Biochemical and Ultrastructural Analysis of the *y10* Mutant of Maize

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The *y10* mutant of maize has been categorized as a carotenoid mutant due to its pale-yellow endosperm color and the albino seedling that develops following germination of the kernel. Analysis of endosperm and seedling leaf extracts revealed that the carotenoid content of endosperm and seedling leaf is significantly reduced compared to wild-type siblings. However, a block at a specific step in the carotenoid biosynthetic pathway was not detected. When *y10y10* seedlings were grown on defined medium containing geraniol or farnesol, which are isoprenoid precursors for both carotenoids and the phytyl chain of chlorophyll, we found a significant increase in the amount of carotenoids in the leaf tissue. These isoprenoid precursors appear to complement the *y10* mutation, although not to wild-type sibling levels. Thus the apparent defect in the *y10* mutant affects a step in isoprenoid biosynthesis that precedes the synthesis of geranylgeranyl pyrophosphate. Examination of seedling leaves by light and transmission electron microscopy revealed that the drastic reduction in photosynthetic pigments in the *y10* mutant has a dramatic effect on the cellular architecture of leaf tissue. In comparison to other albino mutants of maize that have been analyzed at the ultrastructural level, the alterations in plastid architecture of *y10* mutants are quite severe.

Carotenoids function in plant cells as accessory light-harvesting pigments, dissipating excess energy during photosynthesis and protecting chlorophyll from photooxidation (Bartley and Scolnik 1995; Demmig-Adams and Adams 1996; Demmig-Adams et al. 1996). Carotenoids and chlorophylls are incorporated into the core and light-harvesting complexes of photosystems I and II found in thylakoid membranes of chloroplasts and are necessary for the stable incorporation of chlorophyll into thylakoid membranes (Paulsen 1997). In plants, carotenoids are synthesized and accumulate in plastids. All carotenoid biosynthetic enzymes are nuclear encoded, hence they are synthesized in the cytosol and imported posttranslationally into plastids. Several of the genes encoding the carotenoid biosynthetic enzymes have been cloned and characterized from various plants and photosynthetic bacteria (reviewed in Cunningham and Gantt 1998; Hirschberg et al. 1997).

Carotenoids belong to a diverse class of molecules referred to as isoprenoids, as they are synthesized from the condensation of C5 isoprene units called isopentenyl pyrophosphate (IPP). The C20 isoprenoid geranylgeranyl pyrophosphate (GGPP) is synthesized from IPP by three successive additions of IPP to dimethylallyl diphosphate by the enzyme GGPP synthase. The two intermediates formed in this process are the C10 isoprenoid geranyl pyrophosphate and the C15 isoprenoid farnesyl pyrophosphate. The GGPP that is formed in plastids is not only a substrate for phytoene synthase, the enzyme which condenses two GGPP molecules into the first carotenoid phytoene, but also for enzymes which synthesize phytyl pyrophosphate. Chlorophyll synthase esterifies phytyl pyrophosphate to chlorophyllide a, resulting in the formation of chlorophyll a (Lichtenthaler et al. 1997; McGarvey and Croteau 1995; von Wettstein et al. 1995). Plastid GGPP is also utilized in the production of other isoprenoids, including tocopherols and prenyl quinones. The regulation of the different isoprenoid pathways relative to each other is not understood (Kleinig 1989; Lichtenthaler et al. 1997). However, the production of sufficient quantities of GGPP is essential to provide the starting material for these important biosynthetic pathways. The GGPP that is utilized in the plastid is believed to be synthesized within the plastid, although there is some evidence to suggest

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that there may be cooperation between cytosolic and plastidic isoprenoid intermediates (Lichtenthaler et al. 1997).

When carotenoid biosynthesis is blocked, such as in the *w3, al1,* and *cl1* mutants of maize, leaf tissue is albino due to the photoinduced destruction of chlorophyll (Anderson and Robertson 1960). Albinism is a lethal condition and these seedlings die when their endosperm reserves are exhausted. When certain maize albino mutant seedlings are grown under dim light, thus experiencing less photodestruction, many of theses mutants will become pale green due to the synthesis and accumulation of chlorophyll, which is accompanied by the appearance of small grana in leaf plastids (Bachmann et al. 1973; Robertson et al. 1978; Troxler et al. 1969). These observations indicate that many albino mutants are deficient in their ability to synthesize carotenoids, but not chlorophylls. However, it is unlikely that chlorophyll molecules in these mutants are stably incorporated into thylakoid membranes, as it appears that core and light-harvesting complex proteins do not accumulate in the absence of carotenoids, even though the level of transcripts for these proteins is normal (Herrin et al. 1992; Markgraf and Oelmüller 1991).

