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Aberrations in plastid transcripts and deficiency of plastid DNA in striped and albino mutants in maize

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Abstract. To better understand the regulatory roles of nuclear genes in chloroplast genomic expression during leaf development in maize (*Zea mays* L.), we studied a striped mutant, *iil* (iojap 1), two albino mutants, *wl* (white 1) and *w2* (white 2), and their double mutants with *l* (luteus). Homozygous *ij1* plants as a female parent produce albino seedlings, called maternal exceptions, among their progeny, even when the nuclear genotype of the male parent is normal (+/+). In contrast to albinos that are blocked in the biosynthetic pathway of carotenoids, *wl* and *w2* seedlings fail to accumulate chlorophyll and carotenoids up to the normal level even under dim light conditions. In *ij1*-affected plastids, the plastid-encoded proteins and nuclear-encoded proteins that are associated with thylakoid membranes were not detecable. However, the 33-kDa protein of the oxygen-evolving complex and ferredoxin: NADP oxidoreductase, which are localized extrinsically, were accumulated even though the level of the proteins was decreased. Both *ij1* and *wl* albino seedlings contain a normal level of plastid DNA. However, both show similar aberrant patterns among the transcripts of all the plastid genes examined (*psb*B, *psb*H, *pet*B, *pet*D, *atp*A, *psa*B, psbA, and *rbc*L). Not only were additional transcripts detected but some of the normal transcripts were not detectable or were barely detectable by Northern hybridization. These facts indicate that the transcripts of *ij1*- and *wl*-affected plastids may have altered synthesis, processing or stability. Therefore, the block in expression of the plastid genome by the nuclear mutants *ij1* and *wl* may be due to alterations in the transcriptional or post-transcriptional processes. The fact that *ij1* and maternal-exception progeny show almost identical patterns of transcripts indicates that the effects of *ij1* on plastid gene expression persist in the subsequent generation even after the nuclear gene, *Ij1*, restores the normal function. In contrast to *ij1* and *wl*, the levels of all plastid transcripts in *w2* seedlings, whether *l* or +, are uniformly reduced. Compared to normal sibling seedlings, the patterns of the RNA species are relatively unaltered. Relative to the level of a nuclear rDNA, the plastid DNA content of *w2* is decreased 20-fold. Therefore, the limited expression of the *w2*-affected plastids may be due to failure to maintain the copy number of plastid genomes. Thus, albinisms of these mutants result from limiting of expression of plastids due to alteration of transcripts on the one hand, or to lowered DNA content on the other.

Key words: Plastid DNA – Mutant (maize) – Albino – Plastid Transcript – *Zea* (mutant, plastid)

Introduction

Among the nuclear mutants affecting plastid development, many exhibit loss of pigmentation (chlorophyll and-or carotenoids) in leaves. While some of the mutations cause the whole seedling to be albino or yellow, many lead to variegated or striped phenotypes (for review, see Kirk and Tilney-Bassett 1978). Some of the types of variegated or striped plants produce albino seedlings in the subsequent generation even after the function of the nuclear genes is restored. For example, *ij1* (iojap 1) and *cm* (chloroplast mutator) in maize, *as* (albostrians) in barley and *chm* (chloroplast mutator) in *Arabidopsis* have this effect (Rhoades 1943; Stroup 1970; Redei 1973; Knoth and Hagemann 1977).

In maize, the *ij1* mutant has been relatively well characterized among the striping mutants at the genetic, biochemical, and anatomical level (Coe et al. 1988a). One unusual feature of the *ij1* plants is genetic-background-specific variability of the phenotypic expression, even

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Abbreviations: FNR = ferredoxin: NADP oxidoreductase; OEC-33 = 33-kDa protein of the oxygen-evolving complex; Rubisco = ribulose-1,5-bisphopsphate carboxylase–oxygenase

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though the affected plastids show similar morphological structures (Thompson et al. 1983; Coe et al. 1988b). Walbot and Coe (1979) showed that the plastids do not contain ribosomes and rRNAs. Subsequent anatomical work indicated that the defective plastids contain little internal membrane in either light or dark-grown plants (Thompson et al. 1983). Siemenroth et al. (1980) determined that ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) and ATPase of plastids are not detectable.

In maize, the albino mutants with white endosperms have been extensively examined for biochemical studies on the carotenoid biosynthetic pathway and its relationship with plastid development (for review, see Robertson et al. 1978). Those mutants are *w3* (white 3), *lw* (lemon white), and the *vp* viviparous) class of mutants. In contrast, the mutants *w1* and *w2* exhibit white or pale-yellow seedlings and yellow endosperms. Also, a recessive modifier gene, *l*, interacts with the *w1* and *w2* mutants (though not with the other mutants mentioned above) and increases the levels of carotenoids in the leaf tissues. However, very little work has been done with these mutants beyond the early work of Lindstrom (1924) and Demerec (1923).

The objective of this study was to better understand the action of two different classes of nuclear mutations, a striped mutant, *ij1*, and the albino mutants, *w1* and *w2*, on plastid genomic expression by measuring the relative content of plastid DNA and the patterns of the transcripts of monocistronic and polycistronic genes of the affected plastids. We also examined whether the plastid genes in *ij1* plants are expressed differently from those in maternal-exception albino seedlings. Finally, we monitored the accumulation of the nuclear- and plastid-encoded proteins in *ij1* mutants to examine whether the affected plastids show any alteration in transport or processing of the nuclear-encoded plastidic proteins.

Materials and methods

Materials. Since *ij1*, *w1* and *w2* seedlings further accumulate carotenoids when the modifier gene, *l*, is present, each of the mutants was separated into two classes, consisting of yellow or white seedlings. To minimize differences in the genetic background, *ij1* and its maternal exceptions of three different phenotypes (white, yellow, and green) were obtained from single ears by appropriate crossing strategies. Maternal exceptions are wholly chlorophyll-less seedlings obtained following the cross of homozygous *ij1 ij1* maternal parents by pollen from normal parents. The stocks of *w1*, *w2* +, and *w2 l* were obtained from the Maize Genetic Stock Center (Urbana, Ill., USA; E. B. Patterson) in 1987 and 1988. The stocks were maintained by selfing heterozygous plants. The *l* double mutant, *w1 l*, was generated following genetic crosses between the *w1*/+ +/+ stock and Oh51a inbred, which has the genetic constitution *l*/*l* (C-d. Han and E.H. Coe, Maize Genet. Coop. Newsl. 62:51, 1988).

Growth conditions. The seedlings were germinated and grown for 5 d under darkness at 27° C in a growth chamber. Then, the seedlings were exposed to dim light of 0.01 μmol photons · m^{-2} · s^{-1} for 1 d and then to high light of 100 μmol photons · m^{-2} · s^{-1}. Fluorescent bulbs were used as a dim light source. For a high light source, fluorescent and incandescent bulbs were used. The seedlings were harvested and frozen immediately in liquid nitrogen under the same light conditions.

Measurement of pigments. One gram of the frozen tissues was ground in 10 ml of ice-cold 100% acetone under dim light. The solutions were centrifuged in a bench-top centrifuge for 5 min. The supernatant was subjected to absorbance scanning from 700 nm to 400 nm in a UV–VIS Recording Spectrometer (Shimadzu Co., Kyoto, Japan).

Preparation of crude total cellular proteins and immunodetection. Total leaf proteins were extracted by grinding the frozen leaf tissues in a mortar containing the homogenizing buffer at a ratio of 2 ml buffer per g tissue [0.1 M 2-(N-morpholino)ethanesulfonic acid (Na-Mops) pH 7.5; 10 mM NaCl; 1 mM EDTA; 10% sucrose; 5% beta-mercaptoethanol (BME); 4% SDS]. After heat-denaturation at 70° C for 5 min, the extracts were microfuged for 10 min. The supernatants were saved at −20° C until needed. Based on visual assessment of protein concentrations on a gel stained with Coomassie Brilliant Blue, equal amounts of protein samples were loaded into a 10–20% gradient SDS-polyacrylamide gel. The proteins transferred onto nitrocellulose (Schleicher and Schnell, Keene, N.H., USA) were incubated with antiserum against a target protein. To detect the antiserum bound to the protein, goat anti-rabbit immunoglobulin-G peroxidase conjugate (Bio-Rad, Richmond, Calif., USA) was used.

Antisera for the large subunit of Rubisco, phosphoenolpyruvate carboxylase, Rieske protein, P700 apoprotein (CP1), and cytochrome f were obtained from W. Taylor (CSIRO, Canberra, Australia). The antisera against the 33-kDa protein of the oxygen-evolving complex (OEC–33) and ferredoxin:NADP oxidoreductase (FNR) were obtained from J. Bennett (Department of Biochemistry, University of Iowa, Iowa City, Iowa, USA) and R. Malkin (Department of Molecular / Plant Biology, University of California, Berkeley, Calif., USA), repectively.

