ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 4, pp. 430-436. © Pleiades Publishing, Inc., 2006.
Original Russian Text © N. P. Yurina, E. N. Pogulskaya, N. V. Karapetyan, 2006, published in Biokhimiya, 2006, Vol. 71, No. 4, pp. 533-540.

Effect of Photodestruction of Plastids from Norflurazon-Treated Barley Seedlings on Expression of Nuclear Genes Encoding Chloroplast Stress Proteins

N. P. Yurina*, E. N. Pogulskaya, and N. V. Karapetyan

Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskii pr. 33, 119071 Moscow, Russia; fax: (495) 954-2732; E-mail: NYurina@inbi.ras.ru

Received March 1, 2005
Revision received March 31, 2005

Abstract—The effects of photodestruction of chloroplasts in norflurazon-treated barley seedlings on expression of nuclear genes *Elip* and *Hsp32* encoding light and heat stress proteins of barley chloroplasts and also of the *Lhcb1* and *RbcS* genes of photosynthesis proteins were studied. The genes of the photosynthesis proteins were not transcribed upon the photodestruction of chloroplasts. However, transcription of the stress protein genes continued, and the transcription of the heat stress protein gene remained virtually at the control level, whereas the light stress protein gene transcription was markedly (by 30-50%) decreased, and this suggests chloroplast control of the *Elip* gene transcription. Disorders in the processing and a partial disturbance in the import of precursors of Hsp32 and Elip proteins into the plastids of the norflurazon-treated seedlings were shown. Data on protease analysis indicates that photodestruction of chloroplasts is associated with accumulation of stress protein precursors in the plastid envelope.

DOI: 10.1134/S0006297906040110

Key words: chloroplasts, expression of stress protein genes, norflurazon, *Hordeum vulgare*

The majority of proteins involved in the development and functioning of chloroplasts in higher plants and other photosynthesizing eukaryotes are encoded by nuclear genes. Expression of these genes is regulated by light-induced signals that are transmitted through photoreceptors: phytochrome and cryptochrome. However, the plastids themselves also influence the transcription of the nuclear genes of the plastid proteins through signals directed from chloroplasts to the nucleus [1-3]. Blocking of the influence of plastids (photodestruction, mutations, etc.) on regulation of nuclear gene expression would allow us to reveal their role in this process. When plant seedlings are grown in the presence of norflurazon (NF, an inhibitor of carotenoid biosynthesis on the level of phytoene desaturase) the chloroplasts undergo photooxidative destruction in the light, which does not affect other compartments of the cell [4-7]. The bleached plastids also lack the majority of internal structures, such as thylakoid membranes and ribosomes, whereas their DNA and envelope are relatively little damaged [3, 5]. In the cells of photodestroyed seedlings the expression of some nuclear genes encoding chloroplast proteins, such as *Lhcb* (the gene of the chlorophyll *a/b*-binding protein of Photosystem 2 light-harvesting complex) and *RbcS* (the gene of the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase; RBPC, EC 4.1.1.39) is blocked [3-8]. Based on these data, it was concluded that the cells had to possess a signal necessary for expression of these genes and entering into the nucleus from intact chloroplasts.

Stress proteins are known to play an important role in the defense reactions of plant cells [9]. Therefore, it seemed interesting to study the effect of chloroplasts on expression of the nuclear genes encoding stress proteins of barley chloroplasts: the light and heat stress proteins Elip (Early Light-Inducible Protein) and Hsp32 (Heat-Shock Protein 32 kD). In higher plants the Elip family proteins are encoded by the nucleus, synthesized on cytoplasmic ribosomes, and after the translation are transported into chloroplasts as precursors [10]. A spontaneous mechanism of Elip incorporation into thylakoid membranes of barley and *Arabidopsis thaliana* has been detected [11, 12]. Elip are proteins with three transmembrane α-helices, and helices I and III are extremely

* To whom correspondence should be addressed.

homologous with the corresponding regions of the light-harvesting complex proteins (Lhcp) of Photosystems 1 and 2 [12]. Contrastingly to typical proteins of the multigene family Lhcp, which are constant structural components of Photosystems 1 and 2, Elip are accumulated in thylakoid membranes for only a short time. Synthesis of these proteins was earlier found during the greening of etiolated seedlings and also under conditions of light stress [10]. Afterwards, Elip production was shown in response to various stress exposures, including drought and low temperatures [12]. Elip are suggested to protect chloroplasts against photooxidative damage [13]. In barley, two multigene Elip families are found: high- and low-molecular-weight Elip (protein precursors of 24-27 and 16-18 kD, respectively). The molecular weight of the mature proteins is 18.0-18.5 and 13.5 kD for the high- and low-molecular-weight Elip, respectively [10].

