

Comparison of the Polypeptide Complement of Different Plastid Types and Mitochondria of *Narcissus pseudonarcissus*

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The protein complement of chloroplasts, prochromoplasts, chromoplasts, and mitochondria of the daffodil (*Narcissus pseudonarcissus*) has been investigated comparatively by gel electrophoresis. Mitochondria do not share common proteins with plastids. On the other hand, there is a broad overlap of the protein complement of the three plastid types investigated, in spite of their different fine structures. There are, however, remarkable differences regarding the relative amounts of many protein species, and certain plastid proteins are entirely absent either from the chloroplasts (e.g., 2 chromoplast proteins in the 60 kDa range) or the chromoplasts (e.g., LHCP). The protein complement of prochromoplasts, which contain chlorophylls and the LHCP, is nevertheless quite more similar to the protein pattern of the chromoplasts than to the one of the chloroplasts.

Introduction

The higher plant plastids exist in several different forms which are correlated with their different functions [1]. Since chloroplasts are of prime importance for every green plant, they are generally assumed to be the oldest form in terms of phyletic evolution. Chromoplasts, on the other hand, probably represent a relative young plastid type, as their occurrence is restricted to flowering plants [2]. Chromoplasts differ from chloroplasts not only in their pigmentation but also in their internal fine structure and their function. They do not contain chlorophylls or thylakoids and cannot perform photosynthesis. Their main function lies in the attraction of animals for pollination or fruit and seed dispersal.

Despite the many differences between chromoplasts and chloroplasts, they can be transformed into each other [3], and they possess qualitatively identical genetic information [4, 5], although in variable quantity [6] and different states of activity [7]. Certain chloroplast metabolic pathways have been shown also to exist in chromoplasts [8–10].

Abbreviations: LHCP, light harvesting chlorophyll *a/b*-binding-protein; LHC II, light harvesting complex II; RuBPCO, ribulose-1,5-bisphosphate carboxylase oxygenase; LSU, large subunit; SDS-PAGE, sodium dodecyl-sulfate-polyacrylamide gel electrophoresis; kDa, kilodaltons.

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In recent years, numerous chloroplast proteins of different species have been detected and characterized by diverse methods including gel electrophoresis [11]. The protein complement of other forms of plastids has been investigated only occasionally, most often with regard to only one single characteristic protein species [12–14]. On the basis of these data, no comparison is as yet possible of the polypeptide complement of different plastid types.

During their development, chromoplasts pass through a green stage, designated as the prochromoplast [15]. It is not known whether prochromoplasts resemble chloroplasts in their protein complement. In the course of investigations on chromoplast differentiation in the daffodil, the chromoplast polypeptide pattern has been compared with the respective chloroplast and prochromoplast pattern and a corresponding comparison has been made for defined subfractions of these plastid types. As it was not possible to obtain plastidal fractions entirely free of mitochondrial contamination, the proteins of a mitochondrial fraction were also investigated.

Materials and Methods

Daffodils (*Narcissus pseudonarcissus* L. cv. “Golden Harvest”) were cultivated outdoors or bought from the local market.

Isolation of organelles

Chromoplasts and mitochondria from the coronae of open flowers were isolated as described by Lied-



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vogel *et al.* [16], but using another isolation medium (0.33 M sorbitol, 50 mM Tricine/KOH pH 8.0, 3 mM Na₂EDTA, 1 mM 2-mercaptoethanol, 0.2% Polyvinylpyrrolidone). Chromoplast bands (at 15/30% [w/v], and 30/40% of a discontinuous sucrose gradient) and the mitochondrial band (40/50% sucrose) were removed separately, mixed 1:1 with buffer I (0.33 M sorbitol, 50 mM Tricine/KOH pH 8.0, 3 mM Na₂EDTA) and pelleted at $17\,000 \times g$ for 20 min. For further purification the two pellets were resuspended in buffer I and layered onto discontinuous Percoll density gradients consisting of steps of 5, 25, and 50% [v/v] Percoll, each in a solution of 0.28 M sucrose, 50 mM Tricine/KOH pH 8.0 and 3 mM Na₂EDTA. After centrifugation in a fixed angle rotor (30 min, $11\,000 \times g$) the chromoplast band (5/25% [v/v] Percoll) and the mitochondrial band (25/50% [v/v] Percoll) were removed, diluted 1:1 with buffer I, and the mixture spun down at $12\,000 \times g$ for 20 min. The organelles were finally washed once in buffer I.

