UNSTABLE CHLOROPLAST RIBOSOMES IN THE COLD-SENSITIVE BARLEY MUTANT *tigrina-o*³⁴

by

GUNILLA HØYER-HANSEN and GIORGIO CASADORO¹⁾

Department of Physiology, Carlsberg Laboratory
Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Valby

¹⁾Permanent address: Istituto di Genetica, Via Amendola 165/A,
I-70126 Bari, Italy

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When grown at 20 °C the recessive nuclear gene mutant *tigrina-o*³⁴ in barley is deficient in 70S chloroplastic ribosomes, but contains wild-type levels of 80S cytoplasmic ribosomes. When grown at 30 °C the mutant seedlings have wild-type levels of both chloroplastic and cytoplasmic ribosomes. One-dimensional polypeptide patterns of chloroplast ribosomes of the mutant grown at the permissive temperature were the same as those of the wild-type.

Instability of chloroplastic ribosomes in the barley mutant grown at the restrictive temperature of 20 °C was correlated with the deficiency of chlorophyll *a*-proteins 1, 2 and 3, cytochrome *f*, coupling factor subunits α , β , ϵ and two thylakoid polypeptides (31, 20 kD) of unidentified function. These proteins have been shown in other species to be synthesized on chloroplastic ribosomes. Chlorophyll *a/b*-protein 2 synthesized on cytoplasmic ribosomes was present in wild-type amounts in the plastids of the mutant grown at the restrictive temperature. Five additional thylakoid polypeptides, (A, B, C, D, E) with as yet unknown function were also present at the restrictive temperature in normal amounts and are therefore predicted to be synthesized in the cytosol.

Electron microscopical analysis revealed that the chlorophyll *a/b*-protein 2 and other prominent thylakoid polypeptides in the mutant at 20 °C most likely are contained in the large dense grana and other abnormal membrane structures typical for the mutant.

Abbreviations: CF₁ = chloroplast coupling factor; Chl = chlorophyll; Chl-P = chlorophyll-protein; Cyt = cytochrome; DTT = dithiothreitol; kD = kilodalton; PAGE = polyacrylamide gel electrophoresis; SDS = sodium dodecyl sulfate; TMBZ = tetramethylbenzidine; TRIS = tris-(hydroxymethyl)-aminomethane.

1. INTRODUCTION

The nuclear gene mutant, *tigrina-o*³⁴, accumulates at 20 °C in the dark protochlorophyllide in amounts exceeding the wild-type level (16, 28) and is considered a constitutive mutant in a regulatory gene for protochlorophyllide synthesis (28, 32). The mutant also has an altered carotenoid composition at 20 °C in the dark, where it contains roughly only 10 per cent of the amount of β -carotene present in the wild-type leaves and accumulates large amounts of lycopene, the total amount of carotenoids being similar to that in the wild-type (28, 29). In the light the mutant accumulates chlorophyll *a* with an absorption maximum near 742 nm in addition to chlorophyll *a* absorbing at 676 nm (28). When the plant is grown at 20 °C under light-dark cycles its phenotype is *tigrina*, but when grown under constant illumination it is uniformly pale green. When the plant is grown at 30 °C considerably more chlorophyll accumulates and the ratio of chlorophyll *a* 742 to chlorophyll *a* 676 approaches zero, i.e., a rather complete normalization as to chlorophyll content occurs at the higher temperature (28).

The abnormal pigment content is accompanied by abnormal organisation of the thylakoid membranes. Under conditions giving a high chlorophyll *a* 742 and lycopene content the lamellar systems have an aberrant organisation with highly condensed grana (crystalloids) and undulating osmiophilic sheets, whereas under conditions giving a low chlorophyll *a* 742 content the thylakoid structure is like that of wild-type (28).

