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Nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays*

(chloroplast DNA/chloroplast ribosome/differentiation/meristem/leaf development)

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ABSTRACT **The recessive nuclear gene *iojap* of *Zea mays* conditions a permanent, heritable deficiency in the ability of the plastid to differentiate. *iojap*-affected plastids contain a normal genome as evidenced by comparison of the restriction endonuclease digestion patterns of affected and normal plastids. *iojap*-affected plastids contain neither detectable ribosomes nor high molecular weight RNA; the affected plastids do not incorporate exogenous amino acids into protein. The lesion in plastid ribosome content occurs early in organ ontogeny because *iojap*-mediated albino stripes can occupy entire clones within affected leaves.**

In most higher plants such as corn, chloroplasts are inherited uniparentally through the maternal line, whereas a number of genes controlling plastid ontogeny and pigment composition show Mendelian inheritance (1). Homozygous (*ij/ij*) plants typically produce green and white sectored leaves in which the green stripes are apparently normal (2). The albino plastids produced in one generation can persist in subsequent generations in a heterozygous (+/*ij*) or normal (+/+) nuclear background (2). The action of *iojap* results in a permanent change in the ability of the plastid to differentiate to a normal, green organelle. The basis of the chloroplast defect is unknown. Electron microscopy of *iojap*-affected tissue demonstrated only rudimentary, proplastid-like organelles, apparently lacking ribosomes but containing fibrils of DNA-like material (3). This phenotype suggests two alternative explanations of *iojap* action: mutator effect changing the chloroplast DNA, or a permanent loss of plastid ribosomes without change in chloroplast DNA.

MATERIALS AND METHODS

Plant Material. Seedlings were grown in vermiculite at 25°C for 10 days. Plastids were isolated from normal green seedlings (+/+ and +/*ij*) and sibling *iojap*-affected (*ij/ij*) plants resulting in F_2 from +/*ij* plants carrying genetic modifiers that confer extreme expression. White tissues generated in this extreme background with *iojap* during seedling development normally occupy >95% of the affected tissue. The nuclear background of these seedlings was 50% inbred line Oh51a. In addition, whole albino plants (+/*ij*) produced from the cross ♀*ij/ij* × ♂+/+ have been compared to green siblings (+/*ij*); in this case albinism is the result of the presence of the *iojap* gene during the previous maternal generation.

Plastid and Nucleic Acid Isolation. Total nucleic acids were prepared from destarched leaves by phenol extraction (4) or by CsCl extraction of protein and lipid (5).

Purified organelles were prepared from 8 to 12-day-old destarched corn plants at the third leaf stage. One hundred to 500 g of leaf tissue was harvested, chopped into 1-cm pieces, and sterilized by rinsing with 5% Clorox for 15 min followed by three rinses with sterile water. The tissue was then chilled to 4°C by mixing with 5 vol of homogenization medium [0.33 M sorbitol/50 mM Tris/30 mM KCl/10 mM $MgCl_2$/5 mM EDTA/5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid/1 mM mercaptoethanol/0.1% bovine serum albumin, pH 8] and blended by five high-speed bursts of approximately 1 sec duration in a prechilled Waring Blendor. To prepare green chloroplasts, the homogenate was filtered through 80-μm nylon net and centrifuged at 400 × *g* in a Sorvall HS-4 rotor to remove nuclei and debris; the chloroplasts were recovered by centrifugation at 2500 × *g*. Chloroplasts prepared through this step constituted partially purified plastids. For further purification, chloroplasts were resuspended (1 ml/100 g initial weight of plant material) in 50 mM Tris/10 mM $MgCl_2$/0.3 M sucrose, pH 8, and incubated for 1 hr at 4°C with DNase I (50 μg/ml). At the end of the incubation the plastids were washed by centrifugation at 3000 × *g* for 10 min through a 10-ml pad of 50 mM Tris/10 mM $MgCl_2$/10 mM EDTA/0.45 M sucrose, pH 8, in a 30-ml Corex centrifuge tube. The pellet was resuspended in 50 mM Tris/10 mM $MgCl_2$/10 mM EDTA/0.3 M sucrose, pH 8, and the washing procedure was repeated twice more. The final pellet was lysed by resuspension in 2% Sarkosyl/50 mM Tris/10 mM $MgCl_2$/10 mM EDTA, pH 8, and the lysate was extracted twice with an equal volume of phenol/0.1 M Tris, pH 9.