The *y10* mutant of maize was phenotypically described and characterized by Robertson (1961) as a carotenoid mutant due to the pale yellow endosperm color of homozygous individuals. Following germination, homozygous *y10* seedlings are albino. However, *y10* seedlings have not been observed to become pale green when grown under dim light conditions, unlike other carotenoid-deficient mutants including *w3* and *al1* (Bachmann et al. 1973). This may indicate that the *y10* mutation affects both carotenoid and chlorophyll biosynthesis. The purpose of the present study is to characterize the biochemical defect that results in the *y10* mutant phenotype and to determine what effect this has on the structure of plastids in *y10* mutants.

Methods

Extraction and Analysis of Endosperm and Leaf Pigments

All procedures were carried out under yellow light. Pale yellow (*y10/y10*) and yellow (*Y10/y10* or *Y10/Y10*) mature dry kernels were soaked overnight at 4°C in ultrafiltered H_2O. Endosperm tissue from five kernels was removed, immersed in liquid nitrogen, and ground to a fine powder with a prechilled mortar and pestle. Ground tissue (0.4 g) was extracted three times with 1:1 (v:v) acetone:hexane containing 0.01% butylated hydroxytoluene (BHT). The extract was dried under argon, resuspended in 5:3:2 (v:v:v) hexane:acetone:isopropanol and stored under argon gas at −20°C until analysis. For samples that were analyzed immediately following extraction, the extract was resuspended in 200 µl 1:1 (v:v) methanol:methylene chloride and 25 µl was injected into the high-performance liquid chromatograph.

Homozygous *y10* and *Y10/-* seedlings were grown for 1 week in a growth chamber under full-light conditions (160 µmol/m²/s) provided by incandescent and fluorescent light. Leaf tissue was ground to a fine powder in liquid nitrogen by using a prechilled mortar and pestle and extracted with acetone containing 0.01% BHT. The extract was filtered through Whatman #1 filter paper under vacuum. The acetone was then evaporated with argon and the remaining residue was extracted with hexane. The hexane layer was recovered, evaporated to dryness under argon, resuspended in 5:3:2 (v:v:v) hexane:acetone:isopropanol and stored under argon gas at −20°C until analysis.

Leaf pigments were separated by reversed-phase high-performance liquid chromatography (HPLC) on a 4.6 mm × 250 mm C30 Carotenoid® column (YMC, Wilmington, NC), and endosperm pigments were separated on a 3.6 mm × 300 mm C18 Resolve® column (Waters, Milford, MA) using a mobile phase of 90:15:10:0.1:0.05 (v:v:v:v:w) acetonitrile:methylene chloride:methanol:octanol:ammonium acetate (Barua et al. 1993) at a flow rate of 1 ml/min. Run times were 35 min for endosperm extracts and 110 min for leaf extracts. The Waters HPLC system included a model 501 pump, U6K injector, model 484 detector, and an NEC PC equipped with Baseline® software (Dynamic Solutions, Ventura, CA) for chromatogram analysis. Detection was performed at 450 nm or with a Waters model 991 photodiode array detector, which was equipped with Millennium software for chromatogram analysis. Carotenoids were identified based on their retention times compared to authentic standards and by comparing their absorption spectra on the photodiode array detector to published spectra.

Plant Culture and Complementation Studies

The procedure used was modified from Wright et al. (1992). Pale yellow (*y10/y10*) and yellow (*Y10/-*) kernels were soaked for 10 min in a 50% bleach solution, washed twice in sterile, deionized H_2O, and imbibed overnight in sterile H_2O containing 1 mM geraniol, 1 mM farnesol, or no isoprenoids. Kernels were removed and transferred aseptically to test tubes containing Murishige–Skoog (MS) medium, 0.8% phytagar, which was either unsupplemented or had been supplemented with farnesol or geraniol to final concentration of 50 or 200 mM. Seedlings were grown at 27°C for 2 weeks under dim light conditions (0.2 µmol/m²/s). As a control, *Y10/-* seedlings were grown on unsupplemented MS medium. Pigments were extracted and separated as described above.

Microscopy

Homozygous *y10* or *Y10/-* seedlings were grown for 1 week in a growth chamber at 27°C under either dim (0.2 µmol/m²/s) or full (160 µmol/m²/s) light conditions. Leaf tissue was excised 2 cm from the seedling leaf tip using a double-edged razor blade and fixed in 3% glutaraldehyde (buffered in 0.2 M phosphate, pH 7.4) for 24 h and then buffer washed at least four times. Specimens were postfixed in 1% OsO_4 (buffered in 0.2 M phosphate buffer, pH 7.4) for 3 h and buffer washed four times. Following washing, the tissue was gradually dehydrated in a series of ethanol and acetone rinses and then gradually infiltrated and embedded in Spurr epoxy resin. Embedded leaves were sectioned on an RMC MT6000-XL ultramicrotome with glass knives. For light microscopy (LM), thick sections (850 nm) were stained with Richardson's stain (azure II and methylene blue) and examined with bright-field and differential interference contrast illumination on an Olympus BHS compound light microscope. For transmission electron microscopy (TEM), ultrathin sections (90–100 nm) were collected on 1 mm × 2 mm slot grids and dried on formvar support films (Rowley and Moran 1975). Grids were stained in 1% uranyl acetate (10 min) and lead citrate (8 min; Venable and Coggeshall 1965) and then examined using a JEOL JEM-100SX TEM at 80 kV.