It has been reported for two mutants of sunflower blocked in β -carotene synthesis (31), a mutant cold sensitive for carotenoid synthesis in maize (25) and for β -carotene deficient heat-bleached rye seedlings (12) that their chloroplast ribosomes are unstable in light or not formed at all. Since *tigrina-o*³⁴ grown at 20 °C has small amounts of β -carotene, it is of interest to determine if seedling leaves of this mutant grown in the light at 20 °C contain the chloroplast specific 70S ribosomes (10). As we found a considerable deficiency of chloroplast ribosomes in these seedlings, we determined the ribosome content of the mutant seedlings grown at 30 °C, the temperature at which an almost normal seedling leaf colour in addition to

normalized chloroplast membrane structure is attained. The polypeptide composition of the thylakoids in mutant seedlings grown at the restrictive temperature (20 °C) is compared to that of mutant seedlings grown at the permissive temperature (30 °C).

2. MATERIALS AND METHODS

2.1. Plant material

Seeds of wild-type barley (*Hordeum vulgare* cv. Svalöf's Bonus) or the mutant *tigrina-o*³⁴ were germinated in tap water moistened vermiculite. They were grown in continuous white light (1700 lux) at either 20 °C or 30 °C. They were harvested after 7 days when grown at 20 °C and after 6 days when grown at 30 °C to permit analysis at comparable stages of development. The mutant seedlings were distinguished from the wild-type by their paler green colour.

2.2. Isolation of ribosomes

Ribosomes were isolated employing a modification of the procedure described by CHUA, BLOBEL and SIEKEVITZ (4). All glass-ware and solutions were sterilized before use. All operations were carried out at 4 °C. Between 10–40 g of leaves were ground in liquid nitrogen in a mortar. The leaves were ground to a fine powder before isolation buffer, which contained 25 mM-Tris-HCl pH 7.5, 25 mM-MgCl₂, 25 mM-KCl, 5 mM-DTT and 1 or 2% Triton X-100 (v/v) was added. To 12.5 g of leaves 10 ml isolation buffer was added. The paste was homogenized with a motor-driven teflon pestle and centrifuged 10 min at 18,000 $\times$ g in an SS-34 rotor. The supernatant was filtered through a layer of Miracloth and spun at 40,000 $\times$ g for 10 min. Then 29 ml of the resulting supernatant was layered on a 10 ml sucrose cushion consisting of 1.87 M-sucrose, 25 mM-Tris-HCl, pH 7.5, 25 mM-MgCl₂, 25 mM-KCl and 5 mM-DTT in a quick seal tube and spun at 325,000 $\times$ g at 4 °C for 20 hours in a 60 Ti rotor. The pellet, consisting of a mixture of 70S and 80S ribosomes, was washed twice with ice-cold distilled water and resuspended in water. The ratio of A₂₆₀ to A₂₈₀ varied between 1.75 and 1.86.

The 80S and 70S ribosomes were separated on a 10–30 % sucrose gradient which also contained 25 mM-Tris-HCl, pH 7.5, 25 mM-KCl, 5 mM-MgCl₂ and 5 mM-DTT. The gradients were centrifuged at 4 °C for 4 hours at 85,000 × g in an SW 40 rotor. After centrifugation 0.5 ml fractions were collected from the top. Absorption at 260 nm and 280 nm was monitored continuously.

If not used immediately the ribosomal pellets and the fractions from the gradients were stored at –80 °C for later protein analysis on SDS-PAGE.

2.3. Isolation of purified thylakoids

Thylakoids for electrophoretic analysis were purified by flotation in a discontinuous gradient as earlier described (19). If they were not used immediately they were frozen in small aliquots in 0.1 M-Na₂CO₃-0.1 M-DTT at –20 °C. Chlorophyll was determined according to ARNON (2).

2.4. SDS-PAGE

SDS-PAGE was performed according to published procedures (5). The molecular weights of the ribosomal polypeptides were determined as described in (20). The gels were stained with TMBZ-H₂O₂ according to (21) and with Coomassie blue.