Etioplasts, *iojap*-affected plastids, and mitochondria were prepared by a similar procedure. After the second wash the plastids were separated from mitochondria on a sucrose step gradient (6).

Plastid purity was determined by coincidence with triose phosphate isomerase activity (7), and mitochondrial purity was followed by assaying for NADH cytochrome *c* reductase (8). Organelle preparations were also assayed for chlorophyll (9) and protein (10) content.

Agarose Gel Electrophoresis and Restriction Endonuclease Digestion. *Eco*RI (gift of M. Smith), *Bam* I (Bethesda Research Laboratories, Rockville, MD), and *Sal* I (BRL), were used as described by Bedbrook and Bogorad (11). Agarose slab gels (0.75 or 0.85%; 0.4 × 14 × 14 cm) were prepared, run, and stained with ethidium bromide (11) and photographed with Kodak high-contrast copy or Tri-X sheet film, the gel being placed directly on a UV Products transilluminator. Band mobility and stoichiometries were determined by scanning photographs (Tri-X sheet film) of each gel track with a Gilford spectrophotometer fitted with a linear transport apparatus; the area under each peak was determined with a Numonics digitizer. Plasmid DNAs were prepared from *Pseudomonas* spp. and *Acinetobacter* spp. cultures (gifts of R. Colwell), as described by Hansen and Olsen (12). Total organelle nucleic acids were displayed on composite 1% agarose/3% acrylamide/0.2% bisacrylamide slab gels run in 100 mM Tris/20 mM EDTA/0.25% sodium dodecyl sulfate ($NaDodSO_4$), pH 8.1.

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

Polyribosome Isolation. Whole-cell polyribosomes were prepared as described by Beachy *et al.* (13) from macerated leaves frozen in liquid N_2. Organellar polyribosomes were prepared from purified organelles lysed in 1% Sarkosyl and then frozen in liquid N_2 and extracted as from whole leaves. Ribosomal RNAs were prepared by phenol extraction of pellets resuspended in 50 mM Tris/10 mM EDTA/25 mM $MgCl_2$/1% $NaDodSO_4$, pH 8, followed by ethanol precipitation; rRNAs were electrophoresed on 2.4% acrylamide/0.15% bisacrylamide gels as described by Loening (4).

Amino Acid Incorporation Studies. Light- and ATP-stimulated incorporation of amino acid into protein by intact plastids were measured (14, 15) in 0.4-ml reaction mixtures containing 500 μg of plastid protein, 5 mM ATP, 40,000 units of T1 RNase, 5 μg of boiled RNase A, and 1 μCi (1 Ci = 3.7×10^{10} becquerels) of ^{3}H-labeled mixed amino acids (New England Nuclear). The reaction was initiated by addition of the labeled amino acids; 50-μl (63 μg of protein) aliquots were removed at zero time and at 5-min intervals thereafter. The plastids in the aliquot were lysed by addition of Triton X-100 to 2% (vol/vol) and then the samples were spotted onto paper discs processed to remove unincorporated amino acids as described by Mans and Novelli (16) but without the hot acid step. Amino acid uptake was monitored by removing a 5-μl aliquot of plastids after 20-min incubation and pelleting the plastids through 1 ml of 10% (wt/vol) sucrose/50 mM Tris, pH 8 in a Microfuge tube; uptake was determined by assaying the radioactivity in the plastid pellet after lysis in 2% Triton X-100.

Development Measurements. Field-grown *ij/ij* plants in various nuclear backgrounds (in segregating progenies from *ij* crossed or backcrossed with inbreds Ky21, K55, Tr, Mo17 and Oh51a) at the University of Missouri Genetics Farm in the summers of 1977 and 1978 were utilized to measure the extent (number of nodes affected) and width of individual *iojap* conditioned stripes.