Prochromoplasts were isolated from the coroneae of close flower buds as described above for chromoplasts, but using a slightly modified sucrose gradient, the 40/50% [w/v] sucrose step being replaced by a 45/55% [w/v] sucrose step. *Chloroplasts* were isolated from green leaves. 600 g of leaves were homogenized in 500 ml ice cold isolation medium (0.6 M sorbitol, 50 mM Tricine/KOH pH 8.0, 3 mM Na₂EDTA, 1 mM 2-mercaptoethanol) in a Waring Blender (5 × 3 s at maximum speed). After filtration through three layers of fine mesh nylon cloth and centrifugation at $200 \times g$ for 2 min, the chloroplasts were pelleted from the supernatant at $1\,500 \times g$ for 10 min. The pellet was then resuspended in buffer II (0.6 M sucrose, 50 mM Tricine/KOH pH 8.0, 3 mM Na₂EDTA) and layered onto a continuous sucrose gradient (1 M–1.9 M sucrose in 50 mM Tricine/KOH pH 8.0, 3 mM Na₂EDTA). After 2 h centrifugation at $80\,000 \times g$ in a swinging bucket rotor, the lower green band (containing intact chloroplasts) was removed, mixed 1:1 with buffer II, and the chloroplasts pelleted ($3\,000 \times g$, 25 min).

Fractionation of organelles

The organelles were first fractionated into matrix and membranes according to procedures already described for chloroplasts [17] and for chromoplasts [10], the latter procedure was also used in the case

of prochromoplasts and mitochondria. The proteins of the membrane fractions were then separated into integral and peripheral proteins according to Fujiki *et al.* ([18], cf. Fig. 1).

SDS-Polyacrylamide gel electrophoresis

All fractions were dialysed against aq. dest. Dilute solutions were lyophilized if necessary, and resuspended in aq. dest. Proteins were determined according to Bradford [19] with bovine serum albumin as a standard. SDS-PAGE was carried out as described by Chua [20], but using a 4% [w/v] acrylamide stacking gel and an 8–18% [w/v] acrylamide gradient separation gel. Molecular weight determinations were carried out on 10 and 15% [w/v] acrylamide separation gels using RNA polymerase of *Escherichia coli* (M_r 165 000, 155 000, 39 000), bovine serum albumin (68 000), ovalbumin (45 000), chymotrypsinogen (24 000), β -lactoglobulin (18 400), and lysozyme (egg white, 14 300) as markers. Some plastid proteins could be tentatively identified by comparing their electrophoretic mobilities with published data. The comparative protein analysis by partial peptide mapping was carried out according to Cleveland *et al.* [21].

Electron microscopy

Isolated organelles were fixed and sectioned according to Liedvogel *et al.* [16], membrane fractions according to Oleszko and Moudrianakis [22].

Results

Purity of the organelles

EM-evidence showed both chloroplast and mitochondrial fractions used to be free of detectable contamination (Fig. 2a, d). The chromoplast and prochromoplast fractions, however, were found to be slightly contaminated with mitochondria (less than 2% of the particle numbers). In all cases, however, the double limiting membranes of the organelles were preserved during isolation. When observed in the EM, all membrane fractions appeared virtually free of contamination by matrix (Fig. 2e–h). Both closed vesicles and membrane sheets could be detected. Although peripheral proteins could not be observed, the appearance of the various membranes is different, probably due to