2.5. Preparation of monoclonal antibodies and immune-blotting assay

The monoclonal antibody employed was prepared according to (30). A mouse was injected three times with 100 µg protein of a photosystem I particle suspension prepared by Dr. BIRGER LINDBERG MØLLER and described in (18, 27). The third day after the third injection the mouse was sacrificed.

Positive clones were selected 2–4 weeks after cell fusion by an immune-blotting assay essentially as described in (30). The antigen consisted of total thylakoid polypeptides which had been transferred to the nitrocellulose filter (Millipore GSWP, 0.22 µm pore size). Reference strips of the nitrocellulose filter were stained directly after protein transfer in 0.1 % Amido Black. Positive hybridomas were cloned by limited dilution (30).

2.6. Electron microscopy

Specimens for transmission electron microscopy were sampled at different levels along the primary leaf. All tissue samples were fixed for 2 hours in 3 % glutaraldehyde in 0.1 M-cacodylate buffer, pH 6.9, washed in buffer and postfixed for 2 hours in 1 % osmium tetroxide in the same buffer. The samples were dehydrated in a graded series of ethyl alcohol and propylene oxide. Staining with uranyl acetate was carried out while dehydrating with 75 % ethanol. Tissue was embedded in Spurr's resin and sections were cut with a Reichert Om U3 ultra-microtome. Sections were stained with uranyl acetate and lead citrate in an LKB 2168 Ultrastainer, System Carlsberg, before being examined in a Siemens Elmiskop 102 operating at 80 kV.

3. RESULTS

3.1. Ribosome content in *tigrina-o*³⁴ seedlings grown at 20 °C or 30 °C

The crude ribosomes obtained after centrifugation through the sucrose cushion were immediately separated on a 10–30 % sucrose gradient. It was found that the 70S peak of the *tigrina-o*³⁴ seedlings grown at 20 °C was very small, whereas the amount of 80S ribosomes was comparable to that of wild-type (Figure 1). At 30 °C the mutant and wild-type had comparable amounts of both 70S and 80S ribosomes (Figure 1). The MgCl₂ content was lowered to 5 mM in the gradients to achieve a better separation of the 70S ribosomes from their 80S counterparts, as it has been shown in *Chlamydomonas* (4) that the 70S ribosomes, but not the 80S ribosomes, sediment slower when the Mg²⁺ concentration is lowered. We also varied the sucrose concentration in the gradients, but this did not influence the separation of the 70S from the 80S ribosomes. Separation was maximal with centrifugation at 85,000 × g for 4 hours. Increased speed and shorter times or lower speed and longer time (up to 15 hours) resulted in poorer resolution of the ribosomal peaks.

3.2. Polypeptide composition of 70S and 80S ribosomes

Lack of assembly of ribosomes can be due to an altered ribosomal protein. In the case of

temperature-sensitivity a variant ribosomal protein may allow ribosome assembly at one temperature but not at the other, as is known for

temperature-sensitive mutants in bacteria (15, 22). Therefore, the polypeptide patterns of the 70S and 80S ribosomes of *tigrina-o*³⁴ grown at

Figure 1. Separation of 70S and 80S ribosomes from wild-type and *tigrina-o*³⁴ grown at 20 °C and 30 °C on a 10–30% sucrose gradient.

In each experiment 5 OD₂₆₀ units of crude ribosomes were applied. The gradients were collected from the top, and OD₂₆₀ (—) and OD₂₈₀ (---) were monitored continuously. The ribosomal peaks were identified by their characteristic polypeptide pattern.

20 °C and 30 °C were compared with the corresponding wild-type patterns.

It is evident from Figure 2 that the ribosomal

protein patterns from 70S and 80S ribosomes from wild-type grown at 20 °C are clearly distinguishable. Thirty-five polypeptide bands

Figure 2. SDS-PAGE pattern of 70S and 80S ribosomal proteins from wild-type barley and *tigrina-o³⁴* grown at 20 °C and of 70S ribosomal proteins from wild-type and the mutant grown at 30 °C.