RESULTS

Characterization of Plastids and DNA Content of Isolated Plastids. Plastids were prepared from normal green seedlings, etiolated seedlings in which normal plastid development was arrested, and *iojap*-affected albino seedlings. Plastid DNA isolated from each source had a mean (±SD) buoyant density of 1.699 ± 0.001 g/cm^3 after centrifugation to equilibrium in CsCl, as expected for corn chloroplast DNA (17); this result indicates that *iojap*-affected plastids contain plastid DNA and that no gross alterations of DNA composition occur.

Fragmentation of Plastid DNA by Restriction Nucleases. Fig. 1 shows the pattern of terminal digestion products of normal green and *iojap*-affected albino plastids produced by *Sal* I, *Bam* I, and *Eco*RI. The restriction digestion patterns of DNA from *iojap*-affected plastids and normal plastids were nearly identical. The *Sal* I fragments represent 100% of the chloroplast genome, the *Bam* I fragments represent approximately 80%, and the *Eco*RI fragments are about 60% (11). Molecular size of fragments was estimated by comparison with plasmid DNAs or λ DNA digested with *Eco*RI and compared with previous reports (11, 18). DNA of normal etioplasts yielded restriction patterns identical to those of Fig. 1 (lanes A, C, and F). The fragmentation patterns were nearly identical in stoichiometry and major size classes to the previously published plastid DNA restriction pattern of corn chloroplast DNA from a Texas male sterile cytoplasmic line (11); a minor difference was evident in *Bam* I fragment 5 which was slightly shorter in the plastid DNAs used in the current study. Because the plastids are maternally inherited in corn, it is not surprising to find minor differences in various cytoplasmic strains.

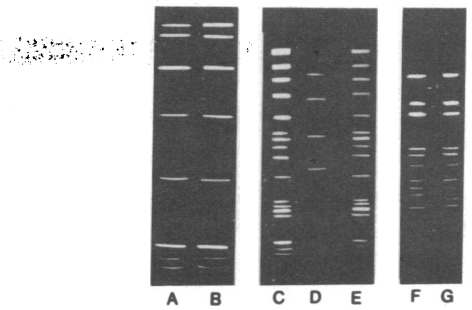

FIG. 1. Fractionation of restriction endonuclease fragments of chloroplast DNA. Lanes: A, green plastid (+/*ij*) DNA digested with *Sal* I, 0.75% agarose gel; B, *iojap*-affected plastid (+/*ij*), *Sal* I, 0.75% agarose; C, green plastid (+/*ij*), *Bam* I, 0.85% agarose; D, undigested plasmid DNAs of *Acinetobacter* (M_r 4.7 and 2.2×10^6) and *Pseudomonas* (M_r 6.6 and 3.2×10^6). E, *iojap*-affected plastid (+/*ij*), *Bam* I, 0.85% agarose; F, green plastid (+/*ij*), *Eco*RI, 0.85% agarose; G, *iojap*-affected plastid (+/*ij*), *Eco*RI, 0.85% agarose (M_r determinations for lane D, courtesy of R. Colwell).

Although the overall restriction patterns of normal and *iojap*-affected plastid DNAs are similar, minor differences in band mobilities and stoichiometry are found. Fig. 1 depicts gels from a single seed lot of Oh51a inbred background. Other lots of this nuclear background and other nuclear backgrounds yielded restriction patterns of normal and *iojap*-affected plastid DNA that differed slightly from those in the figure (data not shown). The figure shows typical differences in the patterns. No consistent differences in stoichiometry or band mobility in the *Sal* I digests of normal and *iojap*-affected plastid DNAs were found in Oh51a background; *Sal* I fragments C, F, and H, which contain the two sets of ribosomal RNA cistrons (11, 19), were always similar in stoichiometry and mobility. Differences in band stoichiometry and mobility were found when the numerous small bands produced by *Eco*RI and *Bam* I were examined. In Fig. 1, *Bam* I fragments 16 and 17 and *Eco*RI fragments H and I are underrepresented in the restriction digests of *iojap*-affected plastid DNA compared with normal plastid DNA; *iojap* plastid DNA *Eco*RI fragment J is overrepresented and of slightly less mobility than the corresponding band from normal plastid DNA. None of these small *Eco*RI or *Bam* I fragments map within or adjacent to the positions of the two sets of ribosomal cistrons in corn chloroplast DNA (11, 19).

