization of RuBPCase. In order to examine this phenomenon around individual veins, we have examined *in situ*, the spatial distribution of C4 proteins and mRNAs in etiolated, light-shifted and light-grown seedling leaves. We have also assayed the accumulation of the light-harvesting chlorophyll a/b binding proteins (LCHP II) of photosystem II (PS II). In C4 plants, most PS II activity is associated with the M cells (Edwards and Huber, 1979). This partitioned activity reflects the cellular distribution of LHCP II polypeptides. Although LHCP II is present in both cell-types, levels are greatly reduced in BS cells (Schuster *et al.*, 1985). Five LHCP II polypeptides are present in maize M cell thylakoids and two are present in BS cell thylakoids (Bassi and Simpson, 1986; Sheen and Bogorad, 1986). In C3 plants, LHCP II polypeptides are abundant in all photosynthetic cells. The results of these experiments are summarized in Table I.

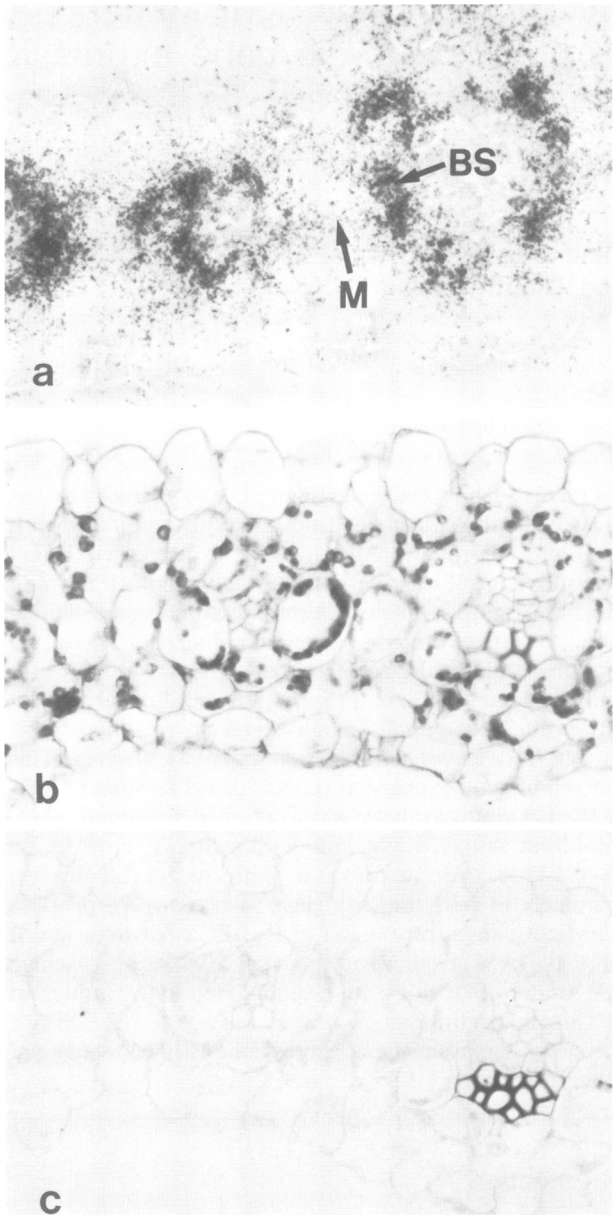

Fig. 1. Localization of RuBPCase and LHCP II in the tip of etiolated seedlings. (**a**) *In situ* hybridization to Lsu mRNA showing accumulation primarily in BS cells but also in M cells. Autoradiographs were exposed overnight. (**b**) Immunolocalization of RuBPCase demonstrating accumulation in both BS and M cells. (**c**) Immunolocalization of LHCP II showing its absence in these conditions. Magnification: ×400.

In etiolated seedlings, RuBPCase mRNAs and protein accumulate in both cell-types (Figure 1), with a lag in protein accumulation (Table I). Levels of Lsu and Ssu mRNAs were much lower in M cells than in BS cells. Since immunolocalization assays are not very quantitative, however, similar assessments of protein levels cannot be made. LHCP II (Figure 1), PPdK, MDH and ME were not detectable by these methods. PEPCase mRNA was detected at low levels at the tip of the seedling, but no PEPCase protein accumu-