Heat stress proteins are produced in plants under conditions of elevated environmental temperature. Low-molecular-weight Hsp are essential for resistance to heat stress and play an important role during plant development [14]. The nucleus-encoded low-molecular-weight heat stress protein Hsp32 is located in chloroplasts [15]. Expression of this protein gene is genetically related with adaptive thermal tolerance of plants.

The purpose of the present work was to study the effect of the plastid signal on expression of the genes of plastid stress proteins, the low-molecular-weight light stress protein Elip and heat stress protein Hsp32, in barley seedlings, and also on expression of genes *Lhcb* and *RbcS* of the photosynthesis proteins.

MATERIALS AND METHODS

Plants and conditions of cultivating. Barley seedlings (*Hordeum vulgare*, Apex cultivar) were grown for 7-8 days in hydroponic culture in the dark at 24°C. To obtain NF-treated seedlings, seeds were soaked in distilled water supplemented with $5 \cdot 10^{-5}$ M NF (SAN 9789, 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl-3-(2H)pyridazinone) and grown in hydroponic culture in the dark at the same concentration of NF.

Heat and light stress conditions. Control and NF-treated intact etiolated plants were incubated in the dark for 2 h (from 06.00 to 08.00) at 42°C in a thermostat. To escape stress caused by water deficiency, vessels with water were placed into the thermostat. Light stress was created by illumination of etiolated seedlings with the light of 300 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ for 24 h. Contents of carotenoids and chlorophylls were assessed by absorption spectra of acetone extracts.

Isolation of RNA and Northern hybridization. The total RNA from the control and NF-treated barley seedlings was isolated as described in [16]. Specimens of leaves (0.3 g) frozen in liquid nitrogen were powdered, the powder was suspended in 1 ml of buffer for extraction (0.6 M NaCl, 10 mM EDTA, 100 mM Tris-HCl (pH 8.0), 4% SDS) and mixed with an equal volume of phenol–chloroform–isoamyl alcohol mixture (25 : 24 : 1 v/v). The aqueous phase was separated by centrifugation, and RNA was precipitated with 8 M LiCl at 4°C. Then the RNA was reprecipitated with ethanol in the presence of 3 M sodium acetate buffer (pH 5.2). The concentration of RNA was determined spectrophotometrically. Samples of total RNA (10 μg) were fractionated by electrophoresis in 1.0% agarose gel in the presence of formaldehyde. By capillary blotting RNA was transferred onto a 0.45-μm Biodyne-B membrane (Pall, Germany), which was UV-radiated (254 nm) for 90 sec. Prehybridization was performed for 2 h at 42°C in the presence of 50% formamide, fivefold Denhard's solution (Denhard's solution consists of 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), fivefold standard salt solution (SSS) (SSS is 0.15 M NaCl in 0.015 M sodium citrate buffer (pH 7.0)), 1% SDS, and salmon sperm DNA (100 μg/ml). The RNA blots were hybridized overnight with ^{32}P-labeled fragments of barley cDNA (*Lhcb1*, *RbcS*, *Elip*, and *Hsp32*) in 10 ml of the solution at 42°C. The membranes were washed successively in twofold SSS supplemented with 0.1% SDS, then in the same solution for 5 min at 60°C, and thrice under the same conditions in 0.1-fold SSS + 0.1% SDS. All cDNA-probes were labeled using a Random Primer Labeling Kit (Stratagene, USA) in the presence of [α-^{32}P]dATP. rRNAs stained with ethidium bromide were used as controls to confirm the application of equal amounts of the total RNA and efficiency of the subsequent transfer of RNA onto the membrane. The linearity of the response was tested by different time expositions with a Bio Max MR-1 film (Kodak, Germany). Radioautographs were scanned and analyzed using the Gel Doc 1000 programs of image analysis (Bio-Rad, USA).

Isolation of plastids from barley seedlings. Intact plastids were isolated from 200-400 g barley seedlings and purified by centrifugation in a Percoll gradient [17, 18].