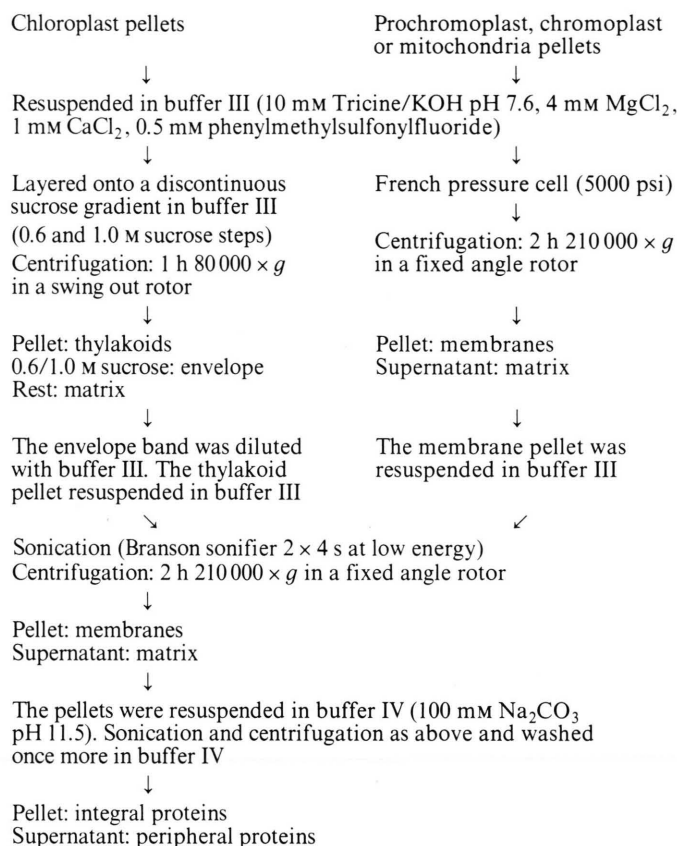


Fig. 1. Flow diagram for the fractionation of organelles from *Narcissus pseudonarcissus* (all steps at 4 °C).

their different protein content and their correspondingly different molecular architecture.

Subfractions of the organelles (see Fig. 3)

A direct comparison of polypeptide patterns of the different subfractions of each organelle in the SDS-PAGE system is shown in Fig. 3. *Chloroplasts* were separated into matrix, peripheral envelope proteins, integral envelope proteins, peripheral thylakoid proteins, and integral thylakoid proteins (Fig. 3a). It appears that many proteins of the matrix (lane B) are bound to the envelope membranes (lane C₂), either specifically or unspecifically. In contrast matrix proteins were not bound to the thylakoids (lane C₁). The integral proteins of the thylakoids and the envelope fraction could be separated into approximately 30 bands in both cases.

The patterns differ distinctly from each other as well as from the peripheral membrane proteins of both fractions.

In the case of *prochromoplasts* the yield of sufficiently pure fractions was too small to permit the separation of 5 subfractions. Thus, only 3 subfractions were separated, the membrane fraction comprising both envelope and thylakoid membranes (Fig. 3b). Some of the bands of the integral membrane proteins (lane D) are also detectable in the peripheral protein fraction (lane C). These bands are either occupied by different protein species (integral and peripheral) or the fractionation method was not sufficient.

In contrast to the *prochromoplasts*, the fully differentiated *chromoplasts* do not contain any thylakoids. Consequently, the "membrane fraction" in this case consists of envelope membranes and