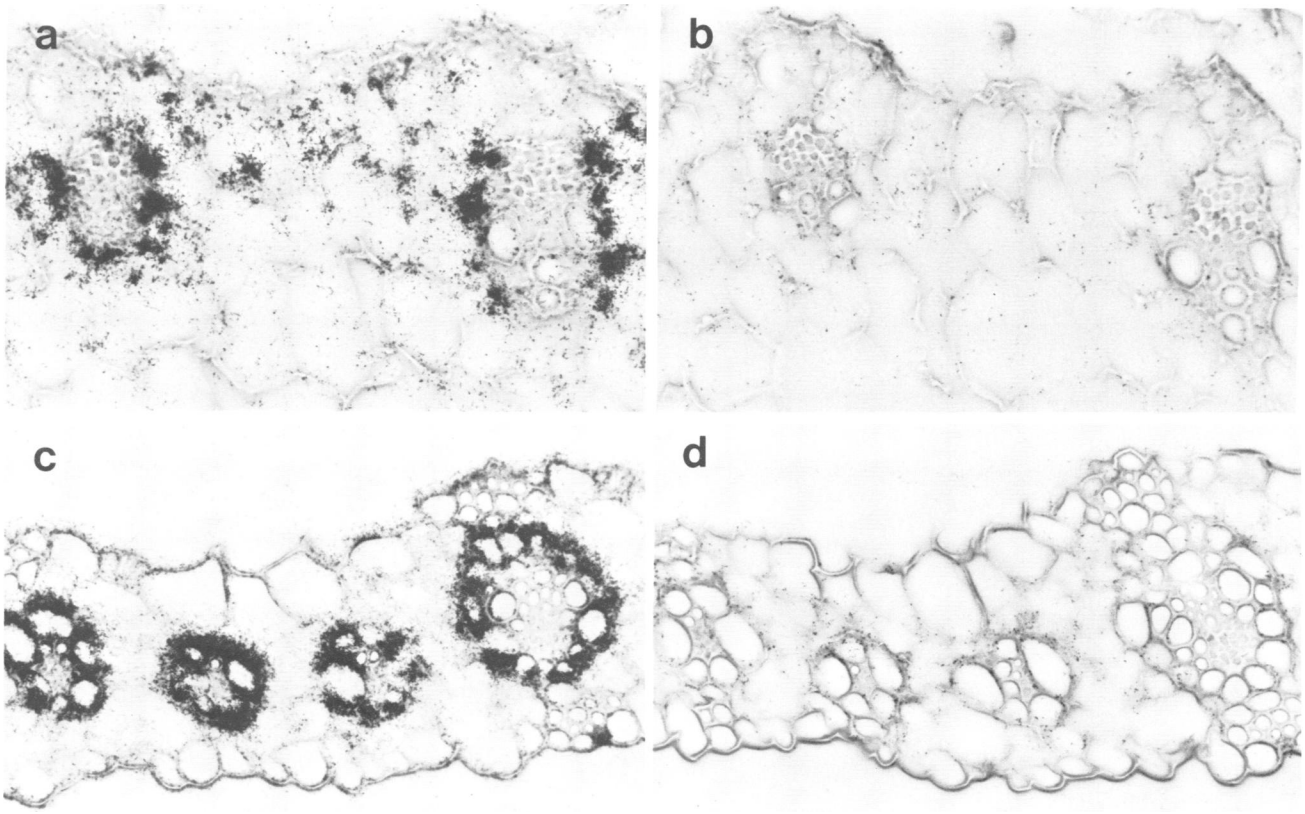

Fig. 2. *In situ* hybridization to Lsu mRNA in light-shifted leaf sheath (**a,b**) and blade (**c,d**) sections. Sections were hybridized with either labeled antisense (**a,c**) or sense (**b,d**) RNA probes. Autoradiographs were exposed for 12 h. Magnification: ×260.

lation was observed. After a 12 h light shift, RuBPCase mRNA was detected in both cell-types in the leaf sheath but cell-specifically (BS) in the blade (Figure 2). All C4 proteins were observed in their appropriate cell-types in the leaf blade but, with the exception of low levels of ME mRNA in the BS, were absent in the leaf sheath. LHCP II polypeptides were detected in M cells in the leaf sheath and in both cell-types in the leaf blade. In light-grown leaf blades, all C4 mRNAs (Figure 3) and proteins were detected in their appropriate cell-types. In the leaf sheath, RuBPCase mRNAs and low levels of PEPCase and ME mRNAs were detected in their appropriate cell-types but no C4 protein accumulation was observed. LHCP II proteins were detected primarily in M cells in the leaf sheath and abundantly in both cell-types of the blade.

In summary, these data demonstrate that RuBPCase is developmentally programmed to accumulate in both cell-types in the dark. Other C4 proteins do not accumulate to detectable levels until, upon exposure to light, RuBPCase levels in M cells are reduced. At this time, all C4 enzymes accumulate in their appropriate cell-types. The reduction of RuBPCase in M cells occurs most rapidly when all M cells are close to a vein (compare RuBPCase expression in light-shifted leaf blades and sheaths). LHCP II proteins are absent in the dark but accumulate rapidly upon exposure to light. In light-grown tissue, RuBPCase does not accumulate in M cells (even at a distance from the vein) and all C4 enzymes are present in their appropriate cell-types.

Previous reports have suggested that maize seedling leaves 1−3 act like typical C3 plants when grown at low light intensity (40 $\mu E/m^2/s$) (Crespo *et al.*, 1979). However, we see normal compartmentalization of C4 enzymes in all of our seedling leaves grown at light intensities of 100, 500 and 2000 $\mu E/m^2/s$. To investigate further, we analyzed C4 gene expression patterns and O_2 inhibition of CO_2 fixation in third leaves of seedlings grown at light intensities of 10, 40 and 600 $\mu E/m^2/s$. C4 carbon fixation is characteristically uninhibited by O_2, wheras C3 fixation is inhibited up to 50% in 21% O_2 (Zelitch, 1975). In seedlings grown at 10 and 40 $\mu E/m^2/s$, chloroplasts of both cell-types were very small but all C4 enzymes were correctly compartmentalized (data not shown). Photosynthetic rates and O_2 inhibition characteristics of the third leaves of plants grown at 40 and 600 $\mu E/m^2/s$ were identical to those seen in a mature foliar leaf grown at 600 $\mu E/m^2/s$ (Table II). All foliar leaves assayed clearly utilized the C4 photosynthetic pathway.