Isolation of total cell proteins. To isolate the total proteins, 100 mg leaves were homogenized in liquid nitrogen and transferred into 1 ml of buffer containing 12.5 mM Tris-HCl (pH 6.8), 2% SDS, 1 mM EDTA, 6 mM dithiothreitol, and 10% glycerol. The extract was heated at 65°C for 15 min and then centrifuged for 15 min at 18,000*g*. The protein concentration was determined by the Lowry method [19], and the proteins were analyzed by SDS-PAGE.

Electrophoresis and immunoblotting. Electrophoresis of the proteins (10 μg per lane) was performed in 12.5% polyacrylamide gel in the presence of SDS according to Laemmli [20]. The proteins were transferred onto a nitrocellulose membrane (Schleicher and Schull, Germany) with pores 0.45 μm in size [21]. The membranes were incubated for 1 h at 4°C in an inhibiting buffer which

consisted of a 5% defatted dry milk in 20 mM sodium phosphate buffer (pH 7.2), 0.15 M NaCl, 0.1% Tween 20, and then incubated overnight at 4°C with primary antibodies (diluted 1 : 2000) to the corresponding protein (Elip, Hsp32, Lhcb, or RbcS). After washing, the membrane was incubated for 2 h at room temperature with anti-rabbit goat IgG conjugated with alkaline phosphatase. The membranes were washed, and specific protein bands were revealed using substrates for development of the alkaline phosphatase activity: 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium. All experiments were repeated at least three times.

Peptide maps obtained by limited proteolysis of proteins. The proteins were digested in polyacrylamide gel as described in [22]. The gel bands of the first direction SDS-PAGE with the proteins under study were equilibrated for 20 min with buffer 1 (0.125 M Tris-HCl (pH 6.8), 0.1% SDS, 1 mM EDTA, 1 mM dithiothreitol) and placed onto the gel of second direction of SDS-PAGE. Then onto the gel buffer 1 supplemented with 10% glycerol, 0.001% Bromophenol Blue, and freshly prepared protease solution were deposited from above for equilibrating. For digestion, chymotrypsin (20 μg/ml) and protease M8 (16 μg/ml) were used. The polypeptides produced by proteolysis were detected by immunoblotting as described above.

Treatment of chloroplasts with trypsin. The chloroplast precipitate was suspended in 0.5 ml of buffer (330 mM sorbitol, 50 mM Hepes-KOH (pH 8.0)) containing trypsin (20 μg/ml; Boehringer, Germany). The sample was incubated with trypsin for 15 min on ice. Then the chloroplasts were diluted with 5 ml of the initial buffer supplemented with the egg inhibitor of trypsin (2 mg/ml; Boehringer) or protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine). The chloroplasts were precipitated by centrifugation, and total proteins of the organelles were analyzed by SDS-PAGE with subsequent immunoblotting.

RESULTS

Photobleaching of NF-treated barley seedlings. Previously the bleaching of barley seedlings grown at varied concentrations of NF (10^{-4}-10^{-7} M) was studied. Even at $5 \cdot 10^{-5}$ M NF, carotenoids and chlorophylls were virtually absent in the seedlings.

Content of stress protein gene transcripts in NF-treated plants. Expression of nuclear genes of stress proteins and genes of photosynthesis proteins was studied by Northern hybridization under conditions of chloroplast photodestruction (Fig. 1). The figure shows that the genes *Lhcb1* and *RbcS* are not transcribed in the NF-treated plants that had been intensely illuminated for 24 h. Under the same conditions, the genes of the plastid stress proteins are expressed as follows: gene *Hsp32* expression is virtually the same in the control and NF-treated plants, whereas the content of gene *Elip* transcripts is decreased in the experimental plants. The relative content of the transcripts in the NF-treated seedlings was 50-70 and 90-95% of the control for *Elip* and *Hsp32*, respectively. Thus, the transcription of the four proteins was differently sensitive to photodestruction of the plastids in the NF-treated seedlings. The photodestruction had the greatest effect on the transcription of genes *Lhcb1* and *RbcS*, whereas the gene *Hsp32* transcription was less affected. By the sensitivity to NF treatment, the genes show the following order: *Lhcb1* = *RbcS* > *Elip* > *Hsp32*.