Results

Analysis of Pigments in Endosperm and Leaf Tissue

The pale yellow color of *y10y10* kernels is suggestive of a reduced synthesis and accumulation of carotenoid pigments in endosperm tissue. Extraction and analysis of colored carotenoids from endosperm

Table 1. Summary of carotenoids in homozygous *y10* mutants[a]

Pigment	Percent of pigment in *Y10/-* endosperm[b]	Percent of pigment in *Y10/-* seedling leaf[c,d]
α-Carotene	81.7 ± 17.6	Not detected
α-Cryptoxanthin	5.9 ± 2.9[e]	Not detected
β-Carotene	79.1 ± 23.2	Not detected
β-Cryptoxanthin	71.6 ± 19.9[e]	Not detected
Zeaxanthin/Lutein	41.2 ± 4.3[e]	3.6 ± 1.3[e]
Violoxanthin	—	0.8 ± 0.1[e]
Chlorophyll a	—	1.6 ± 0.6[e]
Chlorophyll b	—	2.2 ± 0.8[e]

[a] Data is expressed as the percentage of pigment found in *Y10/-* siblings.

[b] Each value represents the mean ± standard error of the mean from four different ears analyzed in duplicate.

[c] Data is expressed as a percentage of carotenoid in *Y10/-* seedling leaf normalized to dry weight (i.e., the tissue remaining following extraction). Each value represents the mean ± standard error of the mean from three different seedlings analyzed in duplicate.

[d] Seedlings were grown under full light (160 μmol/m²/s) conditions.

[e] Significantly reduced compared to *Y10/-* siblings.