Patterns of vein spacing influence photosynthetic gene expression

We predicted, on the basis of two observations, that vein spacing influences the pattern of photosynthetic gene expression in maize. First, the accumulation of C4 enzymes is regulated locally around individual veins (Langdale *et al.*, 1987) and second, RuBPCase is compartmentalized more rapidly in light-shifted leaf blades than in sheaths. In leaf sheaths, wider vein spacing is observed. In order to test this prediction, we have examined C4 protein accumulation, *in situ*, in a number of leaf-like organs. The results are summarized in Table III. In all cases where vein spacing results in more than two or three M cells between adjacent

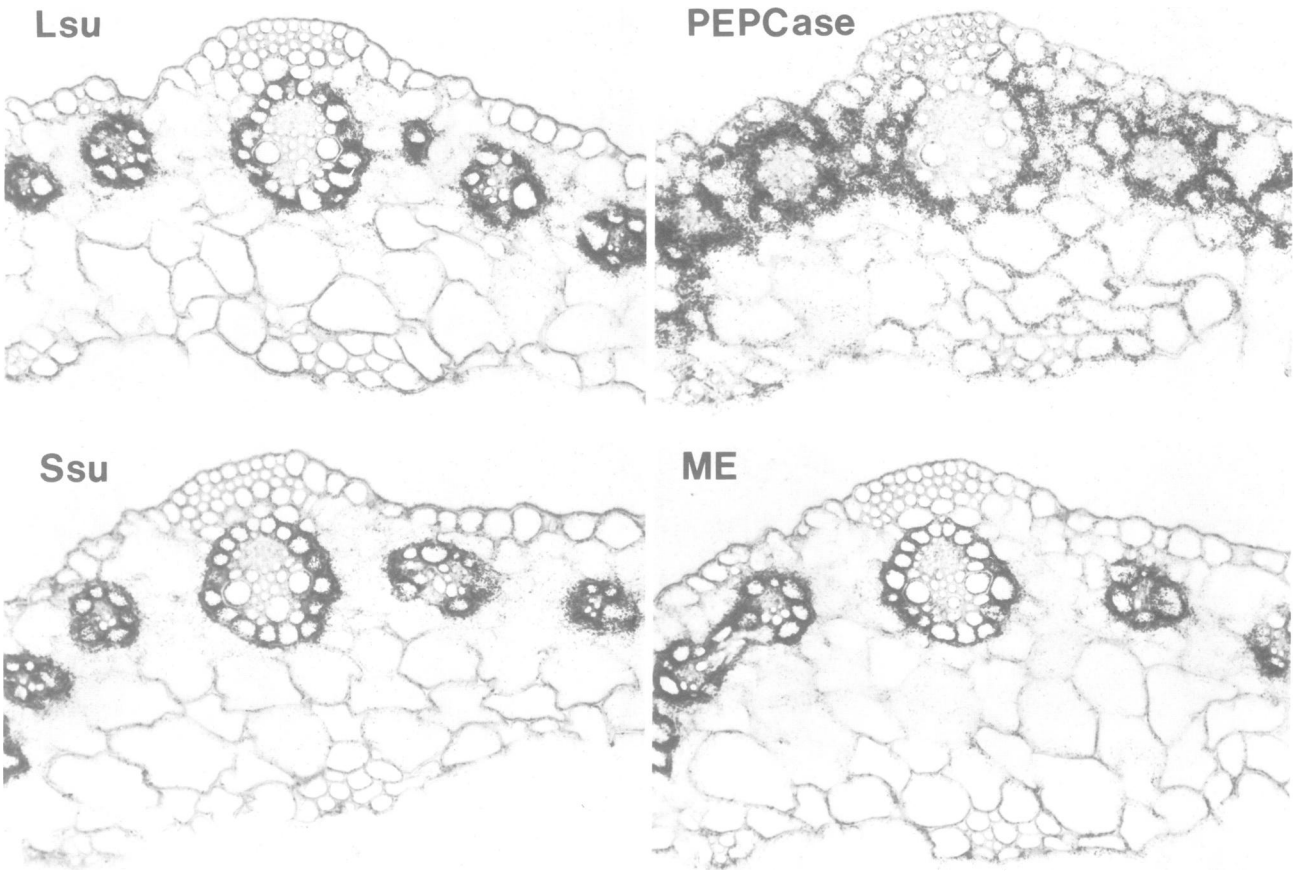

Fig. 3. *In situ* localization of photosynthetic mRNAs in the midrib region of light-grown leaf blade sections. Lsu, Ssu and ME mRNAs were detected in the BS cells whereas PEPCase mRNA was detected in the M cells. Autoradiographs were exposed to ~ equal intensities and do not reflect relative levels of mRNAs in the leaf. Magnification: ×140.

veins, RuBPCase was detected in M cells.

