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Plastid genome structure and plastid-related transcript levels in albino barley plants derived from anther culture

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Summary. Southern analysis of DNA from four albino barley plants regenerated from microspores by direct embryogenesis revealed the presence of plastid genomes which had undergone deletion or alteration of specific restriction fragments (*Δ*ptDNAs). In contrast, a fifth plant appeared to contain an intact plastid genome. All the albino plants studied contained reduced amounts of ptDNA, the most abundant restriction fragments being present at levels between 6% and 20% of those found in the leaves of green seedlings. Steady-state levels of transcripts from plastid and nuclear genes encoding plastid components were estimated by Northern analysis of RNA from albino plants. Transcripts from the plastid genes *rbc*L, *psb*D-*psb*C and the 16S and 23S rRNAs were undetectable or were present at greatly reduced levels in albino plants compared to those found in green leaves. Transcripts from the nuclear genes *rbc*S and *cab*, which encode chloroplast localised proteins, were also present at reduced levels in albino pollen plants. Levels of the nuclear encoded 25S rRNA, which is not a plastid component, were found to be identical in albino plants and green leaves suggesting that only the expression of plastid-related genes may be affected in albino plants. The general reduction of plastid-related transcripts was independent of the different patterns of ptDNA alteration seen in albino pollen plants.

Abbreviations: cDNA, complementary DNA; DNA, deoxyribose nucleic acid; IR, inverted repeat; kb, kilobase; LSC, large single copy; mRNA, messenger RNA; ptDNA, plastid DNA; *Δ*ptDNA, altered ptDNAs; rDNA, ribosomal DNA; rRNA, ribosomal RNA; Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; SDS, sodium dodecyl sulphate; SSC, small single copy

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Introduction

Anther culture is an in vitro technique which relies on the suppression of the normal development of pollen within anthers, the induction of embryogenesis and the subsequent regeneration of whole plants. To achieve this, immature anthers are excised from donor plants and placed under culture conditions which allow optimum regeneration of plants from individual developing microspores (Dunwell 1984). The so-called pollen plants obtained in this manner should be haploid, but in barley spontaneous diploidisation occurs at an early stage in their development, resulting in the production of doubled haploid individuals (Lyne et al. 1984; Sunderland and Evans 1980). During the generation of doubled haploid cereal pollen plants, both green and albino (chlorophyll-deficient) individuals are produced. Whereas green doubled haploids can be used to rapidly establish homozygous lines and are of potential use in plant breeding programmes, albino pollen plants remain in a vegetative state and only survive for relatively short periods in vitro.

Previously it has been shown that albino pollen plants of rice (Sun et al. 1974) and barley (Clapham 1973; Dunford 1989) do not contain mature chloroplasts and lack major plastid gene products such as the 23S an 16S rRNAs (Sun et al. 1979). In addition, Sun's group reported the absence of the abundant stromal enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the large subunit of which is plastid encoded. The most detailed efforts to characterise albino pollen plants at the molecular level have focused on the structure of the plastid genome (Day an Ellis 1984, 1985; Ellis and Day 1986). These studies revealed that albino wheat and barley pollen plants contain extensively deleted forms of the plastid genome (*Δ*ptDNAs), which can be present in both linear

and circular conformations. Detailed analysis of one such linear ⊿ptDNA showed that it was composed of an inverted repeat sequence arranged in a hairpin configuration. These studies used plants regenerated via an intermediate callus stage (Huang and Sunderland 1982). However, recently developed anther culture methodology allows the production of pollen plants by direct embryogenesis (Hunter 1987). To investigate whether there are any differences in the occurrence and extent of ptDNA deletions between plants obtained via these two pathways we mapped the ptDNA of albino barley plants regenerated from pollen by direct embryogenesis. In addition, albino pollen plants provide the opportunity to determine what effect ptDNA deletions and the absence of plastid development may have on the expression of the remaining ptDNA and nuclear genes encoding chloroplast proteins. Hence, we estimated the steady-state levels of RNA transcribed from several plastid genes and two nuclear gene families encoding plastid localised proteins.

Materials and methods

Growth and maintenance of plant material. Barley, *Hordeum vulgare* var. Igri, was used throughout. Albino and green pollen plants were regenerated by direct embryogenesis from barley microspores using the method of Hunter (1987). Regenerated plants were maintained in vitro on Gamborg's B5 medium (Gamborg 1970) with transfer to fresh medium every 4 weeks. Barley seedlings were obtained by germination of surface-sterilised seeds in sterile glass jars on damp filter paper at a density of 100 seeds per jar. Leaf and root tissue from seedlings was harvested after 8–10 days' growth. All plant material was grown under a 16 h light/8 h dark cycle in Fisons Fitotron 600H or Vindon S13F constant environment cabinets at a temperature of 25 °C. All plant tissue was frozen in liquid nitrogen directly after harvest and stored at −70 °C.