Apparent molecular weights are in kD and were determined as in (20).

can be recognized in the electrophoretogram of the cytoplasmic ribosomes. Twenty-nine bands are visible in the pattern of the chloroplast ribosomes. Cross contamination of the two types of ribosomes was unavoidable with the gradients used.

Not enough 70S ribosomes were obtainable from *tigrina-o*³⁴ grown at the restrictive temperature to give a polypeptide pattern characteristic for chloroplast ribosomes. Instead a weak pattern identical with that of the 80S ribosomes was obtained (Figure 2, slot 3), when the relevant gradient fractions were analysed revealing that the majority of ribosomes in these fractions were of the 80S type.

A comparison of the 70S ribosomal polypeptides from wild-type and *tigrina-o*³⁴ grown at 30 °C revealed no difference (Figure 2, slots 5 and 6). Thus, with the limited resolution inherent in the present analysis no variant polypeptides could be spotted in the mutant.

3.3. Polypeptide composition of the thylakoid membrane of *tigrina-o*³⁴ grown at 20 °C and 30 °C

A mutant deficient in 70S ribosomes is expected to be deficient also in the polypeptides that are synthesized on the chloroplast ribosomes. By means of specific inhibitors for protein synthesis on cytoplasmic and chloroplastic ribosomes, and genetic analysis, it has been shown that chlorophyll *a*-proteins 1, 2 and 3 in *Chlamydomonas* (CPI, CPIII, CPIV) are synthesized on chloroplast ribosomes (6, 7, 8), whereas the major light harvesting chlorophyll-protein in the thylakoid membrane, chlorophyll *a/b*-protein 2 in barley is synthesized on cytoplasmic ribosomes (1). As for the chlorophyll *a/b*-protein 2, *in vitro* protein synthesis was employed to show that CF₁ subunits α , β and ϵ of spinach are synthesized on chloroplast ribosomes, and γ and δ subunits on cytoplasmic ribosomes (24).

Figure 4. Staining for heam-associated peroxidase activity with TMBZ-H₂O₂ as in (21).

In each slot 25 µg chlorophyll was applied and the samples were heated prior to electrophoresis. The stained bands are marked with arrows.

Figure 3. Thylakoid polypeptides from wild-type and *tigrina-o*³⁴ grown at 20 °C and 30 °C.

For SDS-PAGE 25 µg of chlorophyll was applied in each slot. In the non-heated samples the chlorophyll-proteins were marked with a steel-thread prior to staining for protein with Coomassie blue. The heated samples were boiled for 2 minutes prior to application on the gel.

In the thylakoid polypeptide pattern of *tig-rina-o*³⁴ grown at 20 °C, chlorophyll *a*-proteins 1, 2 and 3, and all the subunits of CF₁, are present in very low amounts or absent (Figure

3). On the other hand, the chlorophyll *a/b*-protein 2 is present in amounts exceeding those of wild-type, which is to be expected since the same amount of chlorophyll was loaded in each

Figure 5. Immune-blotting assay for identification of chlorophyll *a*-protein 1 using a monoclonal antibody.

The thylakoid polypeptides (25 µg chlorophyll applied) were separated on SDS-PAGE. The polypeptides were blotted over to the nitrocellulose filter. This was then employed in the immune-blotting assay using a monoclonal antibody raised against photosystem I particles containing chlorophyll *a*-protein 1 and as secondary antibody a rabbit anti-mouse antibody coupled to peroxidase. Colour was developed with orthodianisidine and H₂O₂. Reacting polypeptides are marked with arrows.

slot. Also present in normal amounts are polypeptides A, B, C, D and E with as yet unknown functions. When the mutant is grown at 30 °C the green chlorophyll *a*-protein 1 is prominent as are the apoproteins of chlorophyll *a*-proteins 2 and 3. Enough chlorophyll *a*-proteins 2 and 3 was, however, not present in the non-heated samples of the mutant grown at 30 °C to reveal green bands on the gel (Figure 3).