In the coleoptile, where only two vascular bundles are present, RuBPCase and LHCP II proteins were detected in the absence of all C4 enzymes (Figure 4). The accumulation of these proteins, in particular LHCP II, was greatly enhanced if seedlings were grown on filter paper (directly exposed to light) rather than in soil (Figure 4a and b). The accumulation of RuBPCase in this structure is also developmentally regulated, however, since Lsu and Ssu mRNAs accumulate in the coleoptiles of etiolated seedlings (data not shown).

Normal glumes (leaf-like bracts in the male inflorescence) exhibit long rows of epidermal cells between vascular bundles, but all M cells are close to a vein (Figure 5a). In these structures, which are fully exposed to light, all C4 enzymes were detected in their appropriate cell-types. Glumes of the maize morphological mutant *Tunicate* (*Tu*) are expanded so that at least 10 M cells are present between vascular bundles (Figure 5b). As a result of this size increase, these glumes often overlap each other, limiting exposure to light. In these structures, RuBPCase and LHCP II were present in both BS and M cells. In addition, low levels of ME and PEPCase were detected in their appropriate cell-types. PEPCase accumulation was limited to M cells close to the veins. MDH and PPdK were not detected. This pattern reflects incomplete reduction of RuBPCase in M cells and incomplete induction of cell-specific C4 enzymes.

The two other leaf-like organs examined (prophyll and

Table II. Oxygen inhibition of carbon fixation in leaves grown at different light intensities

Leaf number	Light intensity during growth ($\mu E/m^2/s$)	Fixation rate (mg $CO_2/dm^2/h$)			% Inhibition
		21% O_2	1% O_2	21% O_2	
3	40	17.1	17.6	17.5	0
3	600	19.5	19.9	20.2	0
11	600	21.0	20.8	24.0	0

Table III. Summary of photosynthetic-protein accumulation in maize leaf-like organs

Enzyme	Normal glume	Tunicate[a] glume	Prophyll[a]	Coleoptile[a,b]	Husk leaf[a,c]
RuBPcase	BS	B/M	BS/M	BS/M	BS/M
ME	BS	BS[e]	–	–	–
PEPCase	M	M[e]	–	–	–
PPdK	M/BS	–	–	–	–
MDH	M/BS[d]	–	–	–	–
LHCP-II	M/BS	M/BS	M/BS	M/BS	M/BS

[a]Enzyme accumulation was concentrated along the edge of the leaf exposed to the most light.
[b]Seedlings were grown on filter paper, exposed to light.
[c]Data represent the third leaf in towards the ear.
[d]As in [b], Table I.
[e]Low levels of protein detected.