Isolation and analysis of plant nucleic acids. DNA and RNA were extracted from plant tissues according to the methods of Murray and Thompson (1980) and Logemann et al. (1987), respectively. All restriction enzymes were purchased from Bethesda Research Laboratories. Southern transfer and hybridisation were carried out as described by Dalgleish (1987). Northern analysis was carried out essentially as described by Maniatis et al. (1982) using Hybond-N nylon hybridisation filters (Amersham International). Radiolabelled DNA probes were prepared by the random hexamer priming method of Feinberg and Vogelstein (1983). The following DNA fragments were used as hybridisation probes: the *Pst*I-derived inserts of the plasmids pHvCP1-P10 (P1-P10) which represent the entire barley plastid genome (Day and Ellis 1985); *Pst*I + *Pvu*II derived subfragments of pHvCP8 containing barley plastid rDNA; a *Hin*dIII + *Pst*I-derived subfragment of pHvCP4 containing most of the *rbc*L coding region; a *Bam*HI + *Eco*RI-derived subfragment of pBB531-4 containing part of the barley *psb*D-*psb*C gene cluster (Berends et al. 1987); the inserts of pKG4626 and pHvLF2 which encode cDNAs for the small subunit of Rubisco (*rbc*S) and a chlorophyll-a/b-binding protein (*cab*), respectively (Barkardottir et al. 1987; Gollmer and Apel 1983); a *Sal*I-derived subfragment of the plasmid pTA71, which encompasses sequences coding for the wheat 25S rRNA (Gerlach and Bedbrook 1979). Hybridisation filters were washed twice for 15 min in 3 × standard saline citrate/0.1% SDS and twice for 15 min in 0.5 × standard saline citrate/0.1% SDS, at 65 °C (1 × standard saline citrate is 0.15 M NaCl, 0.015 M sodium citrate). Radiolabelled DNA fragments were removed from hybridisation filters prior to rehybridisation by washing in boiling 0.1% SDS followed by incubation at 65 °C for 1 h. Autoradiography was performed at −70 °C for 3–48 h using Kodak XAR X-ray film.

Results

Structure of the plastid genome in albino plants

The ptDNA of five albino barley plants was mapped by Southern analysis of total DNA digested with *Pst*I or *Pst*I + *Xho*I using the cloned ptDNA fragments P1-P10 as probes. Summaries of the restriction fragment data are given in Table 1 and Fig. 1. Four of the five albino pollen plants examined (A301, A307, A308 and A309) had ptDNA restriction fragment patterns that differed significantly from those of an intact plastid genome. The hybridisation patterns obtained with two of the ptDNA probes are shown in Fig. 2. All four albinos contained ptDNA restriction fragments of altered size and some lacked certain fragments altogether. This confirmed that albino barley plants regenerated from pollen by direct embryogenesis contain ptDNAs that have suffered partial or complete deletion of specific restriction fragments. Some ptDNA probes hybridised to multiple restriction fragments of altered size which were present in non-stoichiometric amounts, indicating the presence of mixed populations of ⊿ptDNA molecules. The deletions observed mapped to various parts of the plastid genome but most of the albino plants lacked portions of the large single copy (LSC) region as deduced from the low abundance or absence of restriction fragments from this area. All the albinos retained restriction fragments located in the inverted repeat (IR), and all except A308 retained sequences in the small single copy (SSC) region. All the plants possessed fragments covering the border between P1 and P3 close to one IR (see Fig. 1).

The restriction patterns seen in plant A308 provided an opportunity to predict what form ⊿ptDNA molecules might have in albino plants. The most abundant ptDNA restriction fragments from A308 hybridised to probes representing portions of the IR and LSC regions (Fig. 3). Probe P3 hybridised to major fragments of normal size, while probes P1 and P8 hybridised to major altered fragments of 9.1 kb (P1′) and 3.6 kb (P8′) respectively in both *Pst*I and *Pst*I + *Xho*I digests. Further restriction mapping indicated that P8′ is derived from a *Pst*I + *Pvu*II subfragment of P8 lying adjacent to P3 (data not shown). The absence of major restriction fragments mapping to other ptDNA regions indicated that 75% of the plastid genome had suffered deletions, including the entire SSC region, most of the LSC region and part of the IR. Since P1′, P3 and P8′ are the most abundant fragments in A308 it is likely they comprise a single species that represents the major form of ptDNA in this albino and may have either a linear or a circular conformation. A linear molecule could be generated by deletion events ending in P1 and the copy of P8 adjacent to P3 leaving truncated derivatives of these fragments (Fig. 4 A). The free ends of P1′ and P8′ could explain why restriction fragments of identical size are generated by both *Pst*I and *Pst*I + *Xho*I digests since no *Xho*I sites exist between these free ends and the *Pst*I sites flanking P3. A circular molecule could be formed by a single recombination event occurring between directly repeated sequences in P1 and P8 resulting in their fusion. Since no such fusion fragment is detected