There are several possible explanations that might account for the minor differences in restriction patterns of normal and *iojap*-affected plastid DNAs. As noted above, there is considerable microheterogeneity in the organization of normal plastid DNA in various nuclear backgrounds. Second, *iojap*-affected plastids could contain a heterogeneous sample of chloroplast DNA, with some molecules in which some regions of the genome are deleted and others amplified but with an average composition similar to normal plastid DNA organization. Third, plastid DNA from *iojap*-affected tissue could be variant but contaminated with sufficient normal plastid DNA to give a similar but nonidentical pattern. At present we favor the possibility that normal variation in chloroplast DNA organization is the explanation for the minor differences in normal and *iojap*-affected plastid DNA restriction patterns.

Polyribosome Characterization. Normal green leaves as well as *iojap*-affected leaves contained a high proportion of polyribosomes with large aggregates predominating (Fig. 2 *A* and *C*). Etioplasts from normal leaves and green plastids also contained a high proportion of polyribosomes, but the polyribosomes were considerably smaller with an average of three to five ribosomes each (Fig. 2 *B* and *E*); small polyribosomes are typical of plastids in most species (14). *iojap*-affected

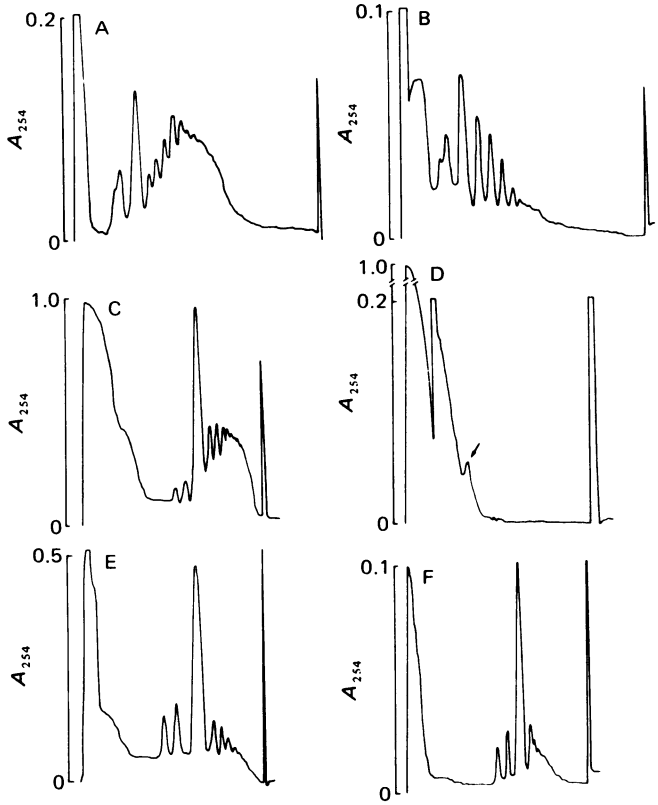

FIG. 2. Characterization of polyribosomes displayed on a 10–40% sucrose gradient centrifuged at 250,000 × *g* (top of gradient is at the left). (*A*) From whole green leaves; centrifuged for 1.75 hr. (*B*) From green plastids (+/+ and +/*ij*); 1.75 hr. (*C*) From *iojap*-affected albino leaves (+/*ij*); 3 hr. To test whether the polyribosomes of this gradient were of cytoplasmic or chloroplast origin, the two peaks left of the major monosomic peak were separately collected and extracted for RNA, and the RNA was displayed on acrylamide gels. The lower S value peak of the sucrose gradient was shown to contain 18–19S RNA and the larger S value peak of the sucrose gradient, to contain 24–25S RNA (data not shown, but compare to Fig. 3 or Fig. 4*C*); (*D*) From *iojap*-affected plastids (+/*ij*); 3 hr. The material in the region of the arrow was collected by ethanol precipitation and shown to be high molecular weight DNA by two criteria: mobility on an agarose/acrylamide composite gel was similar to that of authentic plastid DNA (Fig. 3), and the hyperchromicity at 260 nm was 29% after the pH was changed from 7.2 to 12.5. (*E*) From etioplasts (+/*ij* and +/+); 3 hr. (*F*) From mitochondria isolated from albino leaf sectors (+/*ij*); 3 hr.