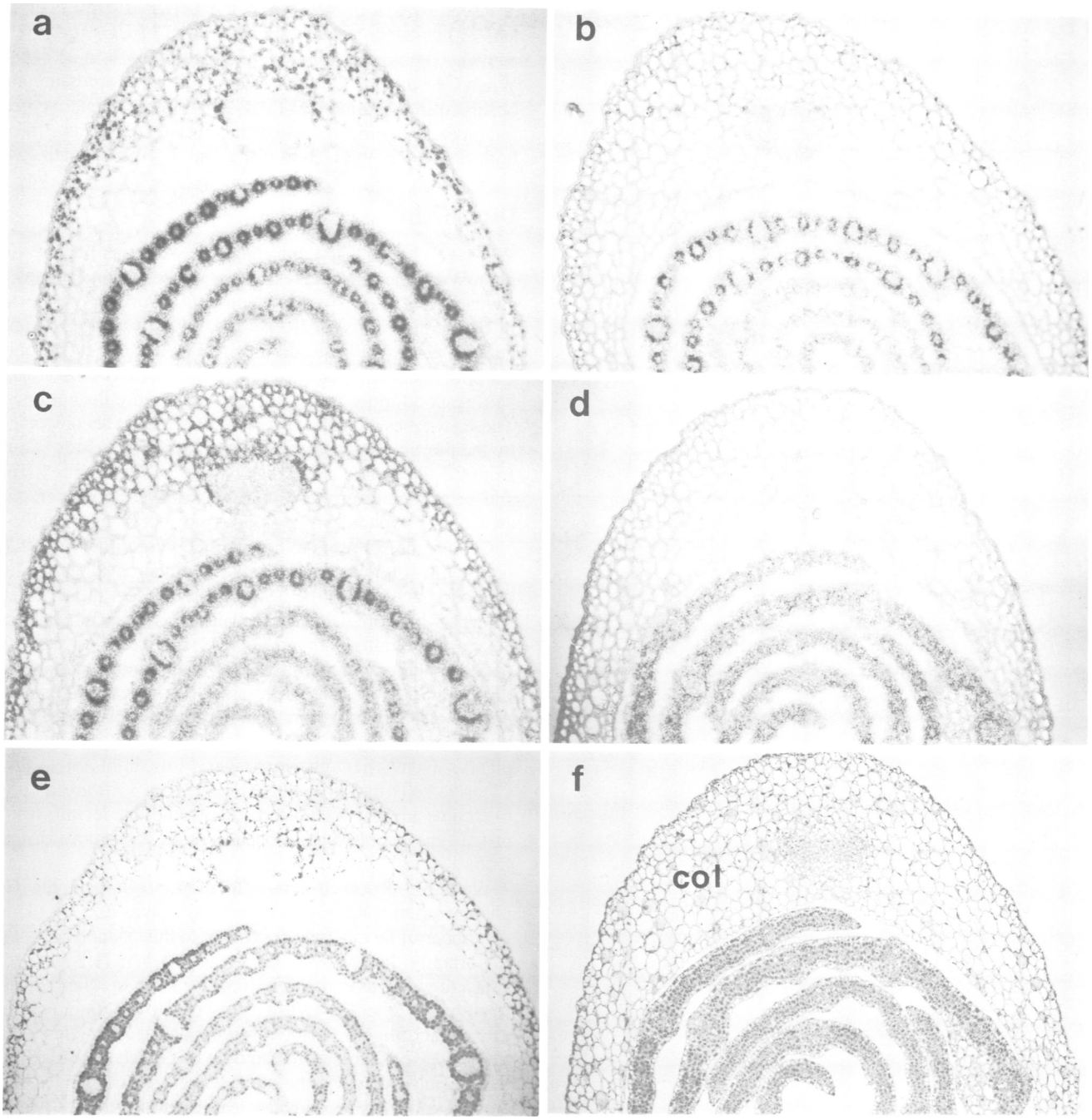

Fig. 4. Accumulation of photosynthetic proteins and mRNAs in the maize coleoptile. Sections illustrate half of the symmetrical structure hybridized with labeled Lsu antisense RNA (**a**) and (**b**), Ssu antisense RNA (**c**), and Lsu sense RNA (**d**). The coleoptile is labeled (col). (**e**) immunolocalization of LCHP II and (**f**) a Fast green stained section. With the exception of (**b**) which was grown in soil, all seedlings were grown on filter paper. Magnification: ×55.

husk leaves) surround the ear. The prophyll (Figure 5c), which is positioned between the normal (foliar) leaf sheath and the ear, exhibits a unique pattern of veins comprising two midribs. The spacing of veins in the prophyll differs across the organ but at least five M cells are positioned between any two veins. In parts of this structure that had emerged above the ear and had therefore been exposed to light, albeit low levels, RuBPCase and LHCP II were detected in both BS and M cells. No C4 enzymes were observed. A pattern identical to this was seen in one of the husk leaves examined. Seven or eight husk leaves overlap around each ear (numbered here 1−8 from outer to inner). The innermost leaves (4−8) receive almost no light and in these tissues C4 proteins were detected only in stomatal guard cells (data not shown). In leaf three, RuBPCase and LHCP II were detected in both cell-types in the absence of any other C4 proteins (Figure 6). A proportion of BS cells in this leaf did not contain chloroplasts and those present in the remaining BS cells were unlike those normally associated with C4 metabolism−they were very small and were not centrifugally arranged. In the outer leaf, RuBPCase and LHCP were present in both cell-types but additionally, C4 enzymes were detected in their correct cell-types (Figure 6). M cell-specific proteins were detected primarily in cells near