Table 1. Summary of restriction fragments found in Southern analyses of ptDNA from albino pollen plants

ptDNA probe	Plant											
	A301		A307		A308		A309		A320		Control	
	P	PX	P	PX	P	PX	P	PX	P	PX	P	PX
P1	20.7	12.0 8.1w 3.8 2.6 2.3	20.7* 11.0	18.6 12.0 3.8 2.6 2.3	9.3	9.3	20.7m	12.0m 3.8m 2.6m 2.3m	20.7m 15.8w	12.0 3.8 2.6 2.3	20.7	12.0 3.8 2.6 2.3
P2	20.1 16.6w 14.8w 13.2w 11.0w 9.8w 5.8w	13.8w 12.4w 11.0w 9.1w 8.3m 6.6m 5.4w 4.6 4.3w 2.8 0.6w	16.6w	12.4w 4.6w 2.8w	20.1 8.3m	12.4 7.4m 0.6w	24.0w 20.1w 17.8m 13.5w 10.7w 7.8w	12.4m 11.8w 8.3w 6.5w 4.6w 4.1w 2.8m 0.6w	20.1 17.8w	12.4 8.3 7.3w 4.6 2.8 0.6	20.1	12.4 8.3 4.6 2.8 0.6
P3	18.9 13.5m	12.4m 10.9w 9.6w 8.3 6.8m 5.6w 4.7m 4.3w 0.6w	12.4m 9.3m 7.6w 6.2* 2.7w 2.6w 1.3w	12.4m 9.3m 8.7w 7.1w 5.6w 4.7* 2.8w 2.6w 2.2* 0.5	18.9 8.3m	12.4 7.4m 4.7	18.9w 16.6m	12.4m 8.3w 4.7m 4.2w 0.6w	18.9	12.4 8.3 4.7 0.6w	18.9	12.4 8.3
P4	13.4 6.7m 4.3w	20.9w 13.0m 12.0w 10.5w 9.6w 6.7 6.3w 4.3w	13.4w	6.7w	22.4w	12.6w	13.4w	6.7w	13.4	6.7	13.4	6.7
P5	11.9	6.5 5.4	–	–	–	–	11.9m	6.5m 5.4m	11.9	6.5 5.4	11.9	6.5 5.4
P6	10.7 5.5	7.1 2.8 1.7 0.8	10.7m 5.5m	7.1m 2.8m 1.7m 0.8m	4.3w 2.0w	15.1w 2.4w 1.3w 0.8w	10.7 5.5	7.1 2.8 1.7 0.8	10.7 5.5	7.1 2.8 1.7 0.8	10.7 5.5	7.1 2.8 1.7 0.8
P7	9.9	6.6 3.3	19.5w 9.9w 8.3w	6.6w 6.0w 3.3w 2.9w 2.0w	–	–	9.9m	6.6m 3.3m	9.9	6.6 3.3	9.9	6.6 3.3
P8	8.1	5.0 3.1	8.1m	5.0m 3.1m	14.5w 8.1w 7.6w 3.6	5.0m 3.6 3.1w	14.1w 8.1	7.1m 5.0 3.1	8.1	5.0 3.1	8.1	5.0 3.1
P9	10.7 5.5	7.1 2.8 1.7 0.8	10.7m 5.5m	7.1m 2.8m 1.7w 0.8m	4.3w 2.0w	15.1w 2.4w 1.3w 0.8w	10.7 5.5	7.1 2.8 1.7 0.8	10.7 5.5	7.1 2.8 1.7 0.8	10.7 5.5	7.1 2.8 1.7 0.8
P10	5.2	5.2	5.2m	5.2m	–	–	5.2	5.2	5.2	5.2	5.2	5.2

All values refer to the size (kb) of restriction fragments hybridising to ptDNA probes P1–P10 in *Pst*I (P) and *Pst*I + *Xho*I (PX) digests of DNA extracted from albino pollen with digests of DNA from the leaves of green seedlings as a control. The level of hybridisation of each restriction fragment to the ptDNA probes is indicated in comparison to that found for A320 except for the control: w, weak; m, medium; no mark, similar to A320. Unusually abundant restriction fragments are marked with an asterisk