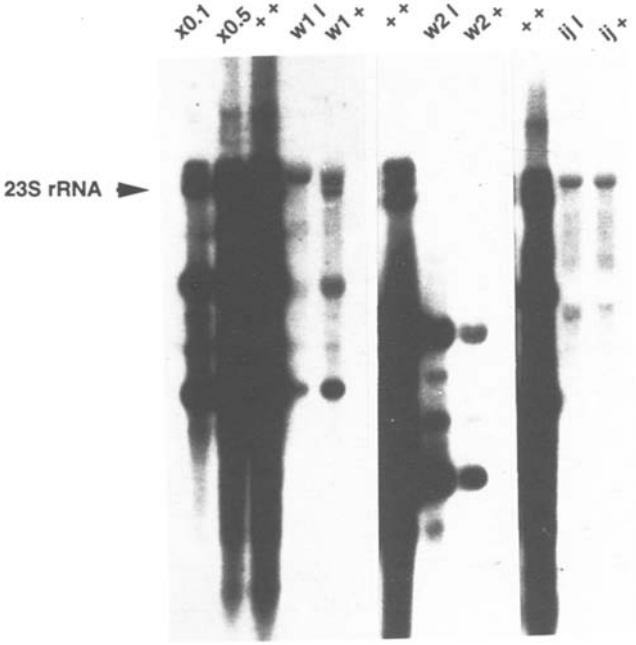

Fig. 1. Northern analysis of 23S plastid rRNAs from mutants and maternal-exception seedlings of maize. The seedlings were grown for 1 d in dim light (0.01 μmol photons · m^{-2} · s^{-1}) after 5 d in darkness. *Lanes* ×*0.1* and ×*0.5* indicate 10- and 2-fold dilution, respectively, of 6 μg total cellular RNAs of normal green seedlings. Figure is a combination of three different Northern blots of each mutant, *w1*, *w2*, and *ij1*. Each lane was loaded with 6 μg of total cellular RNAs, as indicated on the top of panel, from the mutant (*w1* +, *w2* +, and *ij1* +) and its *l* double mutant (*w1 l*, *w2 l*, and *ij1 l*)

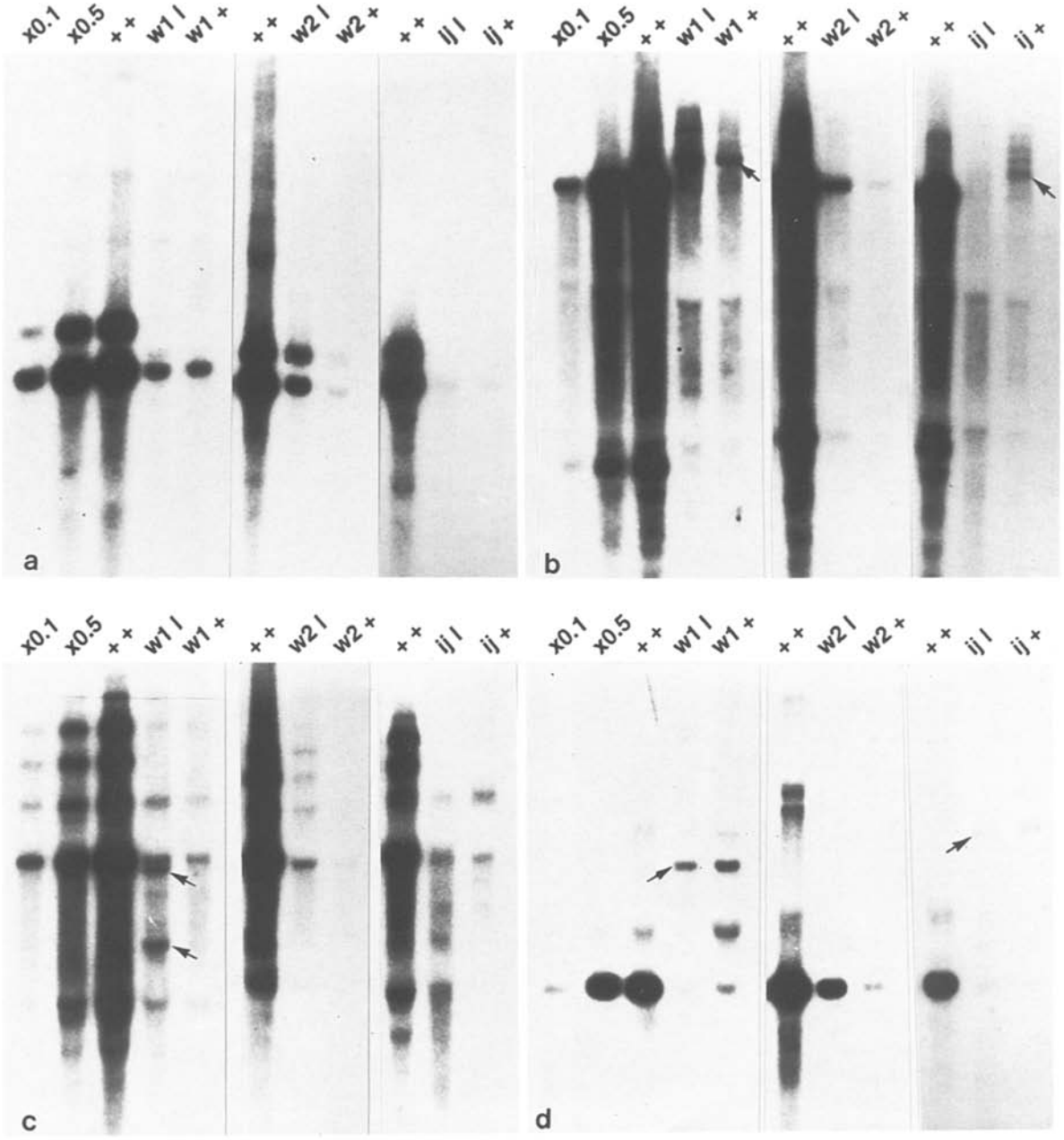

Fig. 2a–d. Northern analysis of the transcripts of *rbc*L (**a**), *psa*B (**b**), *atp*A (**c**), and *psb*A (**d**) of *w1*, *w2* and *ij1* maize seedlings. The seedlings and lanes are as described in Fig. 1. The *arrows* in panels **b** and **d** indicate a larger-size transcript. The *arrows* in panel **c** show extra transcripts

Preparation of RNA, and northern analysis. Total cellular RNA was prepared from the whole leaves by a guanidinium-thiocyanate extraction procedure (Barkan et al. 1986). Six micrograms RNA per sample was fractionated by electrophoresis on 1.3% agarose-formaldehyde gels. After electrophoresis, the relative amount of each RNA sample was re-examined by comparison of the intensity of the rRNA bands in the gels visualized with ethidium bromide. The RNA gel was transferred to nitrocellulose (Schleicher and Schnell). Filters were prehybridized overnight at 42° C in 5 × SSC (25 × SSC is 219 g NaCl and 100 g sodium acetate per liter, adjusted to pH 7.4 with HCl) 50% formamide, 5 × Denhardt's solution (100 × Denhardt's is 2.0 g Ficoll 400, 2.0 g polyvinylpyrollidone 40,000 MW, and 2.0 g bovine serum albumin Fraction V Sigma, in sterile distilled water brought to 100 ml, stored at −20° C until needed, vacuum filtered), 50 mM sodium phosphate (pH 6.5), 0.5% SDS, and 0.1 mg · ml^{-1} denatured salmon-sperm DNA. Probes

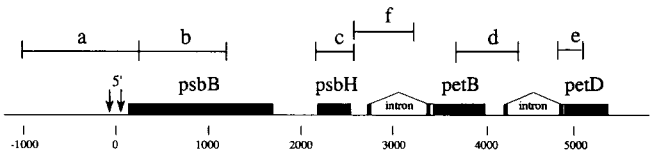

Fig. 3. Locations of the probes for the *psb*B operon of the maize plastid (redrawn from A. Barkan, 1989). *a*, 1.2-kb probe immediately upstream from the 5′ end of the *psb*B gene (including 150 bp of the 5′ end of the psbB gene); *b*, a probe for *psb*B; c, a probe for *psb*H; *d*, a probe for *pet*B (including the intron of *pet*D); *e*, a probe for *pet*D; *f*, a probe spanning the region between *psb*H and the exon of *pet*B