plastids did not contain detectable mono- or polyribosomes (Fig. 2*D*) although mitochondria prepared from the same step gradient did contain polyribosomes (Fig. 2*F*). After purification, the *iojap*-affected plastids were lysed and the high molecular weight material was pelleted; this initial centrifugation was sufficient to pellet some plastid DNA (the peak marked with an arrow in Fig. 2*D*), but no material resembling ribosomal subunits or monosomes was recovered from the *iojap*-affected albino plastids.

Total Nucleic Acid Extraction from Plastids. Purified and partially purified plastid preparations in which plastids were separated from mitochondria by differential centrifugation, but were slightly contaminated by cytoplasmic ribosomes, were used to assess the presence of DNA and rRNA in plastids from various nuclear genotypes. Fig. 3 shows a typical comparison of nucleic acids extracted from plastids from sibling green and albino plants and electrophoresed on a composite agarose/acrylamide gel. Green purified plastids (lane B) contained high molecular weight DNA and 23S and 16S rRNA; partially purified plastids (lane C) were contaminated with cytoplasmic

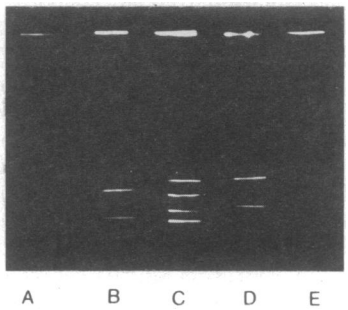

FIG. 3. Characterization of plastid total nucleic acids on composite agarose/acrylamide gels. Plastid preparations were lysed with 2.5% $NaDodSO_4$/50 mM Tris/10 mM $MgCl_2$/5 mM EDTA, pH 8, and the supernatant was cleared by centrifugation for 2 min in a Microfuge. The concentration of DNA was checked in an aliquot of the supernatant by fluorometry and an aliquot containing 0.3–1.0 μg of DNA was loaded onto the gel; the fluorimetric procedure does not detect RNA. Lanes: A, 0.5 μg of purified green chloroplast DNA; B, nucleic acids (0.5 μg of DNA) from purified green plastids; C, nucleic acids (0.3 μg of DNA) from partially purified green plastids; D, nucleic acids (0.5 μg of DNA) from partially purified *iojap*-affected plastids; E, presumptive *iojap*-affected plastid DNA isolated from gradient in Fig. 2*D*.

25S and 18S rRNA. Partially purified *iojap*-affected albino plastids (lane D) had DNA and cytoplasmic rRNA. The limit of resolution of this procedure was sufficient to detect 5–10% of normal levels of rRNAs of correct length.

Because the RNA within the *iojap*-affected plastids might be of abnormal length or present in trace amounts, total nucleic acids were extracted from purified plastids, *iojap*-affected plastids, and whole leaves and the nucleic acids were displayed on 2.4% acrylamide gels. Fig. 4 shows tracings from such gels in which the equivalent of 100 times more material from various plastid types was analyzed. Four rRNA species were found in normal whole leaf tissue (Fig. 4*A*). In the normal plastid total nucleic acids, the 23S and 16S rRNA species as well as some