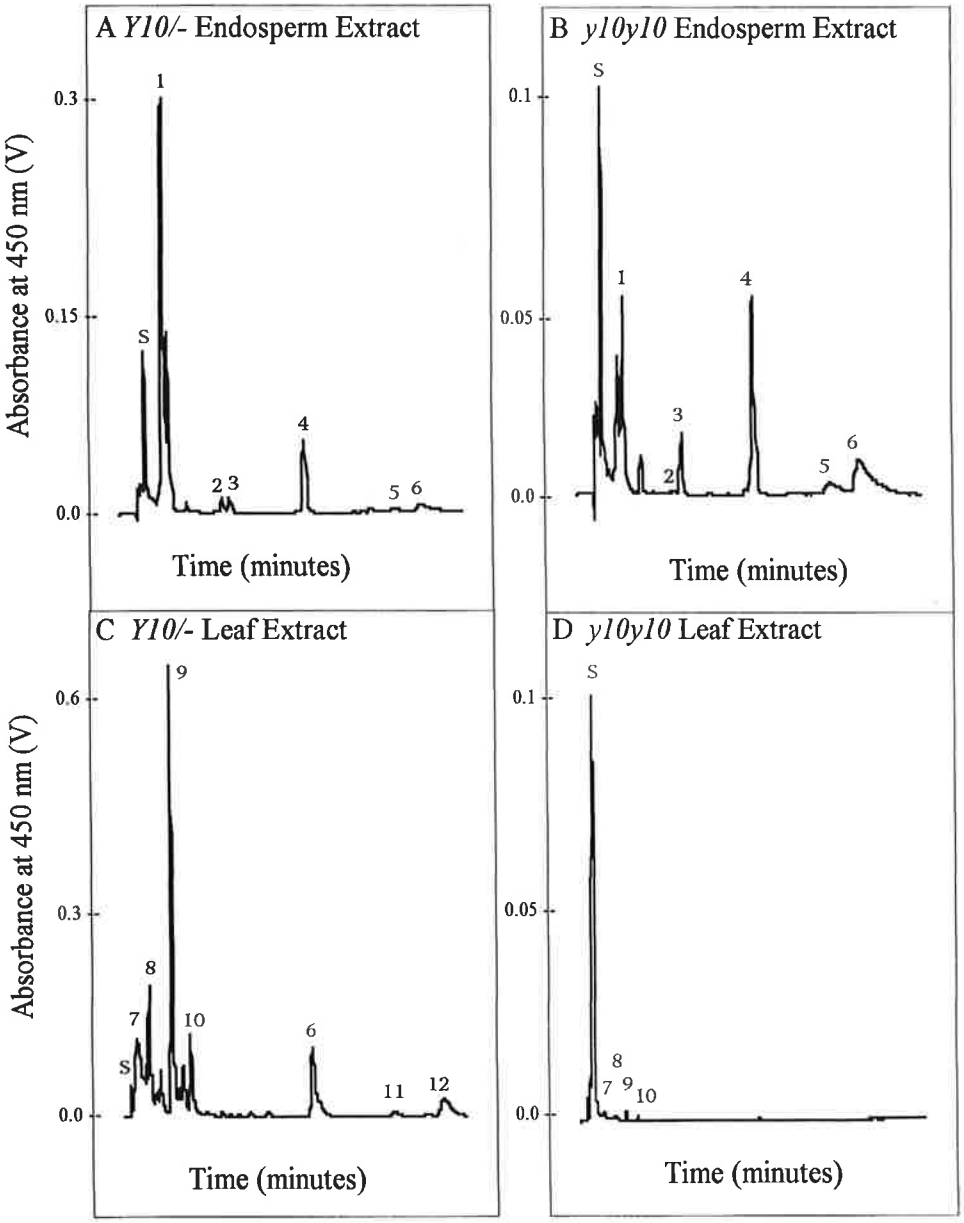

Figure 1. High-performance liquid chromatography of *y10y10* and *Y10/-* tissue extracts. Leaf tissue was analyzed from seedlings grown under full-light conditions (160 μmol/m²/s). Run times for endosperm and leaf extracts were 35 min and 110 min, respectively. **(A)** *Y10/-* endosperm extract. **(B)** *y10y10* endosperm extract. **(C)** *Y10/-* seedling leaf extract. **(D)** *y10y10* seedling leaf extract. S = solvent front; 1 = lutein and zeaxanthin; 2 = α-cryptoxanthin; 3 = β-cryptoxanthin; 4 = xanthophyll monoester; 5 = α-carotene; 6 = β-carotene; 7 = violoxanthin; 8 = chlorophyll b; 9 = lutein; 10 = chlorophyll a; 11 = chlorophyll b; 12 = chlorophyll a.

demonstrated that there is a significant reduction in endosperm of *y10y10* kernels when compared to *Y10/-* siblings (Table 1). Although the distribution of carotenoids is relatively normal, xanthophylls were more dramatically affected than carotenes (Figure 1A,B). When *y10y10y10* endosperm extracts were analyzed on a high-performance liquid chromatograph equipped with a photodiode array detector, no colorless carotenoids such as phytoene and phytofluene were detected in the extracts (data not shown). Thus the reduction of carotenoids in the endosperm of *y10* mutants does not appear to be due to a block at a particular enzymatic step in the carotenoid biosynthetic pathway. Our data suggests that there may be a reduction in the supply of precursor molecules to the enzymes of the carotenoid biosynthetic pathway, or a downregulation of the pathway at a key regulatory step prior to the synthesis of phytoene.

Pale yellow *y10* kernels germinate into albino seedlings under both normal and dim light conditions (Bachmann et al. 1973). Extraction and HPLC analysis of pigments from *y10y10* seedlings grown under full light revealed that there were trace quantities of chlorophyll and carotenoid pigments present (Figure 1D). However, the quantities of chlorophylls and carotenoids present represent less than 1% and 2% of the carotenoids and chlorophylls, respectively, present in *Y10/-* siblings (Figure 1C,D, Table 1). In addition, only two carotenoid peaks (violoxanthin and lutein) and two chlorophyll peaks (chlorophylls a and b) were detected during the analysis of *y10y10* extracts by HPLC (Figure 1D). The identity of these compounds was confirmed by analysis of extracts on a high-performance liquid chromatograph equipped with a photodiode array detector. No colorless carotenoids such as phytoene or phytofluene were present in leaf tissue extracts, suggesting again that the *y10* mutation does not effect a specific step in the carotenoid biosynthetic pathway. In addition, growth of *y10* seedlings under dim light conditions does not result in greening of the seedling leaf tissue, which has been observed in a number of other maize albino mutants such as *w3* and *lw2*. The *w3* and *lw2* mutants accumulate 79% and 31%, respectively, of wild-type seedling levels of chlorophyll when grown in dim light (Bachmann et al. 1973; Robertson et al. 1978). The albino phenotype observed in seedlings that are grown in full light is due to the photodestruction of chlorophylls that results from the absence of carotenoids (Anderson and Robertson 1960). These data suggest that the *y10* mutant is not only deficient in carotenoid biosynthesis, but also in chlorophyll

Table 2. Summary of pigments in supplemented and unsupplemented homozygous *y10* seedlings grown on Murishige–Skoog medium[a]

Pigment	Percent of pigment in *Y10/-* seedlings[b]	
	Unsupplemented	Supplemented[c]
Lutein	0.6 ± 0.5	9.5 ± 2.7[d]
Violoxanthin	1.6 ± 1.0	12.4 ± 4.2[d]
Chlorophyll a	2.3 ± 1.0	5.1 ± 2.2
Chlorophyll b	0.12 ± 0.05	Not detected

[a] Seedlings were grown under dim light (0.2 μmol/m²/s).

[b] Data is expressed as a percentage of pigment found in unsupplemented *Y10/-* seedling leaf normalized to dry weight (i.e., the tissue remaining following extraction). Each value represents the mean ± standard error of the mean from eight different seedlings analyzed in duplicate.