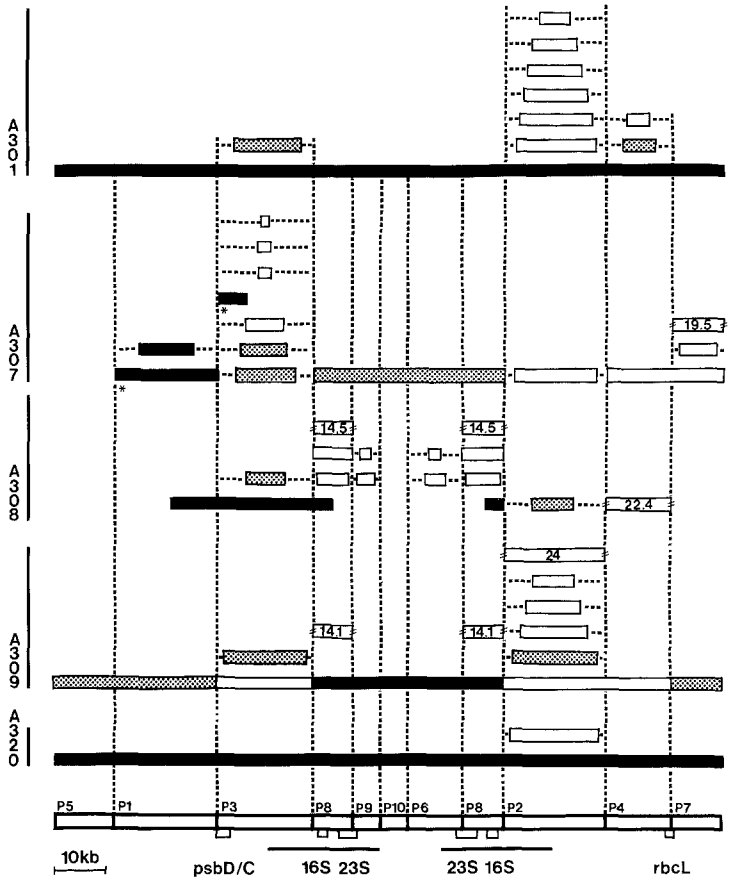

Fig. 1. Summary of *Pst*I-derived ptDNA restriction fragments found in albino pollen plants. The *lower bar* represents a linear map of intact barley ptDNA showing the position of *Pst*I-derived restriction fragments P1–P10. The positions of the inverted repeat sequences are represented by *horizontal lines* below the map. The small single copy (SSC) region is located between the inverted repeat (IR) regions, while the large single copy (LSC) region lies distal to the IR at the ends of the restriction map. The positions of the genes *rbc*L, *psb*D, *psb*C and the 16S and 23S rDNA sequences are indicated by *open boxes* below the intact restriction map. The positions of *Pst*I-derived ptDNA fragment found in albino plants are shown above the map of the intact plastid genome. *Vertical dotted lines* indicate the limits of intact *Pst*I fragments. *Filled bars* represent fragments with levels of hybridization similar to those found for A320. *Dotted* and *open bars* represent fragments with intermediate and weak hybridisation in the same comparison. *Filled bars* marked with an *asterisk* represent fragments that are unusually abundant compared with others. *Bars* flanked by *horizontal dotted lines* represent restriction fragments for which a precise map location on intact ptDNA could not be assigned. *Bars* with *cross-hatched ends* indicate restriction fragments that are larger than the hybridising ptDNA probe. The numbers within the fragments indicate their size (kb)

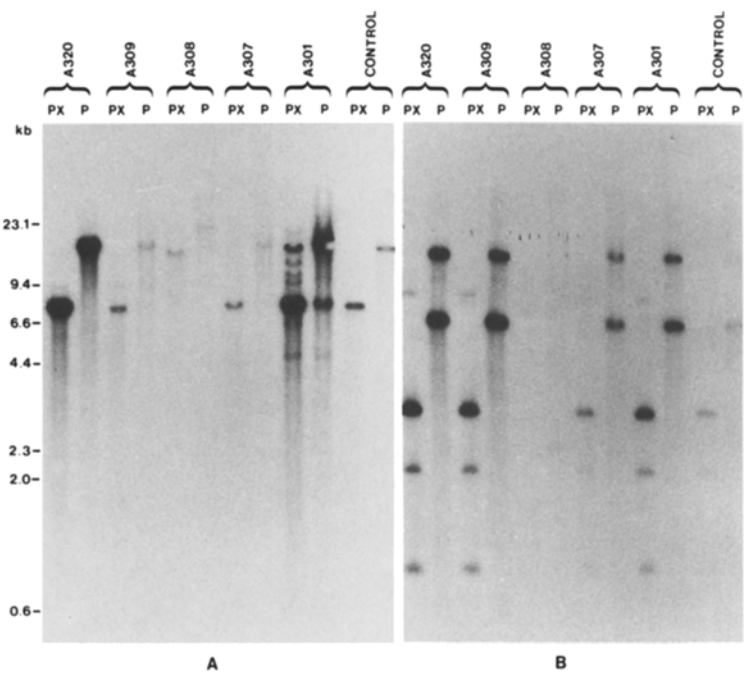

Fig. 2 A, B. Typical patterns of hybridisation to ptDNA probes seen in digests of DNA from albino pollen plants. Autoradiographs obtained from Southern analysis of albino pollen plant DNA digested with the restriction enzymes *Pst*I (P) or *Pst*I and *Xho*I (PX) and probed with the barley ptDNA probes P4 (**A**) or P6 (**B**). Identical digests of DNA from the leaves of green seedlings served as controls in both cases (these lanes were underloaded with DNA in both experiments). Note the altered restriction fragment patterns seen in some albino plants, e.g. A301, and the absence of major restriction fragments when P6 is used a probe against DNA from A308

it is necessary to invoke recombination pathways involving multiple steps. Two alternative pathways are shown in Fig. 4B. The first step of pathway I involves recombination between direct repeats in P1 and P2, resulting in fusion of these fragments and the deletion of most of the LSC region and some of one IR including almost all of P2. The second step involves recombination between small inverted repeats present within the larger IR region. Recombination between directly orientated small inverted repeat sequences in the two copies of the larger inverted repeat would result in deletion of the intervening SSC and IR sequences and fusion of the two copies of P8.