Cytochromes *f* (9) and *b₅₅₉* (33) have been shown to be synthesized on chloroplast ribosomes. Likewise, none of the chloroplast cytochromes (*f*, *b₅₅₉*, *b₆*) were detectable in 70S ribosome deficient leaves of rye (12, 13). Cytochrome *f* and a polypeptide band with an approximate molecular weight of 20 kD can be stained with tetramethylbenzidine (TMBZ) and hydrogenperoxide (21). Figure 4 presents an electrophoretogram of haem stained polypeptides from *tigrina-o³⁴* grown at 20 °C and 30 °C in comparison with their wild-type counterparts. It can be seen that the contents of cytochrome *f* and the 20 kD band are significantly smaller in *tigrina-o³⁴* grown at 20 °C than at 30 °C, thus correlating with the reduction in ribosomes at the lower temperature. The high sensitivity of the TMBZ-H₂O₂ stain (21) may be responsible for the relatively strong staining of the bands present in the pattern of the polypeptides from the mutant leaves containing only few chloroplast ribosomes at the restrictive temperature.

There is a small amount of protein stained with Coomassie blue at the position of chlorophyll *a*-protein 1 in electrophoretograms of *tigrina-o³⁴* grown at 20 °C, but no visible chlorophyll-band (Figures 3, 5). When the solubilized thylakoid membranes are heated prior to electrophoresis the chlorophyll *a*-protein 1 loses its chlorophyll and the apparent molecular weight shifts from 110 kD to 67 kD. The polypeptide in the position of chlorophyll *a*-protein 1 of *tigrina-o³⁴* grown at 20 °C also changed its mobility upon heating (Figure 3). To further prove that the polypeptide in question is chlorophyll *a*-protein 1 it was reacted in immune-blotting assays with a monoclonal antibody raised against a photosystem I particle containing chlorophyll *a*-protein 1 (Figure 5). A clear reaction is obtained with a polypeptide at the position of the apoprotein for chlorophyll *a*-protein 1 from wild-type leaves at both tempera-

Figure 6. Illustration of the positions along the primary leaf where samples have been collected for electron microscopy.

tures and from *tigrina-o³⁴* grown at 30 °C. A weaker reaction is obtained with *tigrina-o³⁴* grown at 20 °C. The antibody, which has been shown to be monoclonal (manuscript in preparation), reacts also with a polypeptide of lower

Figures 7 & 8. Chloroplasts at level 1.

In *tigrina-o*³⁴ grown at 20 °C (Figure 7) and 30 °C (Figure 8) the young chloroplasts show a normal appearance. Starch granules (S) are usually present in these plastids. (Figure 7, $\times 37,000$; Figure 8, $\times 46,000$).

Figures 11 & 12. Chloroplasts at level 3.

In *tigrina-o³⁴* grown at 20 °C (Figure 11) the plastids are abnormal and plastid ribosomes appear absent. *Tigrina-o³⁴* grown at 30 °C (Figure 12) has a normal thylakoid system and plastid ribosomes are present. (Figure 11, $\times 42,000$; Figure 12, $\times 48,000$).

molecular weight (31 kD) and so far unknown function (arrow in Figure 5). This must be due to a structural similarity (26) in the two reacting polypeptides.

3.4. Chloroplast membrane structure of *tigrina-o³⁴* grown at 20 °C and 30 °C

The chloroplast structure was examined along the primary leaf of the mutant grown at 20 °C

Figures 9 & 10. Chloroplasts at level 2.

Figure 9 shows a mixture of normal and abnormally structured plastids for *tigrina-o³⁴* grown at 20 °C. Where grana are visible (right plastid) they have an unusually large diameter and lycopene induced modifications start to appear (arrow). At this level the mutant grown at 30 °C (Figure 10) has normal chloroplasts. (Figure 9, $\times 33,000$; Figure 10, $\times 40,000$).