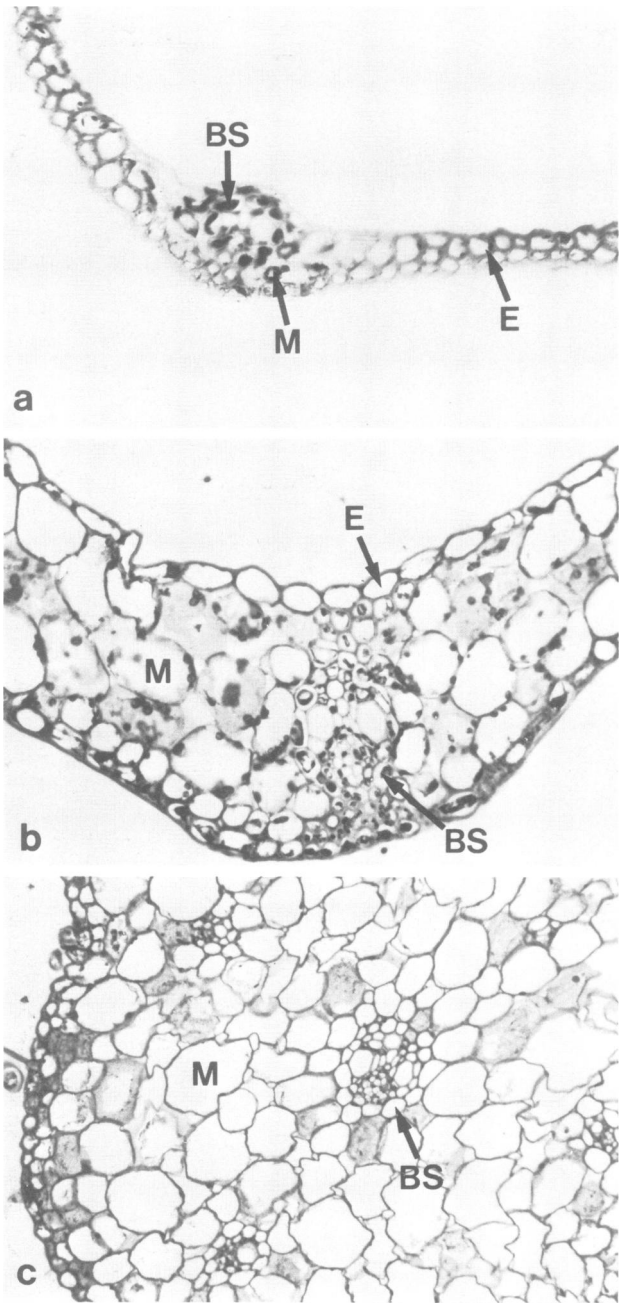

Fig. 5. Fast green-stained sections illustrating the morphology of (**a**) a normal glume, (**b**) a *Tunicate* glume and (**c**) a prophyll. BS, M and epidermal (E) cells are indicated. Magnification: ×240.

to veins (Figure 6h); however, no clear distinction could be made between RuBPCase accumulation in near and distant M cells. BS cell chloroplasts in this leaf were more differentiated than those in the third leaf. The pattern of gene expression observed in the outer husk leaf can be induced in any of the inner leaves by ripping off all outer leaves and exposing them to light for 7 days. Complete compartmentalization of RuBPCase was not induced, however, even after exposure to light for 21 days.

To determine the physiological effect of the observed gene expression patterns, we assayed O_2 inhibition of carbon fixation in a normal foliar leaf (leaf 11) and in husk leaves 1, 2 and 3 (Table IV). Photosynthetic rates in all husk leaves were much lower than that seen in the foliar leaf. The observed pattern of RuBPCase and LHCP II expression in leaf 3, however, reflects direct fixation of CO_2 in the C3 pathway. Photosynthesis was inhibited by 45% in 21% O_2. When C4-type and C3-type patterns of gene expression are observed in the same leaf (leaf 1), an intermediate oxygen tolerance is exhibited, suggesting that CO_2 is being fixed both directly in the Calvin cycle and via the C4 pathway.

Discussion

We have shown that light and cell position influence C4 photosynthetic gene expression in maize. Light induces a switch from C3-type to C4-type gene expression patterns in both etiolated tissue (RuBPCase in both cell-types) and husk leaves (RuBPCase and LHCP II in both cell-types; only C3 photosynthetic pathway functioning). Cells closest to a vein respond more rapidly to light than more distant ones. These switches in gene expression patterns are accompanied by the morphological differentiation of BS cell chloroplasts from C3-like (small and radially arranged) to C4-like (large and centrifugally arranged). In husk leaves, however, the switch to C4 photosynthesis does not occur in all cells.

Cell position appears to be relatively more important than light induction in the regulation of C4 photosynthesis. This deduction is made on the basis of three observations. First, M cells which are distant from veins do not accumulate C4 enzymes even after light induction. Second, different patterns of cell spacing within the same organ elicit distinct patterns of photosynthetic gene expression (compare light-exposed normal and *Tu* glumes and leaf sheaths and blades). Third, light-grown foliar leaves do not exhibit characteristics of C3 plants, even when grown at very low light intensities. Clearly, cells that are situated close to a vein are able to overcome any low light effects that would, by default, result in C3-type gene expression patterns in cells more distant from a vein.

We have observed direct fixation of CO_2 in the C3 photosynthetic pathway in inner husk leaves of a C4 plant. A number of factors may contribute to this phenotype. Most importantly, husk leaves have at least 10 M cells between adjacent veins, as compared to two or three in foliar leaves. In addition, inner leaves are exposed to low light intensities as a result of light filtering through outer leaves. This light-filtering effect enriches the amount of far red light reaching the inner leaves since the outer leaves absorb mainly blue and red light. The second husk leaf in towards the ear (where RuBPCase accumulates in both cell-types) is exposed to only 20% blue (420–480 nm), 35% red (660 nm) and 70% far red (730 nm) of incident light (Taylor, 1988). It is possible that these abnormal light levels contribute, via phytochrome or cryptochrome (or both), to the phenotype observed in these leaves. An enrichment of far red light leads to the accumulation of phytochrome in the Pr form (for a review see Nagy *et al.*, 1988). In etiolated tissue, where RuBPCase is also present in both cell-types, phytochrome also accumulates in this form. The possibility that compartmentalization of RuBPCase in foliar leaves is regulated by light quality is presently being investigated.