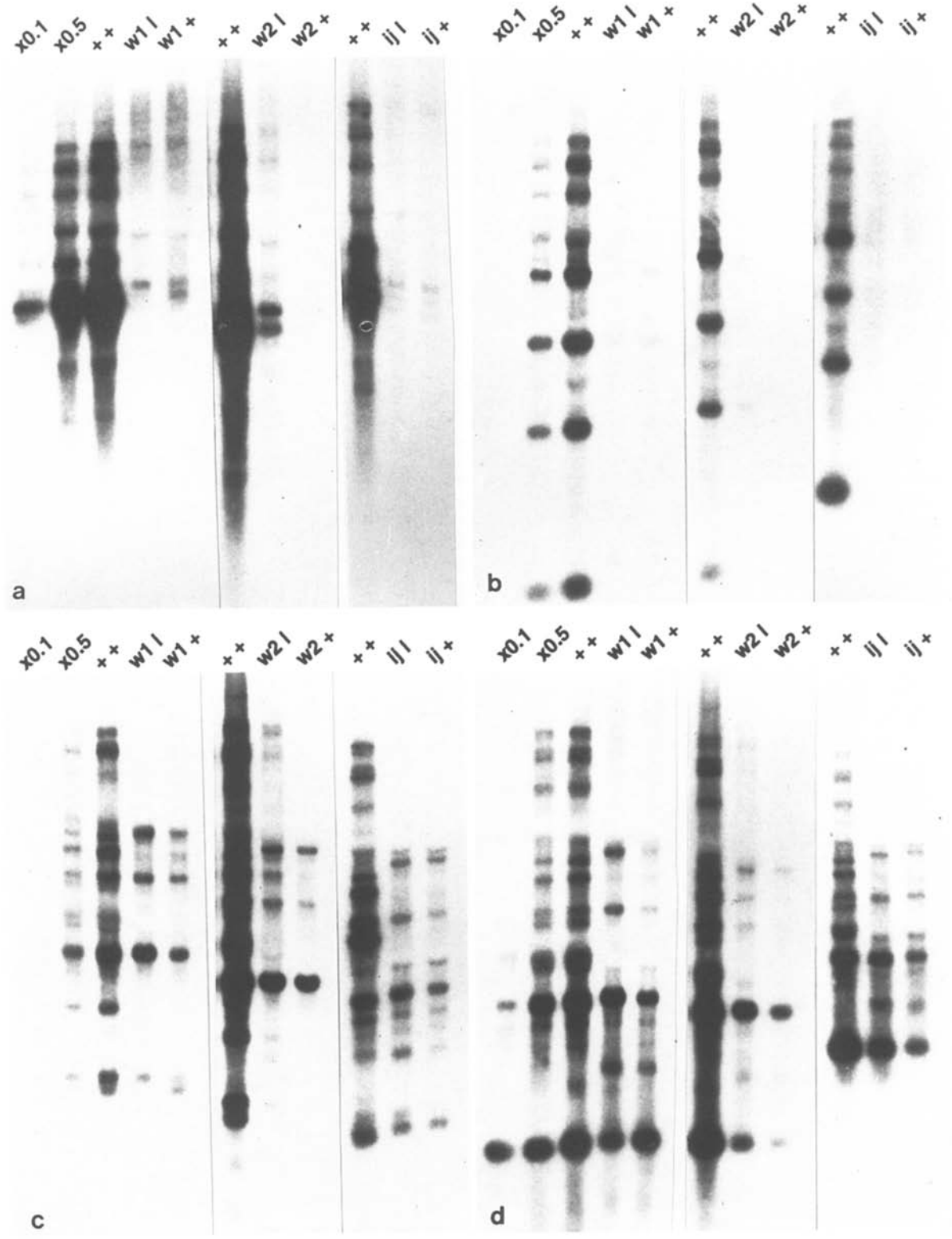

Fig. 4a–d. Northern analysis of the transcripts of *psb*B (**a**), *psb*H (**b**), *pet*B (**c**), and *pet*D (**d**) of *w1*, *w2*, and *ij1* maize seedlings. The probes for *psb*B, *psb*H, *pet*B, and *pet*D are indicated in Fig. 3 (b, c, d, and e, respectively). The samples were loaded in the same way as in Fig. 1

were radiolabeled by random primer labeling and added into the hybridization solution (5 × SSC; 50% formamide; 2 × Denhardt's solution; 20 mM sodium-phosphate buffer, pH 6.5; 0.5% SDS; 0.1 mg · ml^{-1} salmon-sperm DNA; 2–3% sodium dextran sulfate). The hybridization was performed for 16 h at 42° C. Final washes of the filters were done in 0.2 × SSC and 0.1% SDS solution for 45–60 min. at 65° C.

Preparation of DNA, and Southern analysis. Total cellular DNA was extracted from the seedlings according to a procedure provided by Dr. R. Martienssen (Cold Spring Harbor Laboratory). The DNA was digested with *Bam*HI (3 unit/μg DNA) at 37° C for 2 h. The DNA samples fractionated on 0.7% agarose gel were transferred to nylon. The filter was prehybridized and hybridized under conditions similar to the Northern blot, except without formamide, at 65° C.

Probes. Probe pZmL37 for *rbc*L is a 570-bp *Pst*I fragment of maize plastid DNA. Based on the published DNA sequencing data (Rock et al. 1987), the probes for *psb*B, *psb*H, and *pet*B genes were generated from the maize *Bam*HI 9' fragment. pAB6 for *pet*D is the maize 300-bp *Bam*HI fragment. For a gene, *clp*P, immediately upstream from the *psb*B gene, the 1.2-kb *Bam*HI/*Hind*III fragment from maize *Bam*HI 10 was used. Probe pATP1 for *atp*A is the maize 1.1-kb *Sal*I/*Bam*HI fragment internal to the *atp*A coding region. For detection of the plastid-specific rRNAs, the 650-bp *Xho*I fragment from pCB1–12 (23S rDNA from pea) was used as a probe. Probes pMA2 and pSD7 contain *psa*B (1.6 kb *Bam*HI 21' of maize plastid DNA) and spinach *psb*A gene, respectively. Probe pGmr1 contains *Eco*RI fragments of a soybean nuclear rDNA repeat unit. Probes pZmL37, pCB1–12, and pGmr1 were provided by W. C. Taylor (CSIRO, Canberra, Australia), K. Oishi (Molecular/Cell Biology Department, University of Arizona, Tucson, Ariz., USA), and E. A. Zimmer (Smithsonian Institution, Washington, D.C., USA). The rest of the probes were from A. Barkan (Institute of Molecular Biology, University of Oregon, Eugene, Ore., USA).

Results

All three mutants, *ij1, w1,* and *w2,* further accumulate carotenoids when the modifier gene, luteus (*l*), is present. To examine whether the level of carotenoids or *l* itself influences the expression of the affected plastids, the *ij1, w1,* and *w2* seedlings were separated into two classes, yellow (*l*/*l*) and white (+/+ or +/*l*).

Previous biochemical and molecular studies on *ij1* have been with seedlings grown under greenhouse conditions (Walbot and Coe 1979; Siemenroth et al. 1980). Under such conditions, other albino mutants that have been known to be blocked in the biosynthetic pathway of carotenoids tend to lose plastid ribosomes and internal membranes by photodamage (Bachmann et al. 1968; Reiss et al. 1983; Mayfield et al. 1986). To minimize possible photodamage, seedlings were germinated and grown in darkness for 5 d and were exposed for 1 d to dim light of 0.01 μmol photons · m^{-2} · s^{-1}. Under this condition, *w3,* which is known to be blocked in the carotenoid biosynthetic pathway, accumulates chlorophyll at the normal level (Mayfield et al. 1986). However, the chlorophylls of *ij1* and *w1* seedlings were barely detectable (data not shown). The carotenoid content was estimated from the absorbance at 480 nm using the equation of Britton and Goodwin (1971). Under the dim-light condition, the double-mutant seedlings, *ij1 l* and *w1 l,* accumulated carotenoids to 34.5 and 10.0 μg · mg^{-1} fresh leaf tissue, respectively, compared to 10 and 4.5 μg · mg^{-1} for the single mutants, *ij1* + and *w1* +. Under this condition, green seedlings contained 50–65 μg · mg^{-1} fresh leaf tissue. Depending on the genetic background, yellow seedlings usually contain two- to threefold higher carotenoid levels than white seedlings. However, there was little difference in the levels of carotenoids between the *w2* + and *w2 l* seedlings, 35.5 and 39 μg · mg^{-1} fresh leaf tissue, respectively. The original description of *w2* was as an albino mutant (Lindstrom 1924), but the *w2* + stock that we received from the Maize Genetic Stock Center showed strongly yellowish seedlings. Therefore, it is possible that the *w2* + stock contains another modifier or was maintained in the *l* genetic constitution.

To examine the possibility that a block in plastid gene expression is responsible for the deficiency of the pigments, and to obtain information on the possible roles of the nuclear genes *ij1, w1* and *w2* in plastid gene expression, the steady-state level and patterns of plastid transcripts were examined by Northern hybridization analysis. Total cellular RNAs were extracted from seedlings grown under the same conditions as those used for the pigment measurement. Since green stripes are not easily distinguishable from white tissues on the *ij1* seedlings grown under the dim light, RNAs for this study were extracted from maternal-exception seedlings obtained as described in *Materials and methods.* Plants having *w1, w2,* and *ij1* showed a 10 to 20-fold decrease in the level of 23S rRNA (Fig. 1). However, *ij1*-affected plastids contained little of the "breakdown" products of 23S rRNA.

Of the two different transcript sizes typical of the monocistronic gene *rbc*L (Crossland et al. 1984; Mullet et al. 1985), only the small processed transcript was detectable in *w1* and *ij1* (Fig. 2a) even after longer exposure to the film (data not shown). But the *w2* seedlings contained both transcripts. It is interesting that the relative amount of the unprocessed larger transcript in *w2* is slightly higher than that in the normal sibling.