Detection of stress protein gene expression on the protein level in NF-treated plants. The presence of four proteins (Elip, Hsp32, Lhcb1, and RbcS) was detected by Western blotting of cell extracts from the NF-treated and control barley seedlings. Contents of the Lhcb1 and RbcS proteins were decreased in the NF-treated seedlings as compared to the control (Fig. 2). The presence of these proteins in the chloroplasts of the photodestroyed seedlings seems to be due to the proteins synthesized before the photodestruction [23]. The contents of the light and heat stress proteins were insignificantly different in the NF-treated and control plants. However, in the NF-treated barley seedlings additional proteins with higher molecular weight were accumulated in both the case of the heat stress protein Hsp32 and the light stress protein Elip (Fig. 2, arrow). The figure shows that under two stress exposures (heat shock and oxidative stress), an additional 36-kD protein is produced along with the mature Hsp32. In the case of Elip, in addition to the mature 13-kD protein a 17-kD protein is also accumulated (Fig. 2).

To quantitatively assess the accumulation of stress proteins in dependence on the duration of intense illumination of the plants, a diagram was plotted based on data for the protein Hsp32 (Fig. 3). The diagram shows that in the plants subjected only to heat stress, the mature Hsp32 protein (hatched columns) is accumulated, and its con-

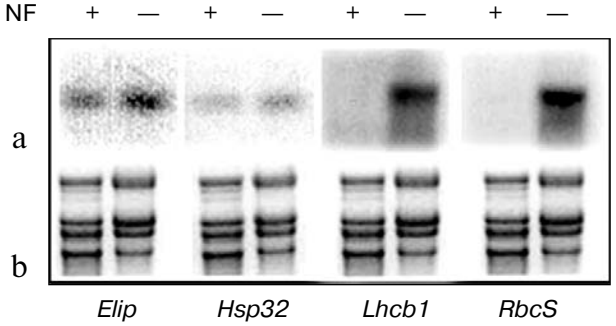

Fig. 1. Radioautograph of the Northern blot hybridization of ^{32}P-labeled *Elip*, *Hsp32*, *Lhcb*, and *RbcS* with the total RNA from the NF-treated (+) and control (–) barley plants (a); b) ethidium bromide-stained rRNA of each specimen presented in (a).

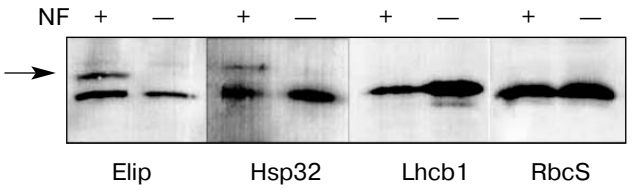

Fig. 2. Western blotting of the proteins Elip, Hsp32, Lhcb1, and RbcS from the NF-treated (+) and control (–) barley plants. Seven-day-old etiolated barley seedlings were illuminated (300 μmol·m^{-2}·sec^{-1}) for 24 h. In the case of Hsp32 the seedlings were previously subjected to heat stress (42°C, 2 h). The arrow indicates additional proteins.

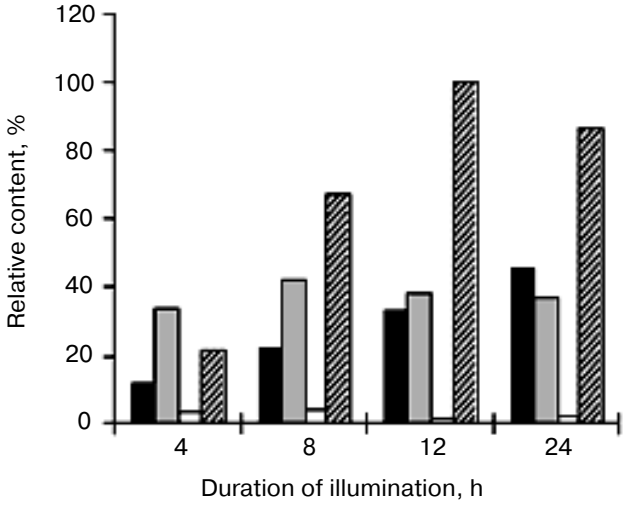

Fig. 3. Immunoblotting analysis of Hsp32 content in the NF-treated (the black column shows the 36-kD protein, the gray column is Hsp32) and control (the white columns present the 36-kD protein, the hatched columns are Hsp32) barley seedlings. The seedlings were incubated for 2 h at 42°C and then illuminated (300 μmol·m^{-2}·sec^{-1}) for 4-24 h. The isolated total proteins were fractionated by SDS-PAGE, and then immunoblotting was performed.

tent increases with increase in the incubation time to 12 h. In these preparations, a trace amount of a 36-kD protein is also found, but its content does not change during the incubation. Another situation is observed in the plants subjected to combined heat shock and oxidative stress. The content of the additional 36-kD protein increased linearly up to 24 h of incubation of the seedlings in the light, whereas the content of Hsp32 flattened out on the early stages of the incubation (Fig. 3, black and gray columns, respectively).