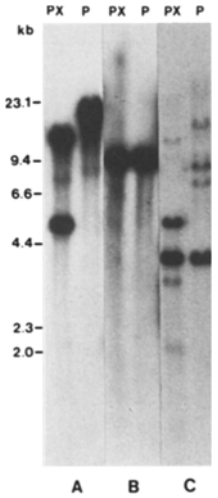

Fig. 3A–C. Major ptDNA restriction fragments seen in albino A308. Autoradiographs obtained from Southern analysis of DNA from A308 digested with the restriction enzymes *Pst*I (*P*) or *Pst*I and *Xho*I (*PX*) and probed with the barley ptDNA fragments P3 (**A**), P1 (**B**) and P8 (**C**). The positions of size markers (kb) are indicated on the *left*

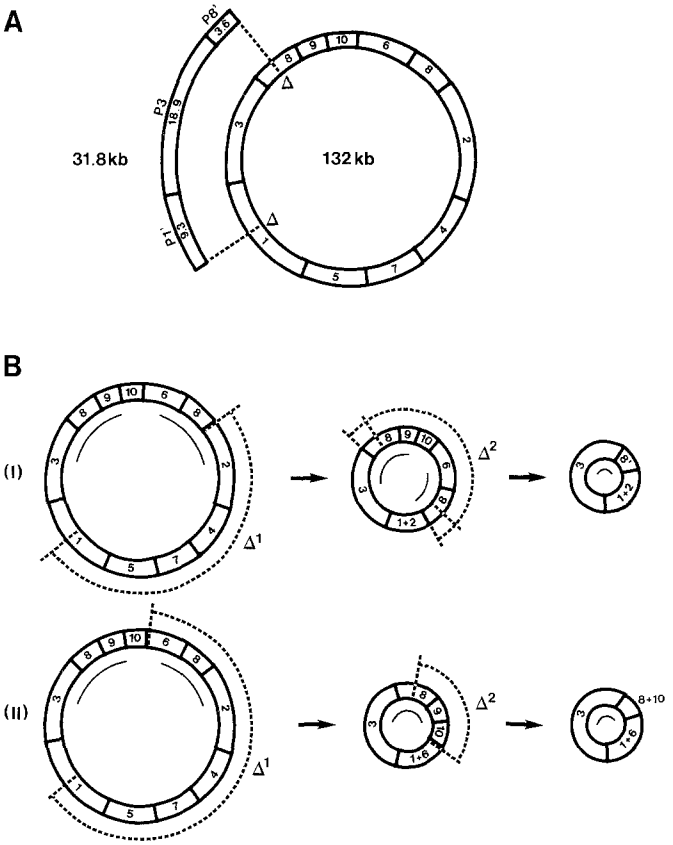

Fig. 4A, B. Possible structures for linear and circular *Δ*ptDNAs in A308. **A** The possible structure of a linear *Δ*ptDNA in A308. The *inner circle* represents a *Pst*I-derived restriction map of the intact plastid genome and the numbers refer to the *Pst*I-derived fragments P1–P10. The *outer segment* represents the possible map positions of the major restriction fragments described in the text. Altered ptDNA fragments are indicated with an *apostrophe*. *Deltas* (*Δ*) mark the end-points of deletions in P1 and P8. (Sizes of intact and altered ptDNA fragments in kb). **B** Two possible pathways for the generation of circular *Δ*ptDNAs in A308 [(I) and (II)]. A simplified restriction map of intact barley ptDNA is shown to the *left* of each pathway. Map positions of the *Pst*I-derived restriction fragments P1–P10 are indicated. The *filled innermost lines* indicate the position of the IR region. *Radial dotted lines* specify end-point positions for an initial deletion event (Δ^1). *Concentric dotted lines* represent sequences removed during this deletion event. The second step shows the remaining ptDNA after event Δ^1 and the end-points for the second deletion event Δ^2. A number of possibilities exist for the position of deletion end-points in P8, pathway (I) and the area which may contain them is delimited by *twin radial dotted lines*. The final step of each pathway shows the remaining ptDNA after event Δ^2. The fusion of fragments P1 and P2, and P1 and P6 are indicated as 1+2 and 1+6, respectively. Fragment 8' refers to the truncated version of P8 generated in pathway (I)

Fragment P8 is known to contain such small IRs within the 16S rDNA (Tohdoh and Sugiura 1982). The first recombination event in pathway II occurs between P1 and P6, resulting in the deletion of one copy of the IR and most of the LSC region. Further recombination between P10 and P8 leads to the deletion of P9.

In contrast to the other albinos examined, the predominant ptDNA restriction pattern seen in A320 was indistinguishable from that of the intact plastid genome following Southern analysis using *Pst*I and *Xho*I and the more frequently cutting enzymes *Bam*HI and *Eco*RI (data not shown). The presence of altered restriction fragments at very low abundance indicated that *Δ*ptDNA molecules may also exist in A320.

Abundance of ptDNA in albino plants

Laser densitometry of bands hybridising to ptDNA probes in digests of equal amounts of DNA indicated that the most abundant ptDNA restriction fragments in albino plants were present at levels 6–20% of those found in normal green leaves. The stoichiometry of ptDNA restriction fragments from A320 was identical to that expected from an intact plastid genome. In plant A301, probes P1 and P3 hybridised to unusually abundant restriction fragments. Probe P1 hybridised to fragments of normal size, while probe P3 hybridised to a *Pst*I fragment of 6.2 kb and *Pst*I + *Xho*I fragments of 4.7 kb and 2.2 kb. Of these, only the 4.7 kb fragment was of normal size. Since P3 and P2 share an extensive region of homology because of IR sequences, these fragments may be located in the LSC region. The fragments hybridising to P1 and the 6.2-kb and 4.7-kb fragments hybridising to P3 were present at approximately 40% of the level of similar size fragments found in normal green leaves. The 2.2-kb P3 hybridising fragment was present at 120% of the level of P3 fragments in normal green leaves.