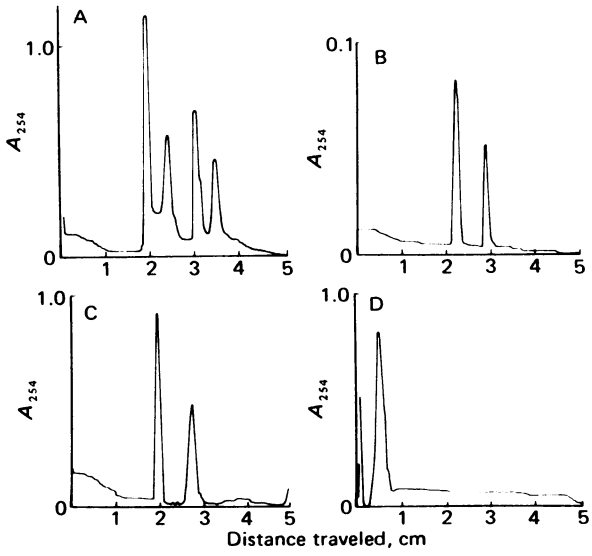

FIG. 4. Comparison of plastid nucleic acids on acrylamide gels. Plastid preparations were phenol extracted and the nucleic acids were precipitated with ethanol; electrophoresis was at 5 mA in a 0.8 × 5 cm cylindrical 2.4% acrylamide gel. (*A*) Whole green leaf (+/+) nucleic acids (75 μg); 4-hr run. (*B*) Authentic plastid rRNA (25 μg); 3.5 hr. (*C*) *iojap*-affected albino leaf (+/*ij*) total nucleic acids (50 μg); 4 hr. (*D*) Purified *iojap*-affected albino plastid (+/*ij*) total nucleic acids (28 μg); 4 hr (the top of the gel was distorted by the large amount of DNA present).

DNA at the top of the gel are visible. Whole leaf nucleic acid preparations from *iojap*-affected leaves contained DNA at the top of the gel as well as cytoplasmic 25S and 18S rRNAs (Fig. 4C); no defined bands were present in the 23S and 16S positions. Nucleic acids from purified *iojap*-affected plastids contained only DNA (Fig. 4D); no rRNA species were detectable. Mitochondrial rRNAs are a minor component of total nucleic acid preparations and coelectrophorese with the cytoplasmic rRNA.

Amino Acid Incorporation. Although the *iojap*-affected plastids contained no ribosomes detectable by conventional procedures, it was still possible that aberrant (but functional) ribosomes existed. An alternative approach was to test for the presence of protein synthetic machinery by assessing the ability of partially purified plastids to incorporate exogenous amino acids into acid-precipitable material. Fig. 5 illustrates a typical experiment in which normal plastids were compared to *iojap*-affected plastids. Normal plastids showed light-dependent protein synthesis (data not shown) and also could utilize exogenous ATP to support protein synthesis in the dark (solid squares, Fig. 5) at the rate of 21,500 cpm/100 μg of protein after 20 min. This amino acid incorporation was linear for 15 min. Incorporation is specific to the organelle because it was insensitive to RNase and to 1 mM cycloheximide (open circles), an inhibitor of cytoplasmic protein synthesis (5). Plastid amino acid incorporation is sensitive to puromycin, an initiation and elongation inhibitor; 0.1 mM resulted in 80% inhibition incorporation (compare solid circles to solid squares). Similarly, chloramphenicol, an inhibitor of plastid ribosome-dependent amino acid incorporation, inhibited the *in vitro* plastid dependent incorporation (data not shown). After isolation on a sucrose step gradient, etioplasts demonstrated a comparable ability for ATP-dependent amino acid incorporation, 25,200 cpm/100 μg of protein after 20 min (data not shown).

iojap-affected plastids (open triangles, Fig. 5) showed no ability to incorporate amino acids into protein. All plastids showed a similar ability to take up exogenous amino acids (approximately 60,000 cpm/100 μg of protein in 20 min); consequently, the lack of measurable incorporation likely reflects the lack of ribosomes and perhaps other components of the protein synthesis machinery. The *iojap*-affected plastids did not contain a diffusable inhibitor of amino acid incorporation because mixing equal amounts of normal and *iojap*-affected plastids resulted in the expected 50% of normal incorporation. Similarly, mixing lysed *iojap* plastids with lysed green plastids in the absence of RNase did not affect amino acid incorporation (data not shown).