The difference in molecular weights of the major and additional protein components and also the immune reaction of both proteins to antibodies to Hsp32 suggest that the protein with the higher molecular weight is a precursor of Hsp32. To test this hypothesis, peptide mapping of the proteins was performed and the influence of NF removal (by washing of roots of the seedlings and placing them into an NF-free medium) on the accumulation of the 36-kD protein was studied experimentally.

Comparison of proteins using peptide maps. Total proteins of the seedlings subjected to heat shock or combined heat shock and oxidative stress were fractionated in SDS-PAGE. From unstained gels of the first direction (the gels were used similar to those the blots of which are presented in Fig. 2) a zone which contained 10-50-kD proteins was cut out, as described in "Materials and Methods", and placed over the second direction gel with the above-deposited protease solution. After electrophoresis in the second direction, the proteins and generated peptides were detected by Western blotting with antibodies to Hsp32. Figure 4 presents results of degradation of the Hsp32 and 36-kD protein with chymotrypsin. In the presence of NF, four main components are detected in electrophoregrams of the 36-kD protein degradation products. Three of these components have the same electrophoretic mobilities as the main components of Hsp32 from the control plants (Fig. 4, the bilateral arrows). The fourth spot with the higher molecular weight corresponds to the incompletely degraded 36-kD protein (Fig. 4, the dotted arrow). Thus, similar peptide maps are obtained for the mature Hsp32 and the additional 36-kD protein that suggests a high homology between these proteins and proves the precursor–product interrelation. Based on these findings, it is concluded that during the seedling growth in the presence of NF the Hsp32 precursor is accumulated. Similar data were obtained by treatment of the mature protein with protease V8 (figure not presented).

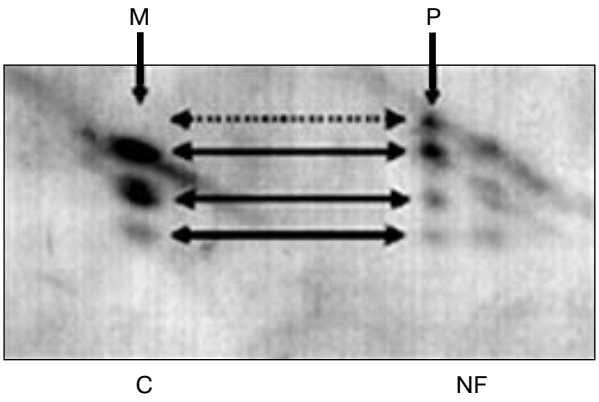

Fig. 4. The peptide map of Hsp32 and the 36-kD protein. The proteins were digested *in situ* with chymotrypsin. The peptides were visualized by Western blotting. The proteins were isolated from the NF-treated (NF) and control (C) barley seedlings after illumination for 24 h. To the right one can see the mature protein peptides in the NF-treated seedlings. The double-headed arrows indicate peptides detected in both specimens. The dotted arrow indicates the precursor position in the NF-treated seedlings. M, mature protein; P, precursor.

Effect of NF removal on contents of Hsp32 and Elip precursors. The precursor–product relation for the Hsp32 and 36-kD proteins would be also proved by a decrease in the amount of the additional product (a hypothetical precursor) with removal of NF. In fact, the content of the 36-kD protein was sharply decreased 16 h after the removal of NF from the medium by twice washing of the plant roots with water (Fig. 5). These data confirm that the 36-kD protein is a precursor of Hsp32.

Figure 6 shows the effect of the plastid photodestruction on the content of the stress protein Elip (13.5 kD). In this case, a 17-kD precursor of the protein is also accumulated, and its molecular weight coincides with that of the earlier described Elip precursor [24]. The accumulation of precursors in the presence of NF is reversible. Protein precursors virtually disappear upon washing of the plant roots free of NF. Some minor immunologically detectable protein bands (Fig. 6) are explained by the presence of the Elip family proteins in the plants.