The *psa*B gene of maize plastids, P700 chlorophyll a

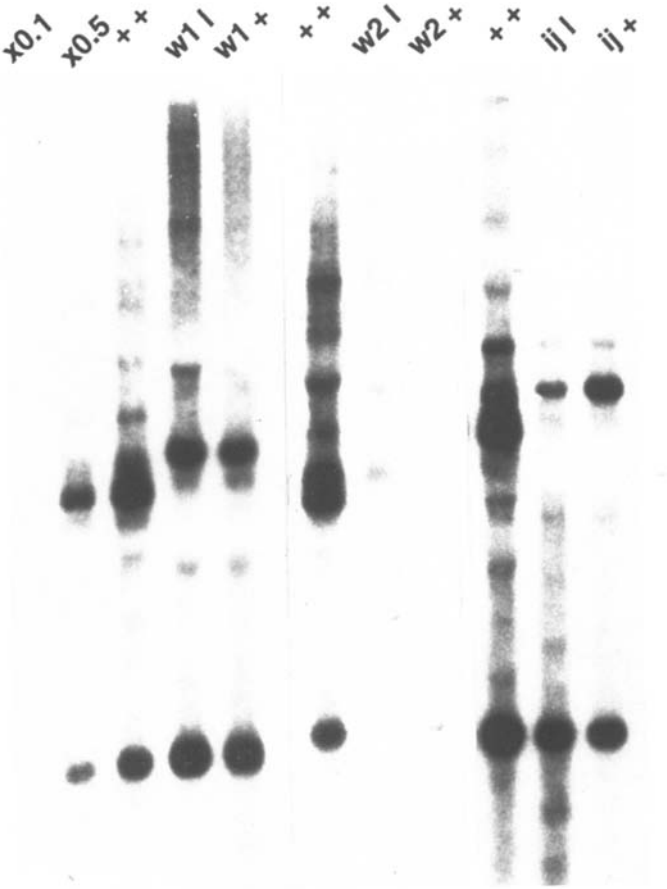

Fig. 5. Detection of transcripts from a *clp*P gene upstream from the 5' end of *psb*B by Northern blot. The probe is indicated in Fig. 3(a). The samples were loaded in the same way as in Fig. 1

apoprotein A2 of PSI, has been sequenced and contains no intron (Fish et al. 1985; Fish and Bogorad 1986). Compared to the RNA species of the normal siblings, *w1* and *ij1* contained a transcript of larger size (arrows in Fig. 2b) that accumulated at a much higher level than the transcript of normal size. But *w2* and its *l* double mutant showed the same pattern of RNA species as the normal seedlings, even though the level of the transcripts was similar to those of *w1* (Fig. 2b).

Among the genes in the *atp* operon (Cozens et al. 1986; Hudson et al. 1987; Barkan 1989), the transcripts from the *atp*A gene were examined (Fig. 2c). The *w1* +, *ij1*, and *w2* seedlings showed transcript patterns similar to those of the normal siblings. Interestingly, two additional hybridized bands were detected in the *w1 l* seedlings (arrows in Fig. 2c).

The monocistronic gene *psb*A, encoding the 32-kDa herbicide-binding polypeptide of PSII (Barkan et al. 1986; Mullet and Klein 1987), was examined by using a spinach probe. An extra transcript was detected with the probe in the *w1* and *ij1* seedlings (arrows in Fig. 2d). The longer transcript was accumulated at a level similar to or higher than that of the normal transcript.

To diagnose the expression of polycistronic transcription units in these mutants, the patterns of RNA species of the *psb*B operon were examined by using four different gene-specific probes (Fig. 3), *psb*B (51-kDa P680 chlorophyll a apoprotein), *psb*H (10 kDa phosphoprotein) of PSII, *pet*B (apocytochrome b–559) and *pet*D (subunit IV) of the cytochrome b6/f complex (Barkan 1988; Westhoff and Herrmann 1988). The transcripts that hybridized with the probes for *psb*B and *psb*H were barely detectable in all the mutants (Fig. 4a, b). But several transcripts of *pet*B and *pet*D were present at much higher levels (Fig. 4c, d) than the transcripts of *psb*B and *psb*H. The largest transcript in the mutants showed aberrant size. Therefore, the highly accumulated transcripts contained only the *pet*B and *pet*D genes but not the *psb*B and *psb*H genes. All three mutants showed almost the same patterns and levels of the transcripts containing *pet*B and *pet*D genes. Alteration in the size and level of transcripts from the *psb*B operon also has been found in the *hcf38* maize mutant (Barkan et al. 1986). However, the altered pattern of the transcripts seen in the *hcf38* mutant is different from that in these albino mutants.

Using the 1.2-kb DNA fragment upstream from the 5′ end of the *psb*B gene (including 150 bp of the coding region of the 5′ end of the *psb*B gene) as a probe (a in Fig. 3), the expression of the gene *clp*P was examined. Two abundant transcripts were detected in *w1* and *ij1* seedlings (Fig. 5). While the smaller transcripts showed similar size to the abundant transcript of normal siblings, the larger transcript was of aberrant size. Since all the *psb*B transcripts were barely detectable (Fig. 4a), the highly abundant transcripts did not contain the noncoding or coding strands of the *psb*B gene. In the case of

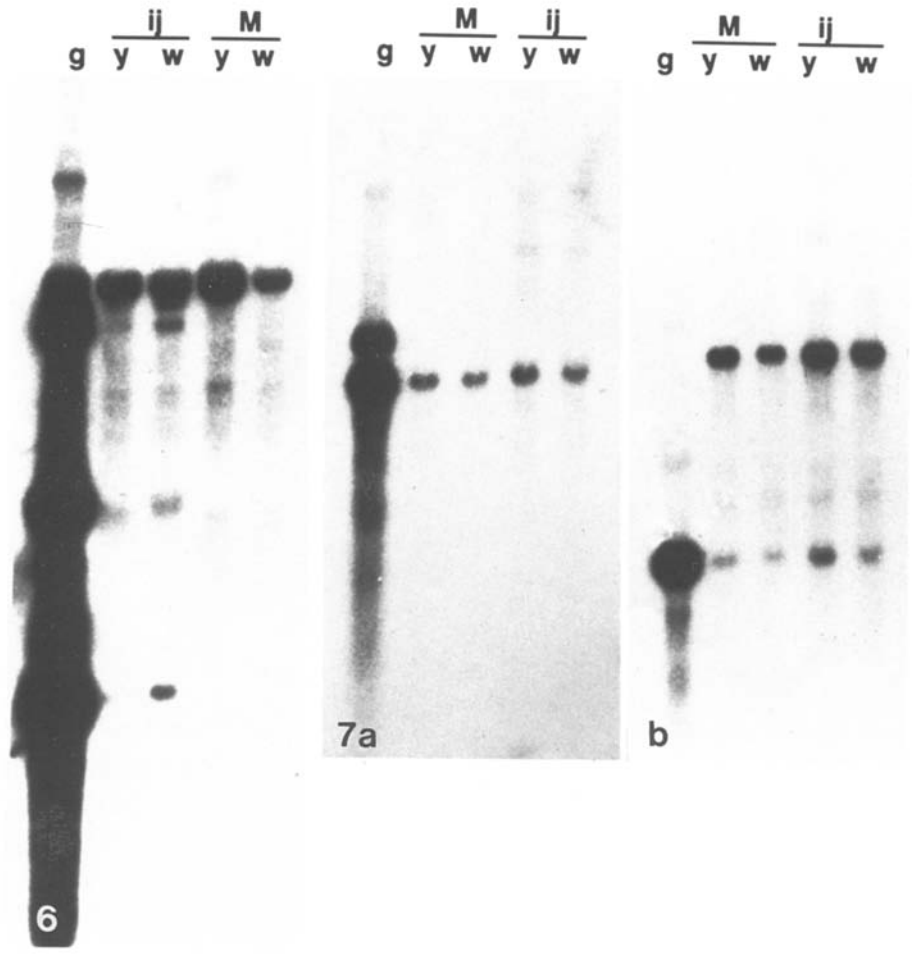

Fig. 6. Detection of 23S plastid rRNA in *ij1* vs. maternal-exception maize seedlings grown in the same sandbench of the greenhouse for 6 d. Based on inspection by unaided eye, only the pure white or yellow tissues on *ij1* striped leaves were considered as *ij1*-affected tissues and were used for extraction of total cellular RNAs. For maternal-exception seedlings, the whole (white) leaf tissues were used for RNA extraction. *Lane g*, normal seedlings. *Lanes y* and *w*, yellow and white *ij1* seedlings *(ij)*, respectively. *Lanes y* and *w*, yellow and white maternal-exception seedlings (*M*), respectively. Six μg of total cellular RNAs was loaded in each lane. The RNA sample of lane 4 was slightly overloaded

Fig. 7a, b. Detection of the *rbc*L (**a**) and *psb*A (**b**) transcripts. The maize seedlings and the growth conditions were as described in Fig. 6. The lanes are as described in Fig. 6