Plastid transcript abundance

RNA samples from the five plants for which ptDNA mapping had been carried out and four additional albino plants (designated A501-A504) were subjected to North-

Table 2. Estimated abundance of different plastid and nuclear encoded transcript in albino pollen plants

RNA	Albino pollen plant								
	A301*	A307*	A308*	A309*	A320*	A501	A502	A503	A504
*rbc*S	≤5%	–	–	≤5%	≤5%	≤25%	≤10%	≤5%	≤10%
cab	ND	–	–	–	–	–	–	–	–
23S rRNA	–	–	–	–	≤1%	≤ 1%	–	–	–
16S rRNA	ND	–	≤1%	–	≤1%	–	–	–	–
*rbc*L	–	–	–	–	≤5%	≤10%	≤ 5%	≤5%	–
*psb*D-*psb*C	–	≤5%	≤5%	≤5%	≤5%	≤15%	≤15%	–	≤15%

All values refer to the estimated level of each transcipt compared with that found in the leaves of green seedlings. A dash (–) indicates that no transcript could be detected; ND = not determined. Albino plants for which ptDNA mapping studies have been carried out are denoted with an asterisk

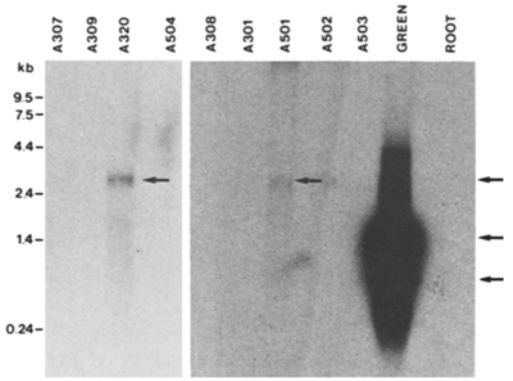

Fig. 5. Detection of 23S rRNA in albino pollen plants. An autoradiograph from a Northern hybridisation experiment using a barley 23S rDNA probe is shown. The source of RNA is indicated above each *lane*. Albino plants are prefixed with A. *Root* and *Green* refer to RNA extracted from the roots and leaves, respectively, of light-grown seedlings; 3.5 μg of total RNA was loaded per lane. The *top arrow* to the *right* shows the position of the intact 2.9-kb 23S rRNA. The *lower arrows* indicate the position of the 23S rRNA fragments. The position of the 2.9-kb band in A320 and A501 is shown by an *arrow*. The position and size of the RNA molecular weight markers (Bethesda Research Laboratories) are shown on the *left*

ern analysis by hybridisation with DNA probes encompassing the plastid 16S and 23S rDNAs, the gene encoding the large subunit of Rubisco, *rbc*L and a portion of the *psb*D-*psb*C gene cluster in order to determine the extent to which these transcripts accumulate in albino tissues (see Table 2).

Hybridisation with the 23S rDNA probe was not detected with most albino RNA samples even after prolonged autoradiography (Fig. 5). This was in contrast to the strong hybridisation seen to RNA from green leaves where it appeared as a heavy smear representing the full length 23S rRNA (2.9 kb) and its two specific processed fragments (1.9 kb and 1.0 kb; Poulsen 1983). A small amount of the intact 2.9-kb 23S rRNA was seen in albinos A320 and A501. The amount of 23S rRNA in these plants was estimated to be ≤1% of that found in green leaves. Barely detectable levels of the two processed fragments of the 23S rRNA were also observed in A320 after prolonged autoradiography (data not shown) suggesting that cleavage of the 23S rRNA had not proceeded to the same extent as seen in green leaves. The 1.6-kb plastid 16S rRNA was also undetectable in the majority of albino plants (Fig. 6). However, plants A308 and A320 did contain small quantities of the 16S rRNA estimated to represent ≤1% of the level found in green leaves. Prolonged autoradiography revealed the presence of a 2.0-kb RNA in A309, A320, A501, A502, A503 and A504 and an additional 1.8-kb transcript in plants A501 and A503.

The 1.7-kb *rbc*L mRNA (Fig. 7A) was present in albino pollen plants at levels ≤5–10% of that found in green leaves or was absent altogether. Similarly, levels of the 3.2-kb and 1.7-kb transcripts originating from the *psb*D-*psb*C gene cluster were also reduced to ≤5–15% of those seen in green leaves (Fig. 7B). Interestingly, albino pollen plants A501 and A502 contained novel transcripts hybridising to the *psb*D-*psb*C probe. Both albinos possessed an additional 2.7-kb transcript while A501 contained a unique 5.5-kb RNA. However, since ptDNA restriction maps of these albinos are unavailable it is unknown whether these novel transcripts were generated as a result of structural alteration of the *psb*D-*psb*C gene cluster.