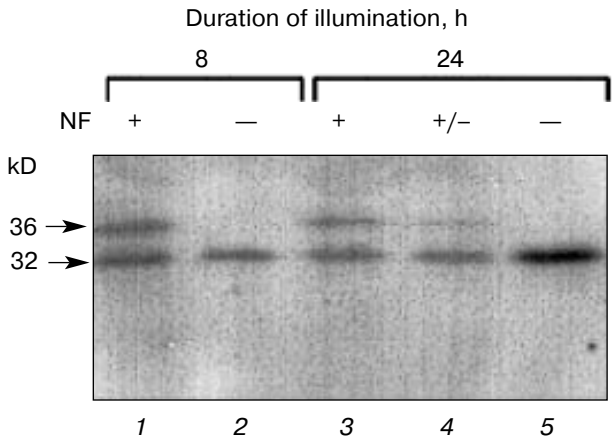

Fig. 5. Effect of NF removal on the Hsp32 precursor content. The NF-treated (+) and control (–) barley seedlings were subjected to heat stress (42°C, 2 h) and then illuminated for 8 h (300 μmol·m^{-2}·sec^{-1}). The roots of the plants were washed, the seedlings were placed onto water, and the incubation was continued in the light for 16 h. The proteins were isolated from the NF-treated seedlings (*1*, *3*), the seedlings placed onto water (*4*), and the control barley seedlings (*2*, *5*).

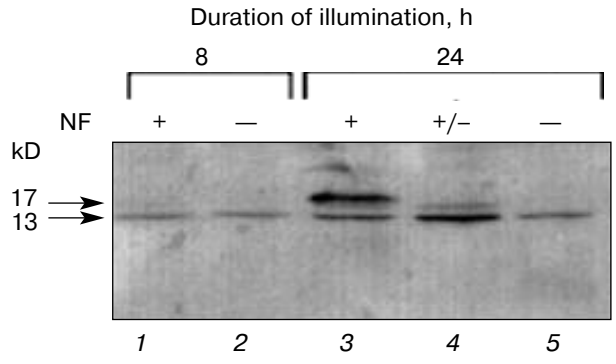

Fig. 6. Effect of NF removal on Elip precursor content. The NF-treated (+) and control (–) barley seedlings were illuminated for 8 h (300 μmol·m^{-2}·sec^{-1}), then the roots of the plants were washed, the seedlings were placed onto water, and the incubation was continued in the light for 16 h. The proteins were isolated from the NF-treated seedlings (*1*, *3*), the seedlings placed onto water (*4*), and the control barley seedlings (*2*, *5*).

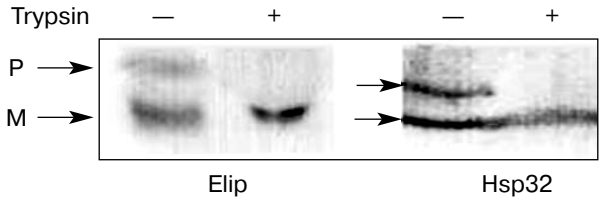

Fig. 7. Effect of trypsin on chloroplasts from NF-treated barley seedlings. Digestion with trypsin (20 μg/ml, 4°C, 15 min) of proteins not imported into the plastids. P, protein precursors; M, mature proteins.

Locating of stress protein precursors. To determine the location of stress protein precursors in the cells of the NF-treated plants, the chloroplasts were fractionated and treated with trypsin. The fractionation revealed association of the stress protein precursors with the chloroplast membrane fraction (figure not presented). The chloroplasts purified in a Percoll gradient were treated with trypsin. The stress protein precursors were sensitive to trypsin, whereas the mature proteins had undergone processing and were located inside the plastids were not destroyed by this treatment of the organelles (Fig. 7). This suggests that not all stress protein precursors in the NF-treated plants were imported across the envelope membrane, and the accumulation of the plastid protein precursors is concluded to be due to affected translocation of the stress protein precursors into the plastids.