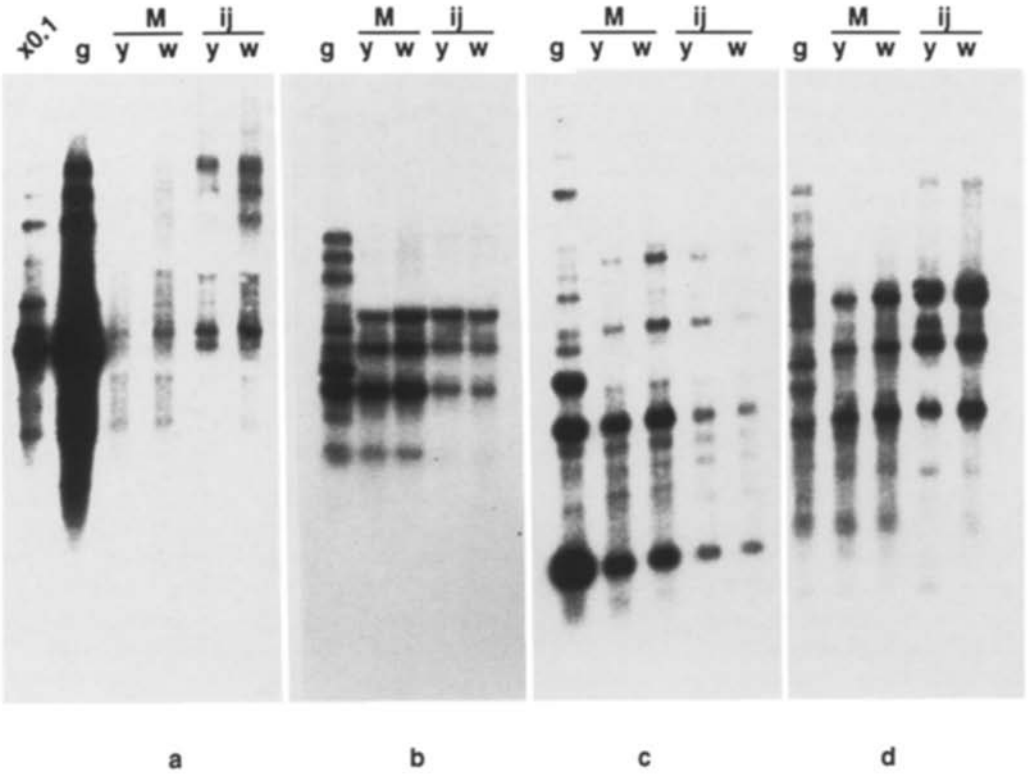

Fig. 8a–d. Northern analysis for the *psb*B (**a**), *pet*B (**b**), *pet*D (**c**), and an intron of *pet*B (**d**) transcripts in *ij1* vs. maternal-exception maize seedlings. The seedlings and the growth conditions were as described in Fig. 6. *Lane g*, normal seedlings. *Lanes y* and *w*, yellow and white maternal-exception seedlings (*M*), respectively. *Lanes y* and *w*, yellow and white *ij1* seedlings *(ij)*, respectively. The probes were as indicated in Fig. 3 (probe b, d, e, and f, respectively). Each lane was loaded with 6 μg of total cellular RNAs

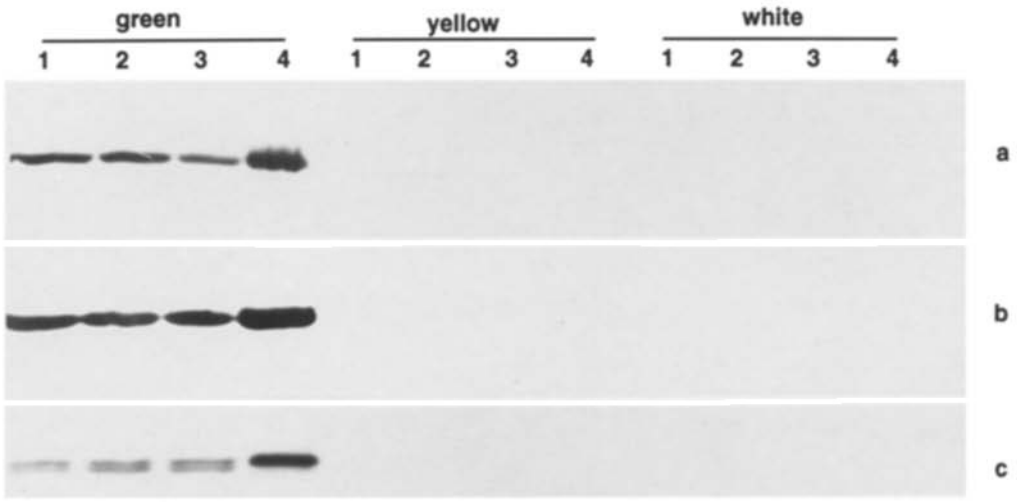

Fig. 9. Immunoblot analysis of CPI (apoprotein of PSI) (*a*), large subunit of Rubisco (*b*), and cytochrome f (*c*) from white and yellow maternal maize seedlings and normal siblings. The growth condition of the seedlings and the lanes in which the protein samples were loaded are described in Table 1. Leaf tissues were ground in 2 ml grinding buffer per g tissue. A 20 μl aliquot of the crude protein extract from the leaf tissues was loaded into each lane. *Green*, normal green sibling; yellow, *yellow* maternal-exception seedlings; *white*, white maternal-exception seedlings

w2, however, all the transcripts were present at very low levels (Fig. 5) just as the *psb*B transcripts were.

To examine whether such alteration in the level and the size of the transcripts in the *ij1*-affected plastid could be influenced by different environmental conditions and whether *ij1* seedlings show the same pattern of the transcripts as the maternal-exception seedlings, *ij1* and maternal-exception seedlings grown in the sandbench of the greenhouse were studied. Under this condition, *ij1* and maternal seedlings were still able to accumulate 23S rRNA (Fig. 6). While *ij1* accumulated "breakdown" products of 23S rRNA, maternal-exceptions did not. From the two different sizes of a monocistronic gene, *rbc*L, only the small transcript was detectable in both *ij1* and maternal-exception seedlings (Fig. 7a). The same patterns of *rbc*L transcripts were found in dim-light-grown *ij1* seedlings (Fig. 2a). For *psb*A, an extra transcript larger than the transcript of normal siblings was

detected in both *ij1* and maternal-exception seedlings (Fig. 7b). The same pattern of *psb*A transcripts was observed in maternal-exception seedlings grown in dim light (Fig. 2d).

Almost identical patterns of transcripts between *ij1* and its maternal-exceptions were observed in the transcripts of other plastid genes. Transcripts for *psb*B, *pet*B and *pet*D were examined and compared (Fig. 8a–c). Except that longer transcripts of *psb*B were present at a slightly higher level in *ij1* than in maternal-exception seedlings (Fig. 8a), the patterns of the transcripts of the *psb*B operon were almost identical between *ij1* and the maternal-exception seedlings. To obtain further information for the transcripts containing *pet*B and *pet*D, the DNA between *psb*H and the open reading frame of *pet*B (f in Fig. 3) was used as a probe. The level and pattern of the transcripts hybridizing with the probe (Fig. 8d) were similar to those with the *pet*B probe (Fig. 8b). Therefore, the transcripts that were abundantly present in *ij1* and maternal-exception seedlings were derived from only the *pet*B and *pet*D genes of the *psb*B operon. Also, the patterns of transcripts of *psa*B gene in *ij1* and maternal-exception seedlings were similar (data not shown).

We conclude that aberration in the patterns of transcripts of plastid genes of the *ij1*-affected plastids is persistent even after the *ij1* gene has been genetically restored. Also, there is little difference in the transcripts between *ij1* seedlings grown under dim light and ones grown in the greenhouse. Therefore, the aberrant patterns of the transcripts are not dependent upon the growth condition given to these seedlings.

Because the plastids affected by *w1* and *ij1* appear to undergo substantial alteration in RNA processing and stability, the possibility was examined whether the defective plastids are able to accumulate the nucleus- and plastid-encoded proteins. For this purpose, maternal-exception *ij1* seedlings were examined by Western analysis. The maternal-exception seedlings were grown under various light regimes to examine whether plastidic proteins are accumulated. The seedlings were grown from very dim light (0.01 μmol photons · m^{-2} · s^{-1} to high

Table 1. The light conditions for growth of maize seedlings from which protein samples were prepared

Lane	Condition
1[a]	5 d darkness + 6 h dim light[b]
2	5 d darkness + 1 d dim light
3	5 d darkness + 2 d dim light
4	5 d darkness + 2 d dim light + 1 d high light[c]

[a] The proteins for immunodetection (Figs. 9, 10) were extracted from the seedlings grown as described and loaded in lanes as numbered

[b] The photon fluence rate of the dim light was 0.01 μmol · m^{-2} · s^{-1} at the level of the seedlings

[c] The photon fluence rate of the high light was 100 μmol · m^{-2} · s^{-1} at the level of the seedlings