Abundance of transcripts from nuclear genes encoding plastid proteins

The expression of nuclear genes encoding chloroplast proteins is reduced in barley plants that fail to develop a functional chloroplast due to treatment with the herbicide Norflurazon (Batschauer et al. 1986). The use of albino pollen plants to study nuclear/plastid interactions is a useful alternative to destruction of the chloroplast compartment with herbicides, which may also have detrimental side-effects on other aspects of plant cell function. To test whether nuclear gene expression was affected in albino barley pollen plants, equal amounts of total RNA were subjected to Northern analysis using cDNA probes encoding the small subunit of Rubisco (*rbc*S) and a chlorophyll-binding protein (*cab*; see Table 2). The *rbc*S and *cab* mRNAs were much less abundant in albino pollen plants than in the green leaves (Fig. 8). In most albinos the *rbc*S and *cab* mRNAs were absent or only barley detectable (≤5% of the levels found in green leaves). However, in some cases the *rbc*S transcript was present at higher abundance as in A502 and A501 where

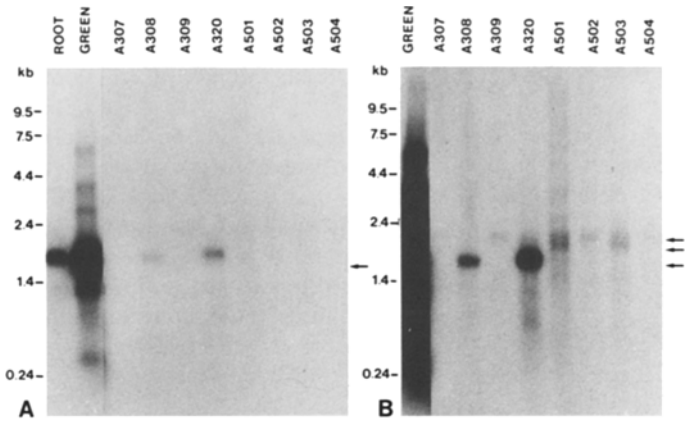

Fig. 6 A, B. Detection of 16S rRNA in albino plants. **A** Autoradiograph obtained by exposing a Northern blot hybridised to a barley plastid 16S rDNA probe to X-ray film for 16 h. **B** A 3-day exposure of the same filter. The source of RNA is indicated above each lane. Albino pollen plants are prefixed with an A. *Root* and *Green* refer to RNA extracted from the roots and leaves, respectively, of light-grown seedlings; 1 μg of total RNA was loaded per lane. An *arrow* indicates the position of the 1.6-kb 16S rRNA band in both *panels*. The *additional arrows* to the *right* of *panel B* show the position of the 2.0-kb and 1.8-kb RNAs described in the text. The position and size (kb) of the RNA molecular weight markers are given to the *left* of each autoradiograph

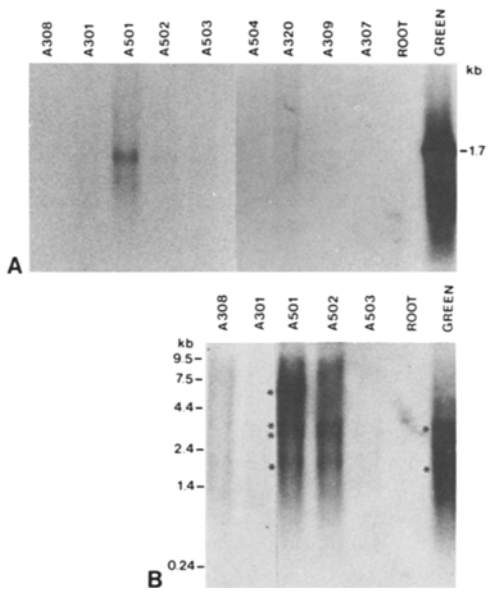

Fig. 7 A, B. Detection of *rbc*L and *psb*D-*psb*C transcripts in albino pollen plants. Representative autoradiographs of Northern blots hybridised with probes for *rbc*L and *psb*D-*psb*C gene cluster are shown in **A** and **B** respectively. The source of RNA is indicated above each *lane*. Albino pollen plants are prefixed with an A. *Root* and *Green* refer to RNA extracted from the roots and leaves, respectively, of light-grown seedlings. Loading of total RNA per lane were 7.0 μg (**A**) and 3.5 μg (**B**). The position of the 1.7-kb *rbc*L mRNA is indicated. The positions of the 3.2-kb and 1.7-kb *psb*D-*psb*C derived transcripts are indicated with asterisk. Additional 5.5-kb and 2.9 kb bands found only in A501 and A502 are similarly marked. The position and size of RNA molecular weight markers are shown on the *left* of the autoradiograph shown in **B**