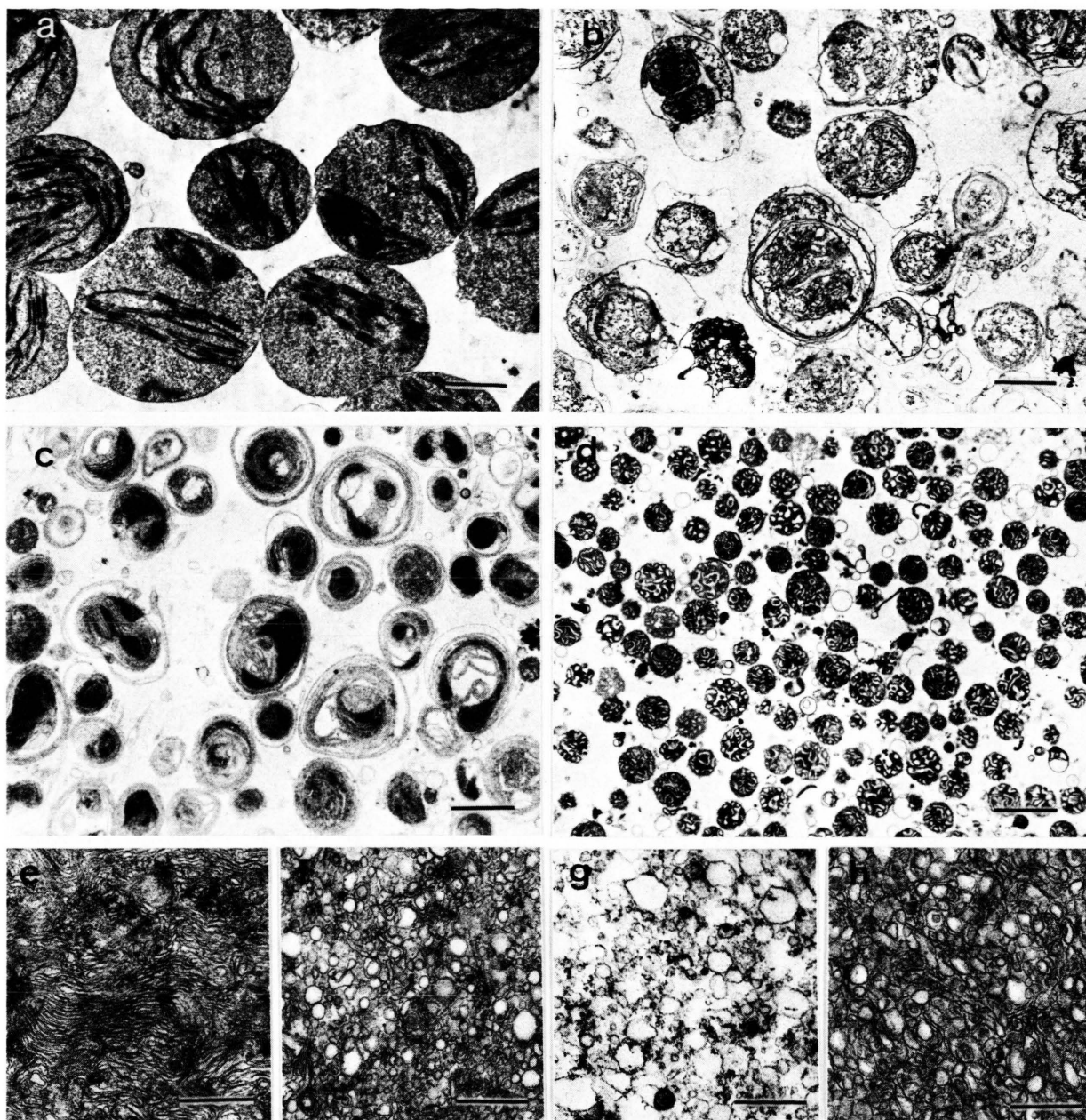


Fig. 2. Isolated organelles (a–d) and membranes washed with sodium carbonate (e–h). Chloroplasts a + e (thylakoids), prochromoplasts b + f, chromoplasts c + g, mitochondria d + h. Magnifications: a–d 4600 \times , e–h 21 600 \times . Bar: a–d 2 μ m, e–h 0.5 μ m.

chromoplast internal membranes (CIMs), most probably derived from the envelope. These membranes are extremely rich in lipid [12]. This made SDS-PAGE of the integral membrane proteins difficult, and certain irregularities could be ob-

served especially in the lower M_r -region (Fig. 3c; lane D). However, as lipid depletion with either chloroform/methanol (2/1) or acetone (80%, v/v) resulted in the loss of some proteins, no lipid extraction was performed. When applied to mito-

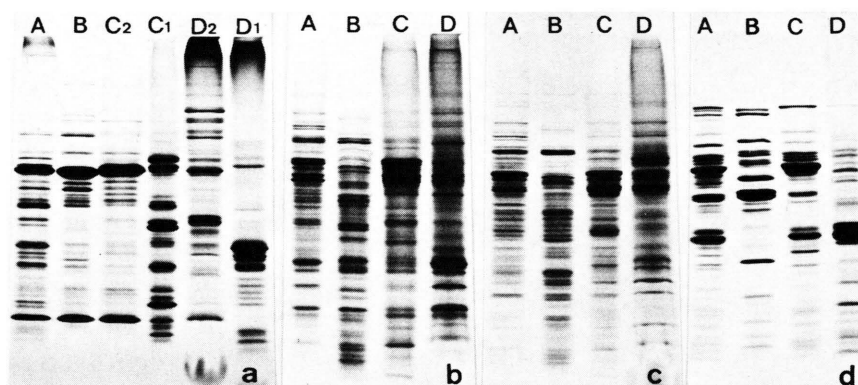


Fig. 3. SDS-polyacrylamide gel electrophoresis of the proteins of chloroplasts (a), prochromoplasts (b), chromoplasts (c), and mitochondria (d). A) whole organelles, B) matrix, C) peripheral proteins of the membranes (1 = thylakoids; 2 = envelope), D) integral proteins of the membranes.

chondria, the isolation and separation procedures yielded remarkably pure fractions, each of which showed a distinctive polypeptide pattern in SDS-PAGE (Fig. 3d).