A number of reports have suggested that CO_2 can be fixed directly in the C3 pathway in maize. These studies assayed RuBPCase:PEPCase ratios (Crespo *et al.*, 1979; Bassi and Passera, 1982) or initial products of $^{14}CO_2$ uptake and photorespiration (Perchorowicz and Gibbs, 1980). When

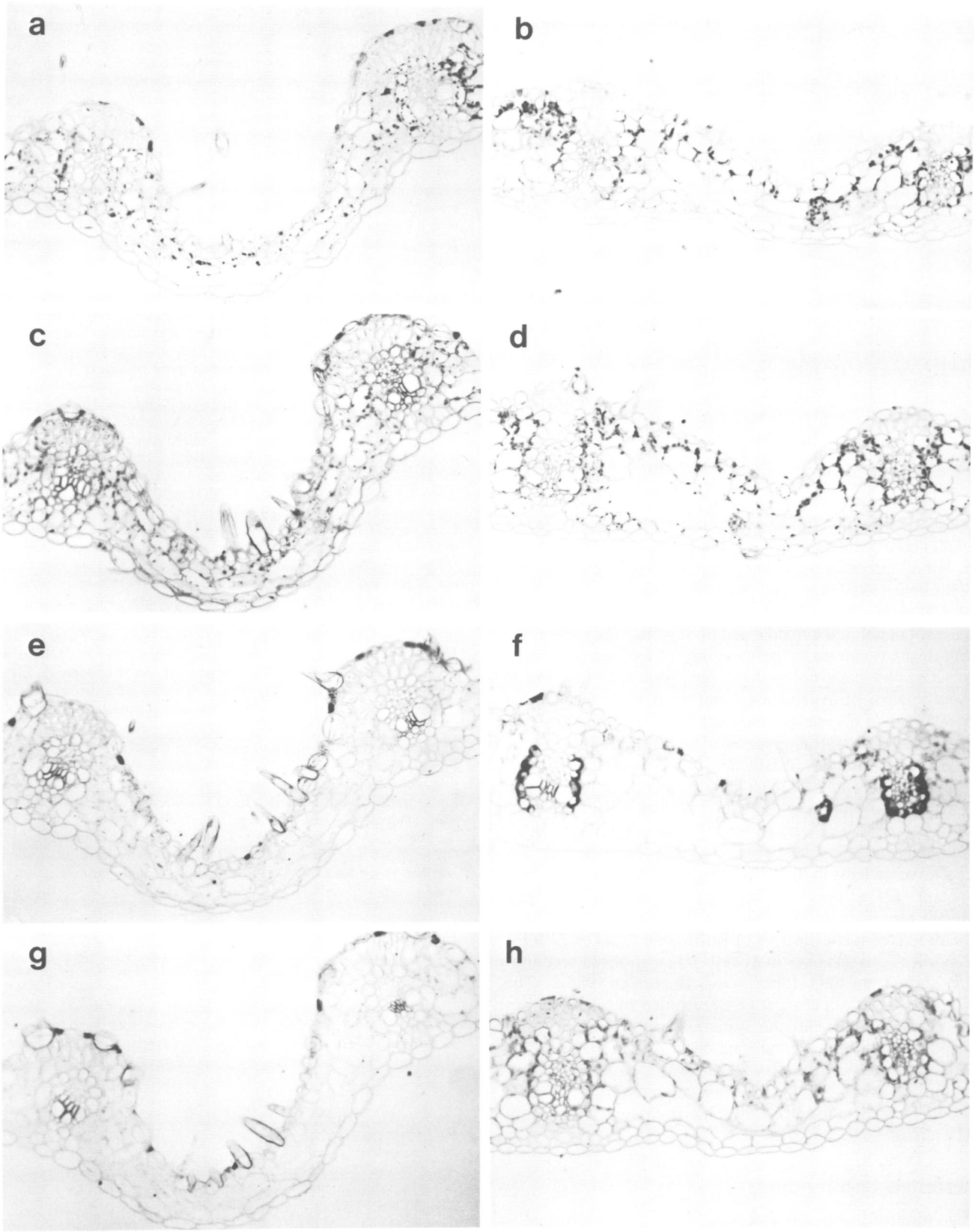

Fig. 6. Immunolocalization of LHCP II (**a,b**), RuBPCase (**c,d**), ME (**e,f**) and PPdK (**g,h**) in inner (leaf 3) (**a,c,e,g**) and outer (leaf 1) (**b,d,f,h**) husk leaf tissue. Magnification: ×70.

plants were grown at low light intensity, C3-type characteristics (PEPCase:RuBPCase of ≤ 1) were detected in seedling leaves 1−3 (Crespo *et al.*, 1979). In our hands, the third leaves of seedlings grown at low light intensities exhibited no C3-type properties. All C4 photosynthetic enzymes were compartmentalized and carbon fixation was

Table IV. Oxygen inhibition of carbon fixation in foliar and husk leaves

Leaf type	Fixation rate (mg CO_2/dm^2/h)			% Inhibition	C4 enzyme composition
	21% O_2	1% O_2	21% O_2		
Normal	21.0	20.8	24.0	0	BS: RuBPCase, ME, PPdK, LHCP-II M: PEPCase, MDH, PPdK, LHCP-II
Husk 1	3.8	4.9	3.8	22	BS: RuBPCase, ME, PPdK, LHCP-II M: RuBPCase, PEPCase, MDH, PPdK, LHCP-II
Husk 2	1.2	1.9	0.9	37	BS: RuBPCase, LHCP-II, (ME) M: RuBPCase, LHCP-II
Husk 3	1.1	2.0	1.0	45	BS: RuBPCase, LHCP-II M: RuBPCase, LHCP-II

uninhibited by O_2. In leaves 1 and 2 of these plants, levels of all photosynthetic enzymes were decreased relative to leaf 3, but all enzymes were compartmentalized. Perchorowicz and Gibbs (1980) reported photorespiratory characteristics of C3 plants in the basal (young) regions of developing leaves, but not in the middle and tip regions. They suggested that this was caused by permeability of the young BS cells to CO_2. We have not assayed photosynthetic function in specific parts of maize leaves, but we have never detected breakdown of C4 enzyme compartmentalization in the basal regions of leaves (Langdale *et al.*, 1987, 1988).