DISCUSSION

In plants grown in the presence of NF, the synthesis of carotenoids is inhibited. The absence of carotenoids results in development of oxidative stress, which causes bleaching and destruction of chlorophyll and also inhibition of Photosystem 2 activity [4, 25]. The light-harvesting complex proteins binding carotenoids and chlorophylls are also destroyed in the absence of pigments responsible for their stabilization [26, 27]. The effect of oxidative stress is more pronounced in etiolated plants transferred from the dark to light. The present work is the first to study the effect of photodestruction of barley chloroplasts on expression of nuclear genes encoding stress proteins and the plastid photosynthesis proteins. The transcription of the four genes under study differs in the sensitivity to photodestruction of the plastids in the

NF-treated seedlings. The transcription of genes *Lhcb1* and *RbcS* was the most sensitive to the plastid signal. The lack of the *Lhcb1* and *RbcS* gene transcripts in the NF-treated seedlings is in agreement with earlier published data on the stronger dependence of expression of these genes on the plastid signals as compared to expression of other genes studied [5, 8, 25]. The plastid signal did not affect expression of the nuclear gene of the chloroplast protein Hsp32, because its transcription is virtually the same in the NF-treated and control plants. Obviously, *Hsp32* exemplifies a gene with expression relatively insensitive to the plastid signal. The decreased transcription of the Elip protein gene upon the photodestruction of chloroplasts suggests that chloroplasts control the expression of this gene. However, *Elip* gene expression depends less on the plastid signal than does the expression of the *Lhcb1* and *RbcS* genes. This is consistent with data on the different inhibition of different gene expression in the seedlings with damaged plastids [28, 29]. Data on the regulation of *Elip* gene expression are also in agreement with the concept that the expression of nuclear genes is more likely uninterruptedly regulated by the functional state of the plastids than discretely, by the "open–shut gate" principle [28]. Possibly, the different sensitivity of transcription of the four genes under study to chloroplast photodestruction is due to difference in the regulatory elements of the corresponding gene promotors.

It is especially interesting that transcription of the genes of the Lhcb1 and Elip proteins of the closely related families is different under conditions of the chloroplast photodestruction. This suggests difference in mechanisms regulating the expression of these genes. Possibly, two different plastid signals are involved in the regulation of expression of these genes. These data are consistent with a report [30] that during the biogenesis of chloroplasts and at low content of chlorophyll the accumulation of Elip always preceded the accumulation of Lhcb1. Chloroplasts seem to have a signaling network which regulates coordinated expression of the nuclear and chloroplast genomes, and the plastid signals can either inhibit the transcription of nuclear genes (as in the case of photosynthesis proteins) or activate it (as shown for the gene *Hsp70*) [31].

Precursors of the plastid proteins encoded by the nucleus are known to be synthesized on cytoplasmic ribosomes, transported across the chloroplast envelope, and undergo endoproteolytic processing in the chloroplast stroma [10, 32]. Photodestruction is associated with accumulation of precursors of these proteins in barley chloroplasts, in addition to the mature proteins Elip and Hsp32 [33]. Precursors of chloroplast proteins of the peripheral antenna of Photosystem 2 and small subunit of RBPC are not detected under these conditions. This is explained by the continued transcription of the stress protein genes along with suppression of the photosynthesis protein transcription.

Under conditions of oxidative stress caused by shortage of carotenoids, protein import into chloroplasts is somewhat affected, and the protein precursors are accumulated in the envelope of the plastids. This is shown by treatment of the isolated plastids with exogenous protease (trypsin). The N-terminal of the stress protein seems to be located inside the plastid envelope, whereas the C-terminal is exposed outwards and subjected to the proteases. It is suggested that in the NF-treated and control plants stress protein precursors are bound to the translocation sites of the chloroplast envelope, but the transfer is partially affected in the NF-treated plants.

Thus, the photodestruction of chloroplasts switches off the plastid signal thus inhibiting the transcription of the light stress protein and results in the accumulation in the plastid envelope of the stress protein precursors synthesized on cytoplasmic ribosomes and imported into the plastids. But further studies are required to elucidate the transmission pathways of the plastid signal and compounds involved in this process.

The authors are grateful to Prof. K. Kloppstech (Botanical Institute, Hanover, Germany) in whose laboratory a part of this work was done. Vectors with the cloned cDNA of barley *Elip*, *Hsp32*, *Lhcb1*, and *RbcS* and antisera to individual proteins used in the work were kindly presented by Prof. K. Kloppstech.