Figures 13 & 14. Chloroplasts at level 4.

In *tigrina-o*³⁴ grown at 30 °C, most of the plastids have a normal appearance (Figure 13), but some abnormal plastids are also observed (Figure 14). The latter show both structures characteristic for lycopene accumulation (arrow) and a strong increase of membrane electron-density of the unusually long grana profiles (double arrows). (Figure 13, $\times 31,800$; Figure 14, $\times 31,500$).

and 30 °C, respectively, as illustrated in Figure 6. The very young chloroplasts at level 1 in *tigrina-o*³⁴ whether grown at 20 °C or at 30 °C have a normal appearance (Figures 7 and 8). At level 2 the mutant grown at 20 °C has a mixed population of chloroplasts (Figure 9), the majority of the plastids having abnormal lamellar structures as already described by NIELSEN (28). Structural modifications typical for lycopene accumulation start to be visible (Figure 9, arrow). At the same level of the primary leaf *tigrina-o*³⁴ grown at 30 °C has normal chloroplasts with internal membrane systems arranged into grana and stroma lamellae (Figure 10).

At level 3 the differences in chloroplast ultra-structure between the mutant grown at low and high temperature is even more striking. *Tigrina-*

*o*³⁴ grown at 20 °C has only abnormal plastids with vacuolated stroma, highly condensed large grana and granular osmiophilic aggregates (Figure 11). The mutant grown at 30 °C displays mature chloroplasts with a normal thylakoid system organized into grana and stroma lamellae (Figure 12). Chloroplast ribosomes are recognizable in these chloroplasts.

In *tigrina-o*³⁴ grown at 30 °C the chloroplasts at level 4 show a further development of their internal membrane system and numerous chloroplast ribosomes (Figure 13). Nevertheless, it is also possible to find at this level some abnormal plastids (Figure 14) with the »undulating osmiophilic sheets« (28) typical for lycopene accumulation and the large dense grana. Chloroplast ribosomes are numerous in these plas-

tids. Close to the leaf tip (level 5) of *tigrina-o*³⁴ grown at 20 °C extremely dense grana, osmophilic aggregates but few thylakoids can be recognized in the plastids and the stroma appears to be completely disorganized (Figure 15). In

mutant seedlings grown at 30 °C the plastid population close to the leaf tip at level 5 is mixed (Figures 16 and 17), and most of the plastids are abnormal in structure. However, the plastid stroma between the large dense grana appears

Figures 15, 16 & 17. Chloroplasts at level 5.

In *tigrina-o*³⁴ grown at 20 °C the plastid stroma is no longer recognizable (Figure 15). In *tigrina-o*³⁴ grown at 30 °C there are some chloroplasts with a thylakoid system still containing typical grana with a small diameter (Figure 16). However, most of the plastids (Figure 17) have only large and electron-dense grana, in which membranes (arrow) are tightly packed; structures indicative of lycopene accumulation are present (double arrow). (Figure 15, $\times 26,000$; Figure 16, $\times 13,600$; Figure 17, $\times 12,000$).

normal in comparison with that of the plastids of *tigrina-o*³⁴ grown at 20 °C (Figure 15).