Comparison of the organelle proteins

(see Fig. 4 and Table I)

Proteins of the matrix (Fig. 4b)

Most of the chloroplast matrix polypeptides (lane A) are also present in the matrix fraction of (pro-)chromoplasts (lane B and C). However, the (pro-)chromoplast matrix fraction yields more bands than the chloroplast matrix. This is probably due to the fact that in all cases an equal amount of protein (by weight) was applied to the gel, but in the case of the chloroplast fraction the two subunits of RuBPCO (lane A: P2 and P5) account for more than 50% of the protein whereas in (pro-)chromoplasts these proteins were only present in very small amounts. Therefore the relative proportion of other matrix proteins in the (pro-)chromoplast fraction is much higher.

Peripheral membrane proteins (Fig. 4c)

Two membrane fractions could be obtained from chloroplasts, namely envelope membranes and thylakoids. Both of them contain the two subunits of RuBPCO as peripheral membrane proteins. In the case of the envelope membranes these are the prevailing protein species (lane A₂: P1 and P2),

whereas among the peripheral thylakoid protein the polypeptides of CF₁-complex predominate. According to published data [23], the respective bands can be tentatively assigned as follows (lane A₁): α -P1; β -P2; γ -P4; δ -P7. In prochromoplasts and chromoplasts, the subunits of the CF₁-complex are present in only small amounts (e.g. lane C: P3, P4). The subunits of RuBPCO could be found in these two plastid types only as peripheral membrane proteins. Two main proteins of the prochromoplasts (lane B: P1, P2) and chromoplasts (lane C: P1, P2) are entirely absent from chloroplasts.

Integral membrane proteins (Fig. 4d)

Among the 7 major proteins of the chloroplast envelope membranes (lane A₂), 3 can be assigned as either the subunits of RuBPCO (P3, P7 [24]) or the phosphate translocator (P6 [25]). Among the thylakoid integral membrane proteins, the LHCP prevails (lane A₁: P2). This protein of the LHC II is also present in the prochromoplast membrane fraction (lane B: P6), but absent from chromoplast membranes (lane C). About 2/3 of the polypeptide species found in chromoplast membranes can also be detected in chloroplast membranes, ca. 2/3 of them in envelope membranes and 1/3 in thylakoids. From this it can be deduced that, during chromoplast development, certain integral membrane proteins are specifically degraded, whereas other are selectively synthesized.