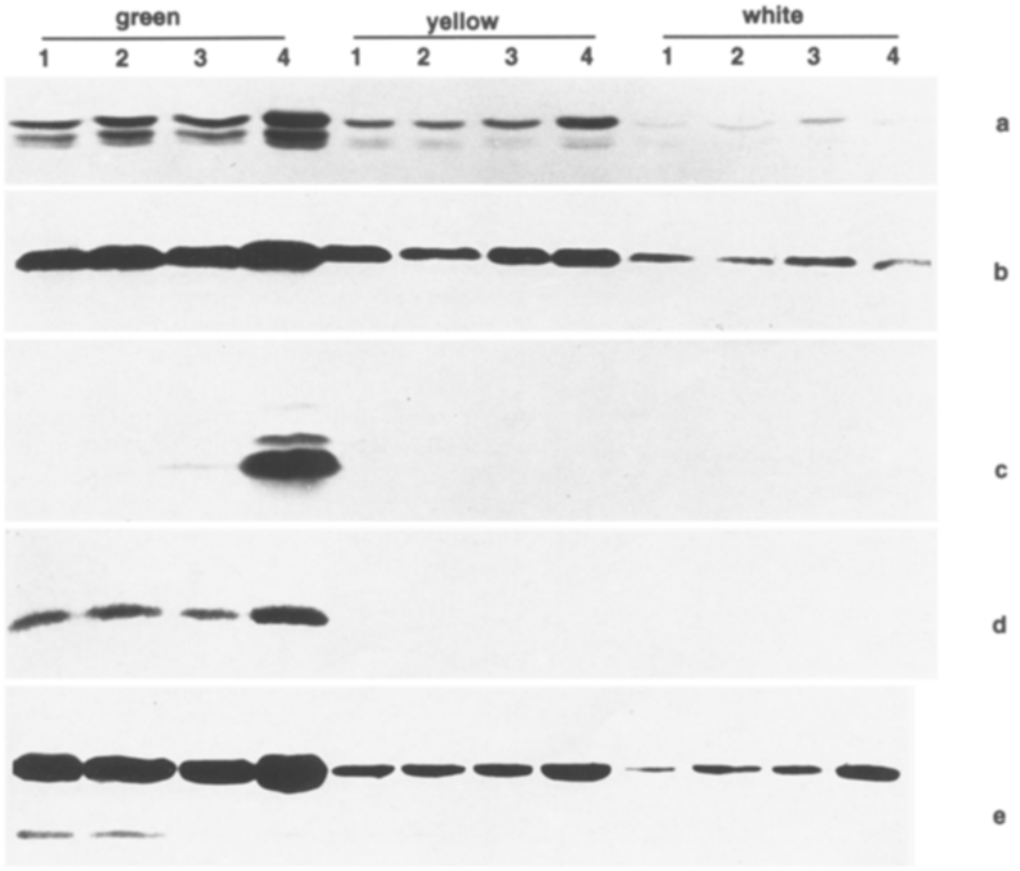

Fig. 10. Immunodetection of FNR (*a*), OEC–33 (*b*), LHCPII (*c*), Rieske protein (*d*) and phosphoendpyruvate carboxylase (*e*). The growth conditions of the seedlings and the lanes in which the protein samples were loaded are as described in Table 1 and Fig. 9

light (100.0 μmol photons · m^{-2} · s^{-1}). Total cellular proteins were prepared sequentially from the leaf tissues of yellow, white, and normal seedlings grown under the different light regimes (Table 1). The following three plastid-encoded proteins were examined by the immunoblotting assay: cytochrome f, CPI (apoprotein of PSI), and large subunit of Rubisco. All three failed to accumulate under any growth condition in yellow or white seedlings (Fig. 9). These results are consistent with the report that *ij1*-affected plastids suffer from ribosome loss or depletion of internal membrane (Walbot and Coe 1979; Thompson et al. 1983).

Using the same immunoblotting technique, the accumulation of the nuclear-encoded proteins was monitored. The light-harvesting chlorophyll a/b protein of PSII (LHCPII) and Rieske protein, a nuclear component of the cytochrome b6/f complex, were not detectable in white or yellow seedlings (Fig. 10c, d). However, OEC–33 and FNR protein accumulated at reduced levels in yellow and white seedlings (Fig. 10a, b). Interestingly, phosphoenolpyruvate carboxylase, localized in the cytosol, was also reduced (Fig. 10e). The reduction in the level of the enzyme could be an explanation for the previous report that the activity of the enzyme was decreased 60% in the white seedlings (Siemenroth et al. 1980). The levels of the proteins in yellow seedlings were slightly higher than in white seedlings. No proteins of aberrant size were detected.

To understand whether the aberrant patterns and the severe reduction of the transcripts are, in part, due to the shortage of plastid DNA templates in the mutants, the relative content of the plastid DNA was measured by Southern blot analysis. Total cellular DNAs were extracted from the seedlings grown under the conditions previously described. *Bam*HI-digested total cellular DNAs were probed at the same time with the nuclear rDNA, pGmr1, and 1.6-kb plastid *Bam*HI fragment containing *psa*B. The signal intensities of the autoradiogram exposed without an intensifying screen were measured with a densitometer. After normalization with the hybridized signals of the nuclear rDNA (a in Fig. 11), the relative contents of the plastid DNAs of the mutants were compared with those of the normal siblings. There was no difference in the contents of the plastid DNAs among the *w1*, *ij1*, and normal seedlings (b in Fig. 11). However, the relative plastid DNA level of *w2* was only around 5% of that of normal siblings (b in Fig. 11).

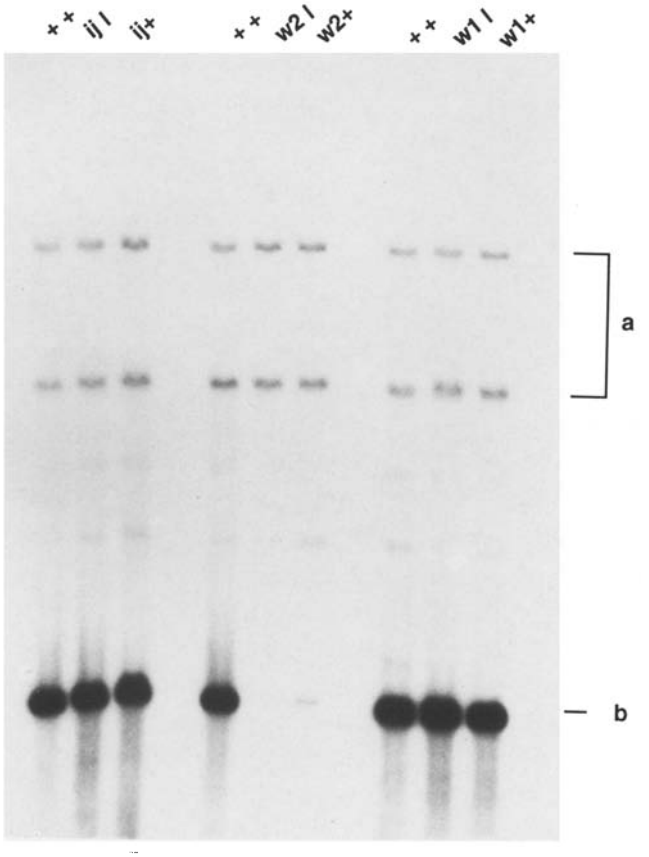

Fig. 11. Southern analysis of the nuclear rDNAs and 1.6-kb plastid DNA containing *psa*B of *w1*, *w2*, and *ij1* maternal-exception maize seedlings. The seedlings were exposed to very dim light (0.01 μmol · m^{-2} · s^{-1}) for 1 d after 5 d darkness. Five μg of total cellular DNAs digested with *Bam*HI was loaded in each lane. The probes for both the nuclear rDNA (pGmr1) and plastid DNA (Bam 21′ fragment) were hybridized at the same time. *a*, *b*, Hybridization signals of the nuclear rDNA and plastid Bam 21′ DNA, respectively. The skewed alignment of the plastid DNA hybridization bands is due to the condition of the gel in electrophoresis

Discussion

The mutants, *ij1*, *w1*, and *w2*, are able to accumulate carotenoids and chlorophylls even though at a low level. The levels and sizes of transcripts or the amount of DNA are substantially altered in the plastids affected by the mutations. These data suggest that albinism of the monogenic mutants is a consequence of the blocking of plastid genomic expression rather than due to a direct genetic lesion in the biosynthetic pathway of the pigments.

The *l* recessive gene specifically interacts with the mutants *w1* and *w2* (Robertson 1975) and striping mutants like *ij1* (iojap 1) (C–d. Han and E.H. Coe, unpublished data, 1988, Maize Genet. Coop. Newsl. 62) in increasing the level of carotenoids. However, *l* does not interact with the albino mutants that have been shown to be blocked in the carotenoid biosynthetic pathway of carotenoids. Therefore, action of the *L* gene appears to be related to the accumulation or stability of the carotenoids. Our data show that there is little difference in the sizes of transcripts of plastid genes between a mutant and its double mutants with *l*. However, it is interesting to note that the additional transcripts of the *atp*A gene are detected only in the *w1 l* genotype, not in *w1* + (Fig. 2c). This is a rather exceptional case because the rest of the transcripts we examined show no difference between the two genotypes. Some of the plastid transcripts of *w2* and some of the nucleus-encoded proteins of *ij1* are accumulated at a higher level in their *l* double mutants. Therefore, the effect of *l* on the genomic expression of plastids affected by the mutants is most likely quantitative.