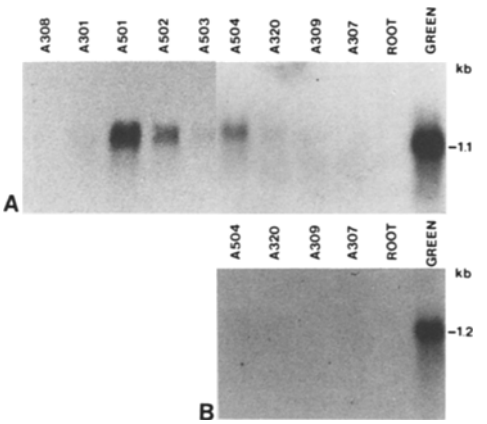

Fig. 8 A, B. Detection of *rbc*S and *cab* mRNAs in albino plants. Representative autoradiographs of Northern blots hybridised with cDNA probes for barley *rbc*S (**A**) and *cab* (**B**) genes are shown. The source of RNA is indicated above each *lane*. Albino pollen plants are prefixed with an *A*. *Root* and *Green* refer to RNA extracted from the roots and leaves, respectively, of light-grown seedlings; 7.0 μg of RNA was loaded per lane. The positions of the 1.1-kb *rbc*S and 1.2-kb *cab* mRNAs are indicated

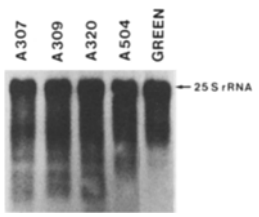

Fig. 9. Detection of 25S rRNA in albino plants. Autoradiograph of a Northern blot probed with the coding region of the wheat 25S rDNA. The source of RNA is indicated above each *lane*. Albino pollen plants are prefixed with an A, and *Green* refers to RNA from the leaves of light-grown seedlings; 7.0 μg of RNA was loaded per lane. The position of the 25S rRNA band is indicated by an arrow on the *right*

the levels were estimated to be around 10% and 25%, respectively of those in green leaves. Southern analysis indicated that this reduction in transcript levels was not due to large-scale alteration or deletion of the *rbc*S and *cab* genes (data not shown).

Abundance of the 25S rRNA in albino pollen plants

Equal amounts of RNA from albino pollen plants and green leaves were subjected to Northern analysis using a probe encoding the wheat 25S rRNA (Fig. 9). Levels of the 25S rRNA were similar in albino pollen plants and the leaves of light-grown seedlings. Indicating that the expression of a nuclear gene that does not code for a plastid-localised product remains unaffected in albino pollen plants.

Discussion

Albino barley pollen plants have been shown to contain plastids which are developmentally arrested prior to the differentiation of proplastids into chloroplasts (Clapham 1973; Dunford 1989). Consistent with data obtained from albino barley pollen plants regenerated from callus, we have shown that those derived by direct embryogenesis contain mixed populations of deleted ptDNAs. Interestingly, the overall pattern of ptDNA deletion did differ between these two groups of plants, in that restriction fragments mapping to the IR and SSC regions were always absent from callus-derived albinos (Day and Ellis 1985) but were often present in those regenerated by direct embryogenesis. We have also shown that most of the remaining ptDNA is less abundant in albinos than in normal green plants, but that certain restriction fragments seem to be present at an elevated copy number. This is reminiscent of the deleted mitochondrial genomes found in so-called petite strains of the yeast *Saccharomyces cerevisiae*, which consist of a short conserved sequence amplified many times (Dujon 1981).

Our Northern analyses have shown that albino pollen plants contain reduced steady-state levels of plastid encoded transcripts, including those derived from the rRNA operon, *rbc*L and the *psb*D-*psb*C gene cluster. Moreover, mRNAs transcribed from two nuclear gene families encoding chloroplast-localised proteins were also found at reduced levels in albino plants. Differences in the pattern of ptDNA deletion appeared not to affect the steady-state levels of these RNAs, since their abundance was uniformly low in all the albinos examined. Obviously, the presence of deletions in the ptDNA of albino plants will abolish transcription of those genes that are lost. One example is provided by albino A308, which contains a truncated P8 fragment from which part of the plastid rRNA operon, including the 23S rDNA, has been deleted (see Fig. 2). This correlates with the observation that A308 contains low levels of 16S rRNA while the 23S rRNA is undetectable. The accumulation of RNAs encoded by intact ptDNA could also be affected by deletions that inactivate genes involved in transcription and RNA stability. Deletions could also change the conformational state of ptDNA, and it is known that differences in the degree of supercoiling of a ptDNA template can affect the extent to which it is transcribed in vitro (Stirdivant et al. 1985; Lam and Chua 1987). Similarly, knowledge of the regulatory pathways which modulate nuclear gene expression in normal plants (Kuhlemeier et al. 1987; Tobin and Silverthorne 1985) offers a number of explanations for the reduced levels of *rbc*S and *cab* mRNA in albino plants. For example, there is evidence to suggest that the presence of a functional chloroplast is required for the activation of *cab* gene transcription in barley (Batschauer et al. 1986) and this could explain why albino pollen plants contain low levels of *cab* mRNA.

An additional Northern experiment demonstrated that the steady-state levels of the nuclear encoded 25S rRNA, which is localised in the cytoplasm as a major component of 80S ribosomes, remains unaffected in albino pollen plants. This result suggests that the reduction in RNA levels seen in albinos may be confined only to those genes that encode products that are plastid components.

Some of the albinos regenerated via both callus and embryogenesis have ptDNA restriction patterns which are indistinguishable from that of an intact plastid genome (Day and Ellis 1985; this study). It would be of interest to subject these apparently intact abino ptDNAs to a more detailed structural analysis in order to determine whether previously undetected mutations hav occurred. Precise localisation of these lesions might then identify ptDNA sequences that have an essential role in chloroplast development.

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