[c] Homozygous *y10* seedlings were grown for 2 weeks on Murishige–Skoog medium, 0.8% phytagar supplemented with geraniol (50 mM or 200 mM) or farnesol (50 mM or 200 mM).

[d] Significantly increased compared to homozygous *y10* siblings grown on unsupplemented Murishige–Skoog medium.

biosynthesis. The *y10* mutation could potentially affect the supply of precursor molecules (i.e., isoprenoids) that are required to synthesize both classes of photosynthetic pigments.

Chemical Complementation of *y10* Seedlings with Isoprenoids Geraniol and Farnesol

To determine whether the *y10* mutant could be chemically complemented by the addition of exogenous carotenoid and chlorophyll precursor molecules, *y10y10* seedlings were grown aseptically on MS medium to which the isoprenoids geraniol (a C10 isoprenoid) or farnesol (a C15 isoprenoid) had been added. The pyrophosphate forms of geraniol and farnesol are both substrates for the plastid enzyme GGPP synthase. If these molecules could be taken up by the seedlings and utilized in plastids of plants which have a reduced or nonexistent pool of these precursors, the plants could potentially utilize them to synthesize carotenoids and/or chlorophyll molecules as long as the appropriate enzymes to do so are present in the plastids. When pigments were extracted from *y10y10* seedlings grown on geraniol- or farnesol-supplemented MS medium, analyzed by HPLC, and compared to those grown on MS medium alone, it was determined that both supplemented and unsupplemented *y10y10* seedlings displayed the same four photosynthetic pigments (violoxanthin, lutein, and chlorophylls a and b; Table 2). Furthermore, it was determined that seedlings supplemented with either concentration of farnesol or geraniol gave similar results, so these seedlings will be discussed together and referred to as "supplemented seedlings."

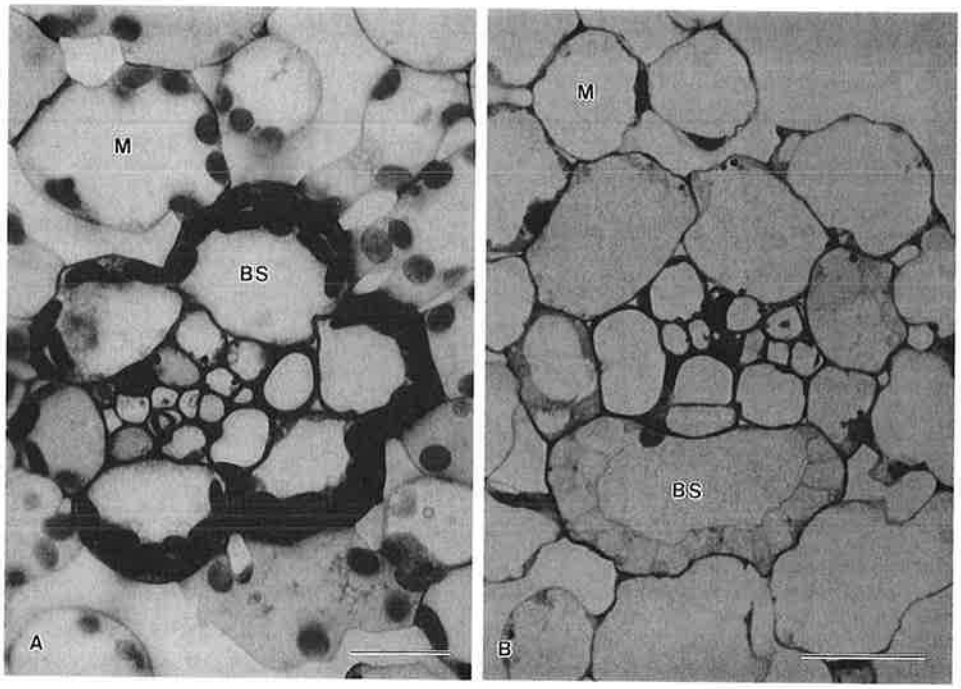

Figure 2. Transverse sections of mesophyll (M) and bundle sheath (BS) cells from leaves of *y10y10* and *Y10/-* seedlings grown under full-light conditions (160 μmol/m²/s). LM; bars = 5 μm. **(A)** *Y10/-* leaf section. **(B)** *y10y10* leaf section. Note the conspicuous absence of chloroplasts in the *y10y10* leaf section.