The distinct characteristic of *ij1*- and *w1*-affected plastids is abberation both in the sizes and levels of the

transcripts of the genes. First of all, the relative levels of transcripts of different plastid genes are highly variable; transcripts of the gene *clp*P upstream from *psb*B are present up to normal levels (Fig. 5), while *psb*B transcripts are barely detectable (Fig. 4a). Secondly, the transcript patterns from individual genes of the affected plastids are different from those in normal siblings; for example, the unprocessed larger transcript of *rbc*L is not detectable but the processed one is present, at a low level (Fig. 2a). Further, compared to levels of transcripts of normal sizes, transcripts of aberrant sizes are accumulated to similar levels or even higher (e.g., Fig. 5 for the gene, *clp*P, located upstream from *psb*B, Fig. 2b for *psb*A, and Fig. 2d for *psa*B). Thirdly, there appear to be no common characteristics among the genes that contain the aberrant transcripts; the accumulation or generation of aberrant transcripts is not dependent upon *1*) whether the gene contains introns or not [e.g., *pet*B or *pet*D (Fig. 4c, d) vs. *psa*B (Fig. 2b)], 2) mono-or polycistronic genes [e.g., *psb*A (Fig. 2d) vs. *pet*B or *pet*D (Fig. 4c, d)], or 3) the orientation of the transcription of the genes, based on the map of maize plastid DNA (S. Rodermel and L. Bogorad, personal communication, 1990, Maize Genet. Coop. Newsl. 64).

These observations suggest that the *ij1*- and *w1*-affected plastids might have altered transcriptional or post-transcriptional processing. Recent studies indicate that transcription of the plastid genes is somewhat differentially regulated in leaf tissues (Deng and Gruissem 1987; Deng et al. 1987; Klein and Mullett 1987). Without data on the transcription activity of the plastid genes in these mutants, it is very difficult to estimate how much the variation in the level of the transcripts results from alteration in the transcription activities of individual genes or operons. However, the levels of the transcripts among the genes are dramatically different, from the normal level to undetectable level. Overall effects of the *ij1* and *w1* mutants on the transcripts of the plastid genes and the plastids (e.g., loss of ribosomes or deposition of pigments) could be, at least in part, due to alteration in the post-transcriptional processes (processing and stability) and their subsequent alteration. The possibility is not ruled out that the affected plastids could generate antisense transcripts.

Because preferential accumulation of some of the transcripts of *pet*B and *pet*D, and transcripts of aberrant size were also observed in *w2*, which shows normal RNA patterns for the rest of the plastid genes examined, alteration of the expression of the *psb*B operon appears to be a phenomenon common to plastids with limited genomic expression. Since the diverse RNA species from this operon are generated mainly by complex post-transcriptional processes from the primary transcripts (Barkan 1988; Westhoff and Herrman 1988), it is appealing to consider the possibility that some of the transcripts undergo turn-over processes different from other transcripts.

While the general patterns of the transcripts in *w2* + and *w2 l* (except for some of the *psb*B operon) are not altered and are comparable to those of their normal siblings, the levels of the transcripts decrease 10 to 20-fold. The distinct characteristic of *w2* is severe reduction of the relative plastid DNA content, that is, 20-fold lower than in normal seedlings. Since there is good correlation between the relative plastid DNA contents and the level of the transcripts in the *w2* mutant, it is reasonable to argue that the low levels of the transcripts in *w2* + and *w2 l* are, at least in part, due to the shortage of DNA templates. Based on light-microscopic observations, the size of the cells and number of plastids per cell of *w2* seedlings do not decrease to this degree (data not shown). Therefore, shortage of the DNA templates is due to severe reduction of the genomic copy number per plastid, not due to decrease in the plastid number per cell.

Taking into account the fact that the copy number of plastid genomes per cell and the plastid population increase during leaf formation and maturation of the leaf tissues (Lamppa and Bendich 1979; Boffey and Leach 1982; Lawrence and Possingham 1986), the significance and role of the multiplicity of plastid genomes in the expression and regulation of the plastid genomes remains puzzling. A possible explanation for the multiplicity of the plastid genome has been proposed in terms of cellular demand for plastid ribosomes during leaf development (Bendich 1987). However, Deng and Gruissem (1987) found a five fold decrease in transcription per molecule of chloroplast DNA during maturation of spinach leaves. Also, it has been reported that the basal region of barley leaves that shows a 10-fold increase in transcription activity per plastid increases the DNA copy number only around 1.5-fold (Baumgartner et al. 1989). In *Chlamydomonas,* a fourfold decrease in chloroplast DNA per cell does not affect accumulation of chloroplast proteins and fixation of CO_2 (Hosler et al. 1989). Even though it still remains to be determined what basal copy number of plastid genomes is necessary for plastid expression, our study on *w2* indicates that a high copy number of the plastid genome is essential for plastid function during leaf development. Also, it is possible that the *W2* gene might be involved, directly or indirectly, in the processes of plastid DNA replication. This is the first mutant in higher plants that shows the effect of deficiency of plastid genomes on expression of plastids.

The failure to detect plastid-encoded proteins even under dim-light regimes might be explained by the depletion of ribosomes and internal membranes characteristic of *ij1*-affected plastids (Walbot and Coe 1979; Thompson et al. 1983). This study shows that the ribosome loss does not result from photodamage, but is one of the characteristics of the plastids affected by *ij1*. Also, this result is consistent with the previous report that Rubisco holoenzyme and ATPase are not detectable (Siemenroth et al. 1980). Neither the plastid-encoded cytochrome f nor the nucleus-encoded Rieske protein were accumulated in the defective plastids. Lack of the nucleus-encoded protein that is associated with thylakoid membranes could be explained, at least in part, by the absence of the corresponding plastid-encoded proteins. It has been documented that the other components fail to be accumulated when one component is missing in a thylakoid protein complex, especially in PSII (Leto et al. 1985; Rochaix and Erickson 1988). The absence of

Rieske protein indicates that a similar mechanism (e.g., proteolytic activity) as postulated for PSII might be involved in the coordinate accumulation of the cytochrome f/b6 complex. In contrast, the extrinsic nuclear proteins, FNR and OEC–33, which are localized outside and inside the thylakoid membranes, are accumulated. Detection of OEC–33 supports the idea that the accumulation of the protein is independent from accumulation of the core proteins of PSII, most of which are encoded by plastid genomes (Sutton et al. 1987). It is interesting to note that yellow seedlings contain a higher level of the proteins than the white seedlings do. The further accumulation of the proteins could be, at least, explained by the protective role of the carotenoids against deleterious factors (e.g., free radicals) that are expected to be present in the abnormal plastids (for review, see Rau 1988; Goodwin 1980). Detection of comparable levels of plastid DNA contents in the yellow and white seedlings of *ij1* and *w1* (Fig. 11) implies that the affected plastids maintain the mechanism for replication or multiplication of the plastid genomes. Taken together, the irreversibly altered plastids appear to maintain the normal transport or processing of the nuclear encoded proteins.

The failure to restore the function of the affected plastids in the subsequent generation has been explained by loss of ribosomes that leads to virtual loss of the translational activity of the defective plastids (for review, see Börner and Sears 1986). This hypothesis has been proposed based on the observation that rRNAs and plastid proteins were not detectable (Walbot and Coe 1979; Siemenroth et al. 1980). However, we found that the *ij1* seedlings and maternal-exception seedlings not only contain transcripts of plastid genes but also show almost identical patterns of plastid transcripts. Therefore, the 'ribosome-less' condition is merely one of the characteristics of the *ij1*-affected plastids. The action of *ij1*, whatever it might be, results in persistent aberration of transcripts even after normal function of the *ij1* gene has been genetically restored. Since sequencing data and biochemical studies indicate that subunits of RNA polymerase of plastids are encoded by the plastid genes (Little and Hallick 1988; Sugiura 1989), accumulation of the transcripts appears to be contradictory to the absence of plastid-encoded proteins and failure of detection of ribosomes that was shown in previous studies (Walbot and Coe 1979; Siemenroth et al. 1980; Thompson et al. 1983). But it is not difficult to imagine that a limited number of ribosomes, which might result from the alteration in plastid genomic expression during transcription or post-transcriptional processing, could be diluted out, before thylakoid membranes are elaborated, mainly because of the rapid cell division and elongation of the developing leaf organ. Recently, it has been suggested that there might be a functional non-chloroplast-encoded RNA polymerase (Hess et al. 1993). Therefore, it is possible that the transcripts present in the *ij1*-affected plastids might be generated by a putative nuclear-encoded RNA polymerase.

Our work on the *ij1*, *w1* and *w2* mutants demonstrates that a monogenic nuclear mutant could affect overall patterns of the transcripts of the plastid genes or could dramatically decrease the level of plastid genomic DNA. Furthermore, we show that the alteration in genomic expression of the *ij1*-affected plastids persists in the subsequent generation even after the *ij1* gene is replaced by the normal allele. The mutants seem to be involved in fundamental and common steps in the expression of the chloroplast genes. Further detailed analysis is necessary to elucidate the primary lesion of the mutations in the expression of the plastids. Mutants like *ij1*, *w1* and *w2*, and access to the genes, should provide valuable opportunities to dissect the complex mechanisms underlying the concerted interaction between the nuclear and plastid genes during chloroplast development. Further understanding of the action of the *ij1* gene and the heritable aberration of the transcripts may be advanced by studies using the clone of the *ij1* gene obtained through a transposon-tagging strategy (C-d. Han and E.H. Coe, 1989 and 1990, unpublished data, Maize Genet. Coop. Newsl., 63 and 64; Han et al. 1992).

