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RESEARCH PAPERS

Changes in the Transcriptional Activity of Barley Plastome Genes under Heat Shock

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Abstract—Plants use a wide range of molecular reactions for adaptation of their photosynthetic apparatus to high temperature. To understand the role of plastome genes in this process, we studied transcription of photosynthetic and household genes in plastids of 7-day-old barley (*Hordeum vulgare* L.) seedlings and found that the genes of both groups are involved in the adaptation to heat shock. Transcription of genes encoding apoproteins of the photosystem I and photosystem II reaction centers was enhanced in 1.5 h after temperature elevation and reduced after 3 h, whereas transcription of some other genes (NADPH plastoquinone oxidoreductase, ribosomal proteins, and multisubunit RNA polymerase) was enhanced after 3 h of exposure to high temperature. Transcription of plastome genes *rpl23-rpl2* and *rps16* was activated by heating either the whole plant or isolated chloroplasts. This permits a supposition that changes in transcription of plastome genes occur not only due to changes in nuclear transcription of the genes encoding plastid transcription factors but also due to changes in the properties of transcription factors inside chloroplasts.

Key words: Hordeum vulgare - heat shock - adaptation - chloroplasts - plastome - transcription

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INTRODUCTION

High temperature is one of the unfavorable factors, which is most frequently encountered by plants. Heat shock suppresses diverse processes [1], in particular damages the photosynthetic apparatus [2–5]. The molecular mechanisms of adaptation of the photosynthetic apparatus to heat shock generate a considerable interest. Plants are known to use chloroplast chaperones [6, 7], proteases [5, 8], and NADPH plastoquinone oxidoreductase complex [9] to maintain the functional activity of the photosynthetic apparatus under hyperthermia. However, in spite of all protective mechanism functioning, proteins of the photosynthetic apparatus are strongly damaged [5, 10] and thus are needed in the replacement.

Many proteins of the photosynthetic apparatus, including apoproteins of PSI and PSII reaction centers, are encoded by plastid DNA. The higher plant plastome comprises two large groups of genes: photosynthetic genes and those encoding the plastid apparatus of gene expression [11]. Expression of both gene groups is required for the maintenance of the photosynthetic apparatus activity because a disturbance in the functioning of the plastid expression machinery hampers or even makes impossible the synthesis of chloroplast electron-transport chain components [12, 13]. Therefore, both these gene groups might be involved in the maintenance of the photosynthetic apparatus activity during heat shock. The levels of mRNAs of major proteins of the photosynthetic apparatus are shown to increase under high temperature [14]. It is also known that the total transcription rate in chloroplasts is enhanced at hyperthermia [15]. However, we failed to find any information concerning the influence of high temperature on transcription of individual plastome genes in higher plants. Therefore, we decided to study possible changes in transcription of plastome genes encoding the photosynthetic complex and those encoding the plastid apparatus of gene expression under conditions of heat shock.

Earlier, it has been demonstrated that 7-day-old barley seedlings are more tolerant to high temperature than seedlings of other ages [16, 17]. At 40°C, these seedlings manifested first signs of disturbed photosynthetic activity only after 3 h [18, 19]. Therefore, in this work, we studied transcription of twenty plastid genes just in 7-day-old barley seedlings in 3 h after the start of their treatment with high temperature. Since transcription of plastid genes might be changed by stress much earlier [20], we also examined transcription of the same genes after 1.5-h exposure to 40°C. The analysis performed

Abbreviations: PEP—plastid encoded RNA polymerase; PS—photosystem; SSC—sodium citrate buffer comprising 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0.

permitted us to suppose that both photosynthetic and household plastid genes are involved in adaptation to hyperthermia.

MATERIALS AND METHODS

Plant material. Experiments were performed on barley (*Hordeum vulgare* L., cv. Gonar) seedlings. The seeds were soaked for 3 h in the diluted $KMnO_4$ solution. Thereafter, seedlings were grown in the solution containing 8 mg/l $Ca(NO_3)_2$, 20 mg/l KH_2PO_4, 2 mg/l $MgSO_4$, and 10 mg/l KCl at an irradiance of 200 μmol quanta/(m^2 s), a 16-h photoperiod, and 21°C.

Hyperthermia. Heat treatment of 7-day-old (since the time of seed soaking) seedlings was performed at 40°C and continuous illumination for 1.5 and 3 h. Heating of chloroplasts isolated from untreated 7-day-old seedlings was performed at 40°C (heat shock) in darkness for 1.5 h. Control chloroplasts were kept at 21°C.

Chloroplast isolation. Barley leaves (10 g) were homogenized in 80 ml of buffer A (0.33 M sorbitol, 50 mM Tricine, pH 8.0, 2 mM EDTA, and 5 mM β-mercaptoethanol). The homogenate was filtered through one layer of cheesecloth and two layers of Miracloth (Calbiochem-Behring, United States) and centrifuged at 2700 *g* for 6 min. The pellet of organelles was resuspended in 1.5 ml of buffer A and fractionated in the discontinuous Percoll gradient (40/70%) by centrifugation at 4000 *g* for 30 min. Intact chloroplasts were collected from the interface between 40 and 70% Percoll. The organelles were washed with buffer A and resuspended in 1 ml of the same buffer. The number of chloroplasts was counted in the Rosenthal–Fuchs chamber [21]. The calculated amount of organelles (5×10^7) was sedimented and resuspended in 40 μl of buffer A. All operations were performed at 4°C.

Run-on transcription assay. For the reaction of run-on transcription, 80 μl of buffer D (50 mM Tris–HCl, pH 7.0, 10 mM $MgCl_2$, 10 mM KCl, and 4 mM 2-mercaptoethanol) and 80 μl of transcription buffer (50 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, CTP, GTP, and ATP 0.2 mM each, 0.01 mM UTP, 2 MBq of α-^{32}P-UTP (110 TBq/mmol) (Amersham Bioscience, Great Britain), 20 units of RNase inhibitor (Fermentas, Lithuania), and 10 mM 2-mercaptoethanol) were added to 40 μl of buffer A containing 5×10^7 chloroplasts and incubated at 25°C in darkness. The reaction was run for 10 min for freshly isolated chloroplasts (hyperthermia in vivo) and 15 min for organelles incubated in the thermostat for 1.5 h (hyperthermia in vitro). The reaction was terminated by the addition of the equal volume of stop-buffer (50 mM Tris–HCl, pH 8.0, 25 mM EDTA, and 5% Na-sarcosyl). ^{32}P-RNA was isolated from the reaction mixture as described in [22].

Creation of the library of hybridization probes. To amplify gene-specific fragments, primers listed in Table 1 were chosen. At the time of the creation of the library of hybridization probes, complete sequence of barley plastome was not still published. Therefore, to prepare primers for some genes, we used sequences of homologous genes of closely related plant species (wheat and rice) using the program Primer 3.

Chloroplast DNA was isolated from barley leaves as described in [23]. For amplification of gene-specific DNA fragments, we used primers selected, barley ctDNA, and