Supplemented seedlings possessed significantly higher quantities of violoxanthin (6 times) and lutein (20 times) compared to unsupplemented seedlings (Table 2). The carotenoids present in the supplemented seedlings represent approximately 10% of the carotenoids found in *Y10/-* siblings grown under the same conditions, but without isoprenoid supplementation (Table 2). In addition, approximately two times the amount of chlorophyll a was present in extracts of supplemented *y10y10* seedlings, although this was not found to be a statistically significant increase. The increased amount of photosynthetic pigments could also be visualized when observing the plants. However, the coloration varied between individual supplemented seedlings and ranged from a very subtle greenish-yellow tinge to an obvious pale green color (data not shown). Thus growth on medium supplemented with isoprenoids does seem to complement, albeit not at a high efficiency, the *y10* mutation. It appears that the defect in the *y10* mutant affects a step in isoprenoid biosynthesis that precedes the conversion of geranyl pyrophosphate to farnesyl pyrophosphate and subsequently to GGPP via three subsequent additions of isopentenyl pyrophosphate by the enzyme GGPP synthase.

Microscopy

The presence and ultrastructure of plastids in *y10y10* and *Y10/-* seedling leaves were examined by LM and TEM. Previous studies on several maize albinos have revealed an abnormal plastid ultrastructure when seedlings were grown in full light (Bachmann et al. 1973; Robertson et al. 1978; Troxler et al. 1969). However, growth of these mutants in dim light could result in plastid architecture that was more similar to wild type. We examined *y10y10* and *Y10/-* seedlings that were grown either under full (160 μmol/m²/s) or dim (0.2 μmol/m²/s) light conditions.

Both mesophyll (M) and bundle sheath (BS) cells in leaves of *y10y10* seedlings grown under full-light conditions did not contain any structures that resembled plastids (Figure 2B). Examination of these tissues with a TEM did not reveal any cellular structures either with double membranes or that remotely resembled plastids or mitochondria (data not shown). The only identifiable cellular structure in these cells were nuclei. These observations indicate that the drastic reduction in photosynthetic pigments in the *y10* mutant has a dramatic effect on the subcellular structure when plants are grown in full light.

Mesophyll and BS cells of *y10y10* plants grown under dim light conditions contained plastids with few internal membranes compared to *Y10/-* siblings (Figure