We propose that RuBPCase is developmentally programmed to accumulate in all photosynthetic cell-types. Light is required to suppress the expression of RuBPCase gene products in M cells and to induce the expression of other C4 photosynthetic genes. These two events may be mutually exclusive, at least in light-shifted husk leaves, since M cell-specific C4 enzymes accumulate in cells that contain RuBPCase. This intermediate state is maintained long after the predicted turnover time of RuBPCase [it takes 72 h for Lsu mRNA to turnover in M cells when etiolated seedlings are shifted to light (Sheen and Bogorad, 1985)]. The described light effect must be transmitted by a mechanism associated with the vascular system, since cells near to veins respond more easily than distant ones. Although this model is based purely on observational data, it is testable. Any factors responsible for RuBPCase suppression in M cells should be present in M cells of foliar leaves and absent from M cells of husk leaves.

Materials and methods

Plant material and growth conditions

All experiments were carried out with an inbred line of *Zea mays* (Pioneer inbred B73). Control seedlings were grown in soil, in a growth chamber, with a temperature/light cycle of 22°C for 16 h in the light (~100 μE/m^2/s) and 16°C for 8 h in the dark. Leaf samples were harvested either 7 days after planting, when the leaves were still enclosed in the coleoptile, or after 21 days when the third leaf blade length was ~15 cm. First and second leaf lengths were ~7 and 15 cm, respectively. Samples were harvested from the leaf sheath and from the middle of all three leaf blades. To examine C4 enzyme expression in the coleoptile, seedlings were grown on moist filter paper under the same temperature/light conditions as described above. Samples were harvested 7 days after planting.

Etiolated seedlings were grown in vermiculite in total darkness for 7 days at 22°C. After this time, the coleoptile length was approximately 9 cm—no leaf blade expansion had occurred. Samples were taken from 5.5–6 cm (base) and 8.5–9 cm (tip) above the seed. Some plants were subsequently shifted to light (500 μE/m^2/s) for a total of 12 h (8 h light, night, 4 h light) prior to harvest. At this time, first and second leaf blades had expanded and were 6 cm and 5 cm in length, respectively.

Plants were grown to maturity under field conditions in Connecticut in the summer of 1987 (~28°C day, 18°C night temperatures; 200 μE/m^2/s) or in a greenhouse in the winter of 1988 (500 μE/m^2/s). Seven weeks after planting, samples of husk leaves, prophylls, and glumes (in the tassel) were harvested. All of the seven overlapping husk leaves were sampled.

In addition, glumes were harvested from heterozygous *Tunicate* (*Tu*) plants. *Tu* is a dominant mutation in maize which expands and elongates glumes in both the tassel and the ear (Mangelsdorf and Galinat, 1964). These glumes, unlike normal glumes, have a full M cell layer between veins.

In situ localization of photosynthetic gene products

Tissue samples were fixed and embedded in paraffin as previously reported (Langdale *et al.*, 1987), with the following exceptions. Husk leaves and prophylls were fixed overnight in FAA (4% formaldehyde, 5% acetic acid, 50% ethanol). Sections (8 μm) were visualized using a Safranin/Fast Green staining procedure modified from Jensen (1962). Samples were deparaffinized in xylenes and rehydrated to 50% ethanol. After a 10-min incubation in 0.5% Safranin O (Sigma, St Louis, MO) in 50% ethanol, samples were washed briefly in 70% ethanol and then counterstained for 30 s in 0.5% Fast Green FCF (Sigma) in 95% ethanol. Finally, samples were washed in 95% ethanol, dehydrated, cleared in xylenes and mounted in Permount (Fisher Scientific, Springfield, NJ). Immunolocalization assays were carried out as previously described (Langdale *et al.*, 1987) using monospecific primary antisera, a biotinylated secondary antiserum and a streptavidin–peroxidase detection system. Reactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB). LHCP antibody was a kind gift from Dr Steve Mayfield (Research Institute of Scripps Clinic, La Jolla, CA). This antibody preparation reacts with both BS and M cell-specific LHCP II polypeptides. *In situ* hybridization experiments were carried out using ^{35}S-labeled riboprobes as previously reported (Langdale *et al.*, 1988).

Photosynthetic rate measurements

Photosynthetic rates and O_2 inhibition measurements were determined as previously described using an open system (Peterson and Zelitch, 1982). Light intensity was 500 μl/l/s, CO_2 concentration ~400 μl/l and temperature 28°C–30°C. O_2 levels were initially held at 21% until three consistent readings were obtained. To test inhibition of carbon fixation by O_2, levels were subsequently dropped to 1% for three consistent readings and then raised back to 21% for an additional three readings.

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