References

Bachmann, M.D., Robertson, D.S., Bowen, C.C., Anderson, I.C. (1968) Chloroplast development in pigment deficient mutants of maize. J. Ultrastruct. Res. **21,** 41–60

Barkan, A. (1988) Proteins encoded by a complex chloroplast transcription unit are each translated from both monocistronic and polycistronic mRNAs. EMBO J. **7,** 2637–2644

Barkan, A. (1989) Tissue-dependent plastid RNA splicing in maize: transcripts from four plastid genes are predominantly unspliced in leaf meristems and roots. Plant Cell **1,** 437–445

Barkan, A., Miles, D., Taylor, W.C. (1986) Chloroplast gene expression in nuclear, photosynthetic mutants of maize. EMBO J. **5,** 1421–1427

Baumgartner, B.J., Rapp, J.C., Mullet, J.E. (1989) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol. **89,** 1011–1018

Bendich, A.J. (1987) Why do chloroplasts and mitochondria contain so many copies of their genome? BioEssays **6,** 279–282

Boffey, S.A., Leech, R.M. (1982) Chloroplast DNA levels and the control of chloroplast division in light-grown wheat leaves. Plant Physiol. **69,** 1387–1391

Börner, T., Sears, B.B. (1986) Plastome mutants. Plant Mol. Biol. Rep. **4,** 69–92

Britton, G., Goodwin, T.W. (1971) Biosynthesis of carotenoids. Methods Enzymol. **18,** 654–700

Coe, E.H., Jr., Neuffer, M.G., Hoisington, D.A. (1988a) The genetics of corn. In: Corn and corn improvement, pp. 81–258. Sprague, G.F., Dudley, J., eds. American Society of Agronomy, Madison, USA

Coe, E. H., Jr., Thompson, D. L., Walbot, V. (1988b) Phenotypes mediated by the iojap genotype in maize. Am. J. Bot. **75,** 634–644

Cozens, A.L., Walker, L.E., Phillips, A.L., Huttly, A.K., Gray, J.C. (1986) A sixth subunit of ATP synthase, an Fo component, is encoded in the pea chloroplast genome. EMBO J. **5,** 217–222

Crossland, L.D., Rodermel, S.R., Bogorad, L. (1984) Single gene for the large subunit of ribulose bisphosphate carboxylase in maize yields two differentially regulated mRNAs. Proc. Natl. Acad. Sci. USA **81,** 4060–4063

Demerec, M. (1923) Inheritance of white seedlings in maize. Genetics **8,** 561–593

Deng, X.-W., Gruissem, W. (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. Cell **49,** 379–387

Deng, X.-W., Stern, D.B., Tonyn, J.C., Gruissem, W. (1987) Plastid run-on transcription: application to determine the transcriptional regulation of plastid genes. J Biol. Chem. **262,** 9641–9648

Fish, L.E., Bogorad, L. (1986) Identification and analysis of the maize P700 chlorophyll a apoproteins PSI–A1 and PSI–A2 by high pressure liquid chromatography analysis and partial sequence determination. J. Biol. Chem. **261,** 8134–8139

Fish, L.E., Kuck, U., Bogorad, L. (1985) Two partially homologous adjacent light inducible maize chloroplast genes encoding polypeptides of the P700 chlorophyll a protein complex of photosystem I. J. Biol. Chem. **260,** 1413–1421

Goodwin, T.W. (1980) The biochemistry of the carotenoids, 2nd edn., vol I. Chapman and Hall, London

Han, C-d., Coe, E.H., Martienssen, R.A. (1992) Molecular cloning and characterization of *ij (iojap),* a pattern striping gene of maize. EMBO J. **11,** 4037–4046

Hess, W.R., Promona, A., Fielder, B., Subramanian, A.R., Börner, T. (1993) Chloroplast *rps*15 and the *rpo*B/C1/C2 gene cluster are strongly transcribed in ribosome-deficient plastids: evidence for a functioning non-chloroplast-encoded RNA polymerase. EMBO J. **12,** 563–572

Hosler, J.P., Wurtz, E.A., Harris, E.H., Gillham, N.W., Boynton, J.E. (1989) Relationship between gene dosage and gene expression in the chloroplast of *Chlamydomonas reinhardtii.* Plant Physiol. **91,** 648–655

Hudson, G.S., Mason, J.G., Holton, T.A., Koller, B., Cox, G.B., Whitfeld, P.R., Bottomley, W. (1987) A gene cluster in the spinach and pea chloroplast genomes encoding one CF1 and CFO subunits of the H+-ATP synthase complex and the ribosomal protein S2. J. Mol. Biol. **196,** 283–298

Kirk, J.T.O., Tilney-Bassett, R.A.E. (1978) The plastids. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands

Klein, R.R., Mullet, J.E. (1987) Control of gene expression during higher plant chloroplast biogenesis. J. Biol. Chem. **262,** 4341–4348

Knoth, R., Hagemann, R. (1977) Structure and function of the genetic information in plastids: XVI. The ultrastructure of plastids and the electron microscopic proof of mixed cells in leaves of plastome mutants induced by the gene mutation albostrians of *Hordeum vulgare* L. Biol. Zentralbl. **96,** 141–150

Lamppa, G., Bendich, A. (1979) Changes in chloroplast DNA levels during development of pea *(Pisum sativum).* Plant Physiol. **64,** 126–130

Lawrence, M.E., Possingham, J.V. (1986) Microspectrofluorometric measurement of chloroplast DNA in dividing and expanding leaf cells of *Spinacea oleracea.* Plant Physiol. **81,** 708–710

Leto, K.J., Bell, E., McIntosh, L. (1985) Nuclear mutation leads to an accelerated turnover of chloroplast-encoded 48 kd and 34.5 kd polypeptides in thylakoids lacking photosystem II. EMBO J. **4,** 1645–1653

Lindstrom, E.W. (1924) Complementary genes for chlorophyll development in maize and their linkage relations. Genetics **9,** 305–326

Little, M.C., Hallick, R.B. (1988) Chloroplast *rpo*A, *rpo*B and *rpo*C genes specify at least three components of a chloroplast DNA-dependent RNA polymerase active in tRNA and mRNA transcription. J. Biol. Chem. **263,** 14302–14307

Mayfield, S.P., Nelson, T., Taylor, W.C., Malkin, R. (1986) Carotenoid synthesis and pleiotropic effects in carotenoid-deficient seedlings of maize. Planta **169,** 23–32

Mullet, J.E., Klein, R.R. (1987) Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. EMBO J. **6,** 1571–1579

Mullet, J.E., Orozco, E.M., Chua, N.H. (1985) Multiple transcripts for higher plant *rbc*L and *atp*B genes and localization of the transcription initiation site of the *rbc*L gene. Plant Mol. Biol. **4,** 39–54

Rau, W. (1988) The function of carotenoids other than in photosynthesis. In: Plant pigments pp. 231–255, Goodwin T.W., ed. Academic Press, New York, NY

Redei, G.P. (1973) Extra-chromosomal mutability determined by a nuclear gene locus in *Arabidopsis.* Mut. Res. **18,** 149–162

Reiss, T., Bergeld, R., Link, G., Thien, W., Mohr, H. (1983) Photooxidative destruction of chloroplasts and its consequences for cytosolic enzyme levels and plant development. Planta **159,** 518–528

Rhoades, M.M. (1943) Genic induction of an inherited cytoplasmic difference. Proc. Natl. Acad. Sci. USA **29,** 327–329

Robertson, D.S. (1975) Survey of the albino and white endosperm mutants of maize: Their phenotypes and gene symbols. J. Hered. **66,** 67–74

Robertson, D.S., Anderson, I.C., Bachmann, M.D. (1978) Pigment-deficient mutants: genetic, biochemical, and developmental studies. In: Maize breeding and genetics pp. 461–494, Walden, D.B., ed. John Wiley & Sons, Inc., New York

Rochaix, J.-D., Erickson, J. (1988) Function and assembly of photosystem II: genetic and molecular analysis. Trends Biochem. Sci. **13,** 56–59

Rock, C.D., Barkan, A., Taylor, W.C. (1987) The maize plastid *psb*B-*psb*F-*pet*B-*pet*D gene cluster: spliced and unspliced *pet*B and *pet*D RNAs encode alternative products. Curr. Genet. **12,** 69–77

Siemenroth, A., Börner T., Metzger, U. (1980) Biochemical studies on the iojap mutant of maize. Plant Physiol. **65,** 1108–1110

Stroup, D. (1970) Genic induction and maternal transmission of variegation in *Zea mays.* J. Hered. **61,** 139–141

Sugiura, M. (1989) The chloroplast genome. In: The Biochemistry of plants. A comprehensive treatise. vol. 15, pp. 133–149, Marcus, A., ed. Academic Press, New York, NY

Sutton, A., Sieburth, L.E., Bennett, J. (1987) Light-dependent accumulation and localization of photosystem II proteins in maize. Eur. J. Biochem. **164,** 571–578

Thompson, D.L., Walbot, V., Coe, E.H., Jr. (1983) Plastid development in iojap and chloroplast mutator-affected maize plants. Am. J. Botany **70,** 940–950

Walbot, V., Coe, E.H., Jr. (1979) Nuclear gene iojap conditions a programmed change to ribosome-less plastids in *Zea mays.* Proc. Natl. Acad. Sci. USA **76,** 2760–2764

Westhoff, P., Herrmann, R.G. (1988) Complex RNA maturation in chloroplasts: the *psb*B operon from spinach. Eur. J. Biochem. **171,** 551–564