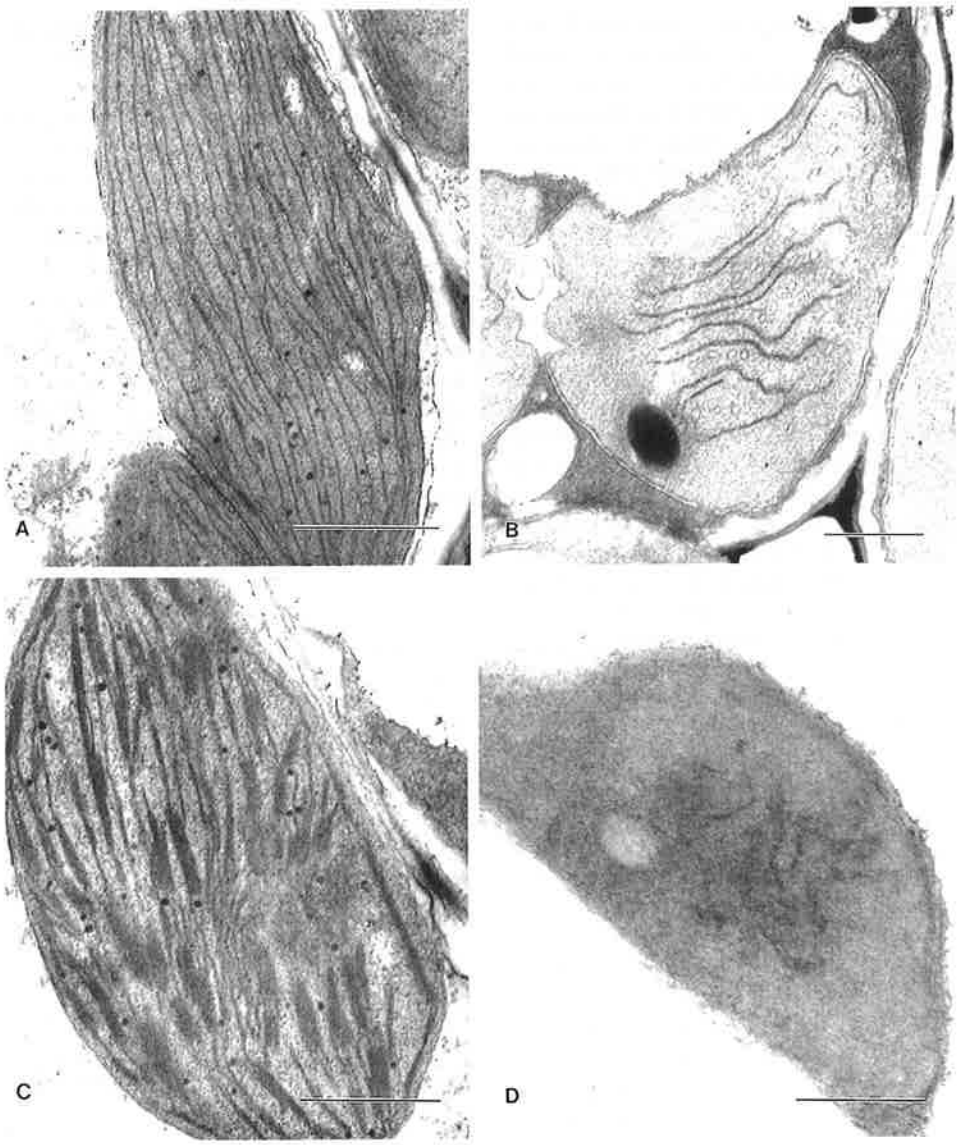

Figure 3. Sections of plastids from bundle sheath cells **(A)** and **(B)** and mesophyll cells **(C)** and **(D)** from leaves of *y10y10* and *Y10/-* seedlings grown under dim light conditions (0.2 μmol/m²/s). TEM; bars = 1 μm. **(A)** Plastid in *Y10/-* bundle sheath cell. **(B)** Plastid from *y10y10* bundle sheath cell. **(C)** Plastid from *Y10/-* mesophyll cell. **(D)** Plastid from *y10y10* mesophyll cell. Note the reduced amount of internal membranes and lack of thylakoid stacking in the plastids from the *y10y10* bundle sheath and mesophyll cells.

3). Bundle sheath cell chloroplasts of *y10y10* plants were irregularly shaped, had few thylakoid membranes, and contained numerous vesicles (Figure 3B). In addition, the stroma was less electron dense than the cytosol, indicating a reduced protein content, and lacked osmiophilic inclusions. Mesophyll cell chloroplasts appeared to have even fewer internal membranes, and those present were not in any discernible order (Figure 3D). In addition, mitochondria lacked cristae and other internal structure (Figure 3B). In comparison to other maize albino mutants that have been analyzed at the ultrastructural level, the alterations in plastid architecture of *y10* mutants is quite severe.

Discussion

The *y10* mutant of maize exhibits a pale yellow endosperm and an embryo that germinates into an albino seedling (Bachmann et al. 1973; Robertson 1961). The albino phenotype in maize is typically attributed to the reduced ability to synthesize and accumulate carotenoids, resulting in the photodestruction of any chlorophyll that is produced (Anderson and Robertson 1960). However, when grown under dim light conditions, which are permissive for chlorophyll accumulation, *y10y10* seedlings do not accumulate chlorophyll and remain albino. These observations, in addition to our findings that only trace amounts (less than 3% of those found in *Y10/-* siblings) of photosynthetic pigments are present in *y10y10* seedlings grown in either dim or full-light conditions, indicate that the *y10* gene influences both carotenoid and chlorophyll synthesis. Since both the carotenoid and chlorophyll biosynthetic pathways require products of the isoprenoid pathway, a possible defect in the *y10* mutant is the supply of isoprenoid precursors to the pathways that synthesize photosynthetic pigments.

Homozygous *y10* seedlings appear to be complemented by the addition to their growth medium of either the C10 isoprenoid geraniol or the C15 isoprenoid farnesol. A 6- or 20-fold increase in carotenoids was observed in supplemented homozygous *y10* seedlings, whereas a twofold increase in chlorophyll was observed. This suggests that the *y10* homozygotes are not defective in GGPP synthase activity, which catalyzes the production of GGPP from dimethylallyl pyrophosphate through the stepwise addition of C5 IPP units (Bartley and Scolnik 1995). Thus the biochemical defect in the *y10* mutant must precede this step. One candidate that the *y10* gene might encode is the enzyme IPP isomerase. This enzyme catalyzes the conversion of IPP to dimethylallyl pyrophosphate and has been shown previously to be a regulatory step in the synthesis of photosynthetic pigments during the transformation of maize etioplasts to chloroplasts (Albrecht and Sandmann 1994). IPP isomerase has been cloned from *Arabidopsis thaliana* (Campbell et al. 1997) and *Clarkia breweri* (Blanc and Pichersky 1995) but not from maize. Alternatively, *y10* may encode a protein that influences the activity of early isoprenoid biosynthetic enzymes or a transcription factor which regulates the expression of isoprenoid biosynthetic enzymes.

A drastic reduction in isoprenoid biosynthesis could result in a decreased production of carotenoids and chlorophylls since GGPP is the immediate precursor for both carotenoids and the phytyl chain of chlorophyll. The addition of the phytyl chain is one of the last steps in the production of chlorophyll molecules (von Wettstein et al. 1995). Without the addition of the phytyl chain to the chlorophyllide porphyrin ring, there is no incorporation and stabilization of the incomplete chlorophyll into thylakoid membranes. These molecules are likely to be dangerous to the plastids in which they are found, and possibly the entire cell, since unbound chlorophyll and precursors can

be easily photooxidized. Free porphyrins can readily generate free radicals, such as singlet oxygen, in the presence of light (Arakane et al. 1996). This may account for the lack of internal structure in plastids of M and BS cells in leaves of *y10y10* seedlings grown in full light as compared to those grown in dim light.