4. DISCUSSION

Chloroplast biogenesis is dependent on two protein synthesizing machineries, one in the cytosol and one in the chloroplast. Two methods have been employed to establish where a polypeptide constituent of the photosynthetic membrane is synthesized. These are the use of specific inhibitors for protein synthesis on chloroplastic and cytosolic ribosomes, respectively, (7, 23) and in vitro protein synthesis with templates derived either from the nucleus or the chloroplast (1, 9, 24, 33). FEIERABEND and coworkers have used chlorotic seedling leaves of rye grown at 32 °C and lacking the 70S ribosomes to determine which membrane polypeptides are synthesized on cytoplasmic ribosomes (11, 12, 13, 14). The polypeptide pattern of the internal membranes of plastids isolated from heat-bleached rye leaves was similar to that of the etioplast membranes and thus only a small amount of chlorophyll *a/b*-apoprotein 2 was present (14). The ultrastructural organisation of the heat-bleached plastids is similar to proplastids or chlorophyll deficient mutant plastids. In this respect the chloroplast ribosome instability which develops when the barley mutant *tigrina-o*³⁴ is grown at the restrictive temperature in the light provides an advantage as a considerable more extensive thylakoid system is present in the plastids. The polypeptide pattern of *tigrina-o*³⁴ grown at 20 °C bears no resemblance to that of the etioplast (19). All the polypeptides found are characteristic for the wild-type chloroplast thylakoid polypeptide pattern and in contrast to heat-bleached rye (14) no additional polypeptides are present. In electrophoretograms of thylakoid polypeptides from *tigrina-o*³⁴ grown at 20 °C only polypeptides that have been shown in other species to be synthesized inside the chloroplast (7, 9, 23, 24, 33) are deficient or lacking. These are chlorophyll *a*-proteins 1, 2, 3, cytochrome *f*, a protein of 31 kD, and the CF₁ subunits α , β and ϵ . On the other hand the chlorophyll *a/b*-protein 2, which is synthesized on cytoplasmic ribosomes (1) is present in normal amounts and it can be assumed that other bands present in prominent amounts in *tigrina-o*³⁴ grown at

20 °C (Figure 3), notably A, B, C, D, and E are synthesized in the cytosol. Exceptions are the γ and δ subunits of CF₁, which originate from 80S ribosomes, but are not detectable in patterns of *tigrina-o*³⁴ grown at 20 °C. This can be explained, if it is assumed that CF₁ is not bound to the thylakoid membrane until the whole molecule is assembled. The differences in the thylakoid polypeptide pattern of heat-bleached rye and *tigrina-o*³⁴ grown at 20 °C could be accounted for by different developmental patterns. In the development of the rye leaves at the restrictive temperature the assembly of the ribosomes may be blocked from the start. In *tigrina-o*³⁴ at 20 °C the ribosomes are destroyed secondarily, possibly by photodynamic action, when considerable thylakoid synthesis has taken place. In the extended leaves the deficiency of the polypeptides synthesized by chloroplastic ribosomes then reflects the dilution of these polypeptides in the membrane or diminished turn-over synthesis.

The primary temperature-sensitive step for the formation of 70S ribosomes in rye is not yet known (12) although several biosynthetic reactions have been studied (3, 17). From the electron microscopy data it is deduced that in the very young plastids ribosomes are present in *tigrina-o*³⁴ grown at 20 °C, in agreement with the small peak of 70S ribosomes obtained after isolation of ribosomes from the total leaf. With the exception of the normal young plastids located close to the intercalary meristem, the mutant contains at the restrictive temperature plastids with large dense grana, undulating osmiophilic sheets and aggregates of osmiophilic granules but highly disorganised stroma consistent with the loss of 70S ribosomes. The large amounts of chlorophyll *a/b*-protein 2 present in these plastids most likely is located in one of the three membrane and grana derived structures (28). It can also be concluded that the chlorophyll *a* absorption at 742 nm displayed by the mutant leaves grown at the restrictive temperature (28) must be due to a special organisation of the chlorophyll *a/b*-protein 2, as this is the only major chlorophyll-protein present in the plastids. When *tigrina-o*³⁴ is grown at 30 °C the modification of the thylakoid membranes is retarded and occurs only in the old plastids close to the tip of the leaves. Further studies are necessary to

elucidate if the abnormal carotene composition in the mutant at the restrictive temperature is responsible for the abnormal membrane organisation and loss of 70S ribosomes in the light.

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