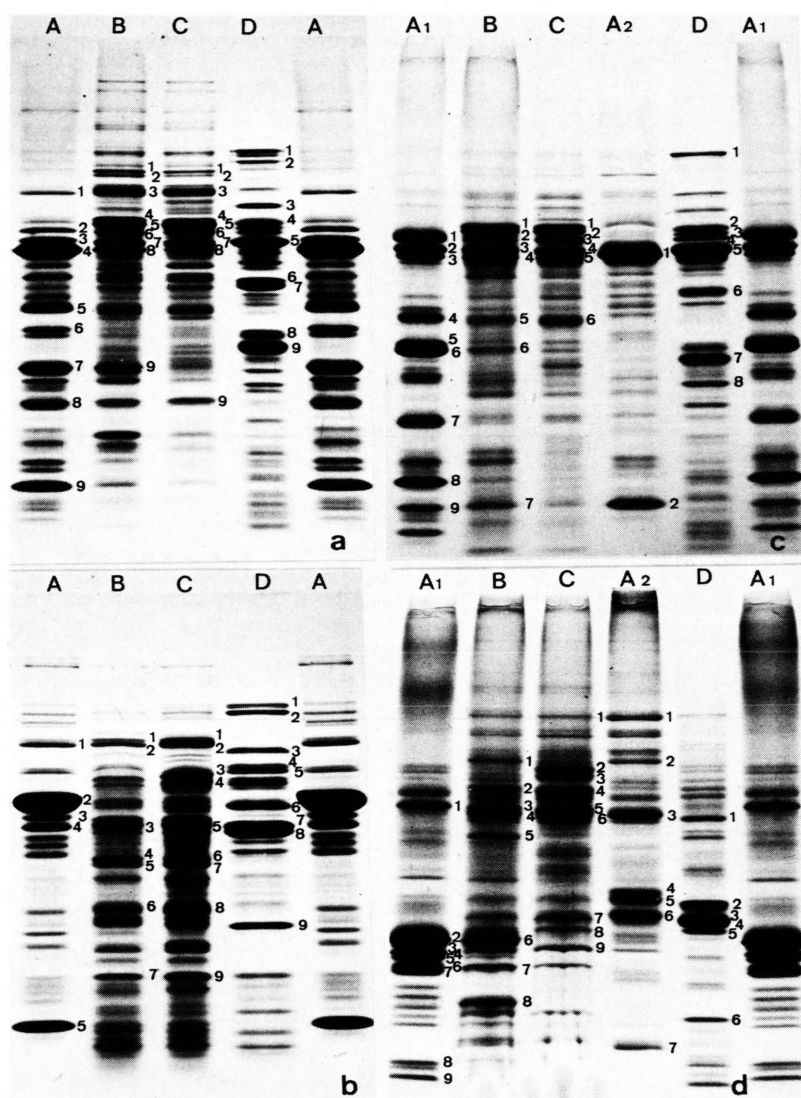


Fig. 4. SDS-polyacrylamide gel electrophoresis of the proteins of whole organelles (a), of the proteins from the matrix (b), of peripheral (c) and integral (d) proteins of the membranes. A) chloroplasts (1 = thylakoids, 2 = envelope), B) prochromoplasts, C) chromoplasts, D) mitochondria.

Discussion

In recent years, numerous chloroplast proteins have been characterized in some detail (*cf.* [11]). This is particularly true for the major functional complexes of the thylakoid membrane, *e.g.*, the CF₁-complex, photosystems I and II together with their respective light harvesting complexes, and the cytochrome *b₆/f*-complex (*cf.*, *e.g.*, [26–28]). The membranes of the chloroplast envelope have also

been investigated with improved technique [24, 29]. However, only 3 out of seven major polypeptides from envelope membranes could be definitely assigned so far, namely both subunits of RuBPCO [24] and the phosphate translocator [25].

On the basis of data in the literature, some of the polypeptides mentioned expectedly could also be found in the chloroplasts of the daffodil, namely both subunits of RuBPCO, the subunits of the CF₁-complex, the major polypeptides of the light

Table I. Molecular weights ($M_r \times 10^{-3}$) of some main proteins of the different organelles. Letters and numbers as in figure 4.

No.	Whole Organelles				Stroma				Membranes (peripheral)				Membranes (integral)					
	A	B	C	D	A	B	C	D	A ₁	A ₂	B	C	D	A ₁	A ₂	B	C	D
1	77.0	94.0	94.0	101	77.0	80.5	80.5	100	58.0	52.0	62.0	62.0	101	55.0	107	73.0	107	49.5
2	58.0	88.0	88.0	97.5	52.0	77.0	77.0	97.5	54.0	14.5	60.0	60.0	62.5	25.0	73.0	60.5	68.5	30.0
3	54.0	77.0	77.0	73.0	46.0	42.5	63.0	73.0	52.0		54.0	58.0	59.0	24.5	52.0	53.5	64.5	29.0
4	52.0	62.0	62.0	62.5	42.5	37.0	61.0	67.0	35.0		52.0	54.0	57.0	24.0	32.0	52.0	60.5	28.0
5	35.0	60.0	60.0	53.0	14.5	36.0	41.5	62.5	31.0		35.0	52.0	53.0	23.5	31.5	44.0	53.5	26.5
6	31.0	58.0	58.0	41.0		28.5	37.0	48.0	29.5		29.5	35.0	41.5	23.0	29.0	25.0	52.0	17.0
7	25.0	54.0	54.0	40.0		21.0	36.0	41.0	23.0		14.5		29.0	22.5	14.5	23.0	29.0	
8	23.0	52.0	52.0	30.0			28.0	40.0	17.0				27.0	13.0		18.0	26.0	
9	14.5	25.0	23.0	29.0			21.0	26.5	14.5					11.0			24.0	