In addition to carotenoids and chlorophylls, GGPP is a precursor for a variety of biomolecules synthesized in plastids, including tocopherols and quinones which are inserted into thylakoid membranes and participate in electron transport (Kleinig 1989; Lichtenthaler et al. 1997). The leaf tissue of *y10* homozygotes may be more dramatically affected by a limited production of isoprenoids as compared to endosperm tissue, since there are greater demands on the isoprenoid pool in leaf tissue. For instance, large amounts of photosynthetic pigments and quinones are required for photosynthesis in chloroplasts. Fewer of these compounds could be synthesized if the supply of isoprenoid precursors is severely limited. This effect would be exacerbated if isoprenoids were preferentially incorporated into chlorophylls, as these pigments would be destroyed in the absence of carotenoids. Thus leaf tissue would be expected to be albino while endosperm tissue could still accumulate low levels of carotenoids, which is consistent with the *y10* mutant phenotype.

Although the efficiency of incorporation of exogenous isoprenoids was low in our study, this is not surprising since the isoprenoids needed to be absorbed by the plant and transported to the site of pigment biosynthesis in leaf plastids. Others have also reported a low efficiency of incorporation of farnesol into whole cut leaf (Albrecht and Sandmann 1994) and into in vitro plastid preparations (Fraser et al. 1994) when studying carotenoid biosynthesis. This may indicate that there is a difficulty for these precursors, which are normally synthesized within plastids, to enter into plastids.

The most striking observation made of the structure of plastids in *y10y10* seedlings is the presence of few internal membranes. Assembly and formation of thylakoid membranes depends upon the accumulation of photosynthetic pigments, as well as core and light-harvesting complex proteins, within the plastid. Prior to insertion into thylakoid membranes, chlorophylls are believed to be bound either to a protein carrier or to the last enzyme in the chlorophyll biosynthetic pathway, chlorophyll synthase. Chlorophyll synthase catalyzes the addition of phytyl chain to chlorophyllide a to generate chlorophyll a (Paulsen 1997). Carotenoids are required for the assembly of photosynthetic apparatuses, although only a small amount is needed to stabilize pigment-protein complexes (Markgraf and Oelmüller 1991). In *Chlamydomonas* mutants that are completely devoid of carotenoids, core and light-harvesting proteins of photosystem I and II do not accumulate at all (Herrin et al. 1992). This may be due to a decreased ability to import nuclear-encoded proteins into plastids, as seen in carotenoid-deficient etioplasts. These plastids can bind but have a much reduced ability to translocate plastid precursor proteins (Dahlin 1993; Dahlin and Franzén 1997). This, in combination with the possible destruction of biomolecules in the plastid due to porphyrin-generated free radicals, may account for the sparse internal membrane structure that is seen in plastids of *y10y10* seedling leaf cells.

Mitochondrial structure is also altered in BS and M cells of *y10* seedlings. No mitochondria were observed in *y10y10* seedlings grown in full light, while very few cristae were observed in the mitochondria of *y10y10* seedlings grown in reduced light. Although plastids appear to be autonomous in their production of isoprenoids, it is not clear to what extent isoprenoid intermediates are shared among plastid, mitochondrial, and cytoplasmic compartments (Kleinig 1989; Lichtenthaler et al. 1997). Mitochondria require, but do not produce, IPP. IPP is used by mitochondria for the synthesis and incorporation of quinones into their inner membranes, where these molecules participate in electron transport (Lichtenthaler et al. 1997). A generalized reduction in cellular isoprenoid pools may limit mitochondrial inner membrane synthesis and function. Altered chloroplast function in *y10y10* seedlings might also effect the availability of other metabolites to mitochondria. Therefore the altered mitochondrial structure in the *y10* mutant is likely a pleiotropic effect resulting from reduced isoprenoid levels in the chloroplast and cytoplasm.

Several maize carotenoid mutants, including *vp2, vp5, vp7,* and *vp9,* display vivipary (i.e., precocious germination) due to a reduced ability to synthesize abscisic acid (ABA; Neill et al. 1986). Zeaxanthin, the carotenoid precursor for ABA, is reduced in kernels of these mutants (Neill et al. 1986; Zeevaart and Creelman 1988). We have not observed vivipary in homozygous *y10* kernels, even though the amount of carotenoids is significantly reduced in their endosperm. This suggests that sufficient quantities of ABA are produced to allow for kernel dormancy. We were not able to resolve zeaxanthin from lutein in our HPLC system. However, the levels of β-cryptoxanthin (the immediate precursor to zeaxanthin) in *y10* endosperm was 79% of the amount found in wild-type endosperm, whereas α-cryptoxanthin (the immediate precursor to lutein) levels were 5.9% of those found in wild type. This may reflect a preferential production of β,β-carotenoids, including β-carotene, β-cryptoxanthin, and zeaxanthin in the homozygous *y10* endosperm, thereby permitting adequate production of ABA and kernel dormancy.

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