Sector Measurement. Leaf ontogeny in corn results from the mitotic activity of the apical meristem; individual leaves differentiated after seed germination are produced by 16–35 cells in the meristem, with upper leaves composed of 16–20 clones and lower leaves, of 30–35 clones (20). Because plant cells are locked together by the cell wall, the derivatives of an individual meristematic cell will form a lengthwise strip of the leaf (21). In addition, the same meristematic cell will produce a sector of the stem subtending the node at which the leaf is elaborated (20). Events that mark a meristematic cell can be viewed in the leaf as a stripe traversing the length of the leaf and extending through the node and down the stem to the next node. Events occurring after the separation of leaf and stem initials will be found in only one of the two organs; similarly, events occurring after expansion of individual clones from one cell to many will result in progressively narrower stripes within the clone.

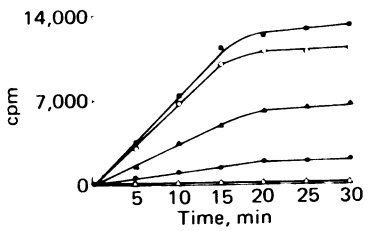

FIG. 5. Amino acid incorporation by partially purified plastids. ■, Normal plastids; ○, normal plastids with 10 mM cycloheximide; ▲, equal parts of normal plastids and *iojap*-affected plastids incubated in the complete reaction mixture; ●, normal plastids with 0.1 mM puromycin added; △, *iojap*-affected plastids.

Table 1. *iojap*-conditioned stripe width compared to clone size

	Less than clone size	Clone size ± 10%	More than clone size	*n*
Upper leaves	28	54	18	1200
Lower leaves	27	58	15	300
Mean	27.5	56	16.5	

Fifteen different field-grown 1977 plants (*ij/ij*) in four nuclear backgrounds (Mo17, Oh51a, Tr, K55) were analyzed by measuring leaf width and the position of leaf stripes in relation to subtending node events and to other leaves of the plant. Leaves with 15–40% albinism were selected. Upper leaves were arbitrarily divided into 16 sectors at midpoint (mean leaf width ± SD, 73 ± 10 mm; mean sector width, 4.5 ± 0.5 mm) and lower leaves were divided into 32 sectors (mean leaf width, 82 ± 10 mm; mean sector width, 5.2 ± 0.5 mm). Life-size photographs of leaves were made and albino stripe width was measured with a magnifying cursor and Numonics digitizer (accuracy, ±0.01 mm). Stripes on a given leaf were evaluated with respect to the expected clonal size of that leaf.

In an effort to determine the time of *iojap* action, 1500 leaf stripe widths were measured and, for 510 leaf stripes, coincidence with stem stripes was assessed. Approximately 55% of the *iojap* events scored were the width expected if an entire clone were affected (Table 1). About 30% of the events were less than 90% of a clone; many of these were half and quarter clone events. Fifteen percent of events covered greater than one clone. Because the leaves examined ranged from 15 to 40% in extent of albino striping, the chance of independent events in adjacent clones is high (26% in leaves that are 40% albino). This evidence suggests that the *iojap* event occurs independently in each clone early in leaf ontogeny, perhaps in the meristematic cell. However, when individual leaves (30–40% albino) and the subtending stem with a similar extent (20–40%) of striping were compared (Table 2), only 8%—less than expected by chance (0.153–0.256 total coincidence)—of stripes matched in extent and position.

The analysis of sectoring suggests that the *iojap* gene acts early in leaf ontogeny such that entire clones derived from a single meristematic cell can be affected to give a white stripe of clonal size. The meristematic cell itself is not changed, however, because coincidence of stripes or of extent of striping is not found in a leaf and stem of the same node, nor are successive leaves similarly striped.

Table 2. Coincidence of leaf and stem stripes

	Distribution of albino leaf stripes		
Leaves	Matching stem stripe in position	Expected by chance	*n*
15–20% albino	12 (0.029)	0.041–0.072	410
30–40% albino	8 (0.080)	0.153–0.256	100

In part, the low coincidence of leaf and stem stripes is due to the lower frequency of stem albinism compared to leaf albinism in most stems examined in this survey. These data are based on the leaves differentiated after seed germination. *iojap* events also occur in leaves differentiated during embryogeny.