This work was supported by the Russian Foundation for Basic Research (project Nos. 03-04-49051a and 05-04-48526), the DFG project, and the Molecular and Cellular Biology program of the Presidium of the Russian Academy of Sciences.

REFERENCES

1. Surpin, J. M., Larkin, R. M., and Chory, J. (2002) *Plant Cell*, **14**, S327-S338.
2. Rodermel, S., and Sungsoon, P. (2003) *BioEssays*, **25**, 631-636.
3. Gray, J. C., Sullivan, J. A., Wang, J.-H., Jerome, C. A., and MacLean, D. (2003) *Phil. Trans. R. Soc. Lond. B*, **358**, 135-145.
4. Lekhotsky, E., Rakhimberdieva, M. G., and Karapetyan, N. V. (1982) *Fiziol. Rast.*, **29**, 682-686.
5. Mayfield, S. P., Nelson, T., Taylor, W. C., and Malkin, R. (1986) *Planta*, **169**, 23-32.
6. Karapetyan, N. V. (1993) in *Photosynthesis: Photoreactions to Plant Productivity* (Abrol, P., Mohanty, P., and Govindjee, J., eds.) Oxford and IBH Publishing Co, New Delhi, pp. 181-198.
7. Yurina, N. P., Karakashev, G. V., Odintsova, M. S., and Karapetyan, N. V. (1989) *Dokl. Akad. Nauk SSSR*, **306**, 739-742.
8. Oelmuller, R., Levitan, I., Bergfeld, R., Rajasekhar, V., and Mohr, H. (1986) *Planta*, **168**, 482-492.
9. Nover, L., Hellmund, D., Neumann, D., Scharf, K. D., and Serfling, E. (1984) *Biol. Zbl.*, **103**, 357-435.

10. Grimm, B., Kruse, E., and Kloppstech, K. (1989) *Plant Mol. Biol.*, **13**, 583-593.
11. Kruse, E., and Kloppstech, K. (1992) *Eur. J. Biochem.*, **208**, 195-202.
12. Adamska, I., Kruse, E., and Kloppstech, K. (2001) *J. Biol. Chem.*, **276**, 8582-8587.
13. Hutin, C., Nussaume, L., Moise, N., Moya, I., Kloppstech, K., and Havaux, M. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 4921-4926.
14. Wehmeyer, N., and Vierling, E. (2000) *Plant Physiol.*, **122**, 189-198.
15. Kruse, E., Liu, Z., and Kloppstech, K. (1993) *Plant Mol. Biol.*, **23**, 111-122.
16. Potter, E., Beator, J., and Kloppstech, K. (1996) *Planta*, **199**, 314-320.
17. Belkina, G. G., Yurina, N. P., and Odintsova, M. S. (1991) *Biokhimiya*, **56**, 1822-1831.
18. Baumgartner, B. J., Rapp, J. C., and Mullet, J. E. (1989) *Plant Physiol.*, **89**, 1011-1018.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
20. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
21. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350-4354.
22. Cleveland, D. W. (1983) *Meth. Enzymol.*, **96**, 222-229.
23. Susek, R., Ausubel, F., and Chory, J. (1993) *Cell*, **74**, 787-799.
24. Potter, E., and Kloppstech, K. (1993) *Eur. J. Biochem.*, **214**, 779-786.
25. Taylor, W. C. (1989) *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40**, 211-233.
26. Apel, K., and Kloppstech, K. (1978) *Eur. J. Biochem.*, **85**, 581-588.
27. Montane, M. H., Petzold, B., and Kloppstech, K. (1999) *Planta*, **208**, 519-527.
28. McCormac, A. C., and Terry, M. J. (2004) *Plant J.*, **40**, 672-685.
29. Kusnetsov, V., Bolle, C., Lubberstedt, T., Sopory, S., Herrmann, R. G., and Oelmuller, R. (1996) *Mol. Gen. Genet.*, **252**, 631-639.
30. Montane, M.-H., Tardy, F., Kloppstech, K., and Havaux, M. (1998) *Plant Physiol.*, **118**, 227-235.
31. La Rocca, N., Rascio, N., Oster, U., and Rudiger, W. (2001) *Planta*, **213**, 101-108.
32. Richter, S., and Lamppa, G. K. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 7463-7468.
33. Yurina, N. P., and Kloppstech, K. (2001) *Plant Physiol. Biochem.*, **39**, 807-814.