harvesting complex II and the phosphate translocator. Much less is known about chromoplast proteins. Chromoplasts of the tubulous type possess one major protein in the 32 kDa range associated with the chromoplast "tubules" [30]. This protein is essential for the formation of these "tubules". According to inhibitor experiments it is coded for in the nucleus [31, 32]. Two major proteins (30 kDa, 68 kDa) are associated with chromoplast lipid globules [33]. The chromoplast internal membranes of membranous chromoplasts exhibit a complex polypeptide pattern with no single species prevailing [16]. In the present study it could be shown that even full-grown chromoplasts in their final state of differentiation contain many polypeptides that, judging from their electrophoretic mobility, might correspond to certain chloroplast proteins involved in photosynthesis. This holds particularly true for both subunits of RuBPCO and several polypeptides of the CF₁-complex, the identity of which has been proven by peptide mapping (Fig. 5). This finding corresponds well with the recent demonstration of a potentially active chlorophyll synthetase in chromoplasts of the daffodil [10]. This enzyme is likely to be bound to thylakoids in chloroplasts [34]. Entirely absent from chromoplasts (but not from prochromoplasts) are the main polypeptides of the light harvesting complex II. The situation is reminiscent of that encountered in etioplasts which are also devoid of thylakoids and do not contain the proteins of the light harvesting complex II, but do contain subunits of the CF₁-complex and the photosystem I reaction centre protein [13]. Less surprising is the identity of many of the integral membrane proteins of the plastid envelope in both chloroplasts and chromoplasts.

On the whole, the differences in the protein complement of chromoplasts and chloroplasts are not as conspicuous as one would expect, at least with respect to qualitative diversity. There are, however, remarkable quantitative differences. It would thus appear that there exists a relatively complex complement of fundamental plastid polypeptides present in every form of plastid, albeit in varied proportions. Nevertheless, the differentiation of a particular plastid type as, e.g., chloroplasts or chromoplasts, may be correlated with the accumulation of certain protein species that consequently may be looked at as "specific" proteins of that particular state of plastid differentiation. For ex-

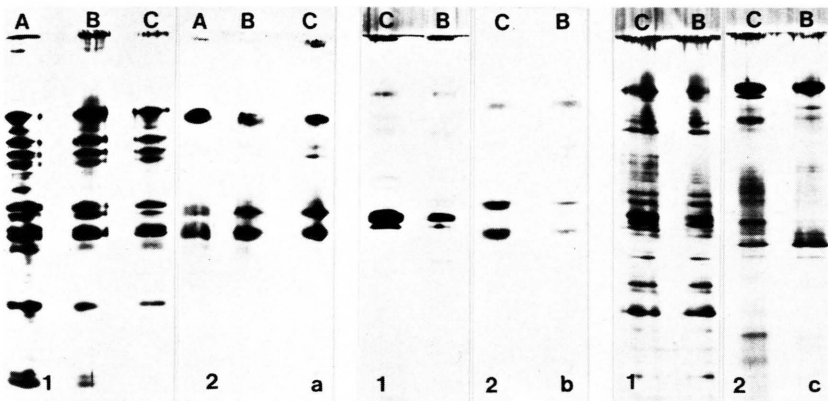


Fig. 5. Partial peptide maps of the LSU of RuBPCO (a), α -SU (b), and β -SU (c) of the CF_1 derived from the matrix of chloroplasts (lane A), membranes of chromoplasts (lane B), and thylakoids (lane C). Gels 1: *Staphylococcus aureus* V8 protease; gels 2: Papain.

ample, 2 main polypeptides of both prochromoplasts and chromoplasts (P4 and P5, cf. Fig. 4a) can only be found as traces in chloroplasts. On the other hand, chromoplasts are entirely lacking the LHCP, which is not only present in chloroplasts but also in prochromoplasts (where it however lacks chl b; cf. [15, 16].

Mitochondria have been shown, in the present study, to contain no (major) polypeptides of equal electrophoretic mobility to those of plastid polypeptides. This demonstrates that contamination of plastid fractions with mitochondria was negligible. It furthermore proves that posttranscriptional transport of organelle proteins, synthesized on 80S ribosomes in the cytoplasm, is highly specific. As far as a transfer of genetic information between plastids and mitochondria has taken place [35, 36], there seems to be no expression of the respective

sequences in the "wrong" organelle. On the other hand, there are some indications of similar (although not identical) electrophoretic mobilities of analogous proteins of plastids and mitochondria (e.g., polypeptides of the ATP-synthase complex: 50–58 kDa; several integral membrane proteins of the organelle envelopes 28–33 kDa). Conceivably this points to a phyletic relatedness of plastids and mitochondria.

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