DISCUSSION

Characteristics of *iojap*-Affected and Other Albino Plastids. The albino plastids of *iojap*-affected tissues have been shown to contain a normal chloroplast genome on the basis of DNA buoyant density, high DNA molecular weight on gel electrophoresis, and *Sal* I, *Bam* I, and *Eco*RI digestion patterns. Analysis of the fraction of cleavage sites conserved between two closely related DNAs has been used to estimate total sequence divergence and the confidence limits of having detected base changes (24). Plastid DNAs from normal and *iojap*-affected albino plastids share 38 or 39 (*Eco*RI fragment J is of different mobility) restriction sites compared in this study; this tested for identity at 234 bases and for the creation of new restriction sites within existing fragments. On the basis of Upholt's equations (24), the fraction of base changes detected between the two plastid DNAs is 0.022 ± 0.02. If differences in chloroplast DNA are generated as a result of *iojap* gene activity, such changes are not resulting in major deletions or rearrangements of the genome, nor is *iojap* a "mutator" gene causing multiple errors in DNA replication.

The phenotype of the *iojap*-affected plastids is ribosome-less as demonstrated by the lack of polyribosomes, of 16S and 23S chloroplast rRNAs, and of protein synthetic capacity. These observations support the earlier ultrastructural studies in which ribosomes were reported as being absent (3). *iojap*-affected plastids have also been reported to be missing ribulosebisphosphate carboxylase large subunit (22) and α and β subunits of plastid coupling factor (23); the lack of these proteins is not attributed to gene mutation but to the lack of plastid ribosomes *per se* (23).

Ribosome-less plastids have been reported in other higher plants, including *Pelargonium* (25–27) and *Hordeum vulgare* (28, 29). In these cases, as with *iojap*-affected *Zea mays* plastids, the plastid compartment contains DNA. Such albino plastids contain numerous proteins, presumed to be nuclear gene products (cf. ref. 28). The albino plastids are found in all tissues or cells examined (this study and refs. 27 and 28), indicating that albino plastid replication and DNA synthesis keep pace with the cell cycle and that the important functions of plastid compartment maintenance, growth, and division are continued in the absence of plastid protein synthesis.

Expression of *iojap* during Development. The *iojap* gene is recessive to wild-type alleles, and *iojap* activity can only be found in homozygous *ij/ij* individuals. These features of *iojap* action suggest that the *iojap* allele fails to provide activities necessary for normal plastid ontogeny, perhaps by directly affecting plastid ribosome biogenesis or maintenance. *iojap* activity, or lack of wild-type activity, has as its earliest and most frequent target the individual stem and leaf progenitor cells as evidenced by the preponderance of *iojap* events that fill entire leaf clones which, in turn, fail to match stem events.

One hypothesis is that *iojap* activity results in a mixture of normal and affected plastids in the meristem or its derivatives and, consequently, the albino stripes are the result of sorting out of n plastid units. In this hypothesis, stem and leaf would be expected to be complementary in albino striping, which is not the case; also, as the number of plastids increases, affected areas would become increasingly narrower, less than clone size, which is not true in the cases examined.

One hypothesis is that a catastrophic loss of plastid ribosomes occurs in leaf progenitor cells concomitant with plastid differentiation and the increased rate of cell division of the progenitors (30). The frequency with which whole albino clones or white stripes of any width are produced would thus depend on various nuclear, and possibly plastid, genes which allow recovery of normal plastid ribosome content. This model assumes that, should plastid ribosome content fall below a minimum required to manufacture plastid proteins required for plastid ribosomes, the plastid ribosome content would fall to zero.

We favor the hypothesis that all plastids in stem and leaf progenitor cells are affected in *ij/ij* plants but that a variable number of plastids recover a normal ribosome content. The results of *iojap* activity are not entirely random, however, because stripe frequency is higher in leaf margins than in the center of the leaf (unpublished data); cells in leaf margins undergo considerably more mitotic events during early leaf ontogeny than do cells close to the midrib (30). Consequently, either *iojap* activity is more likely in most rapidly dividing cells or the chances of restitution are least in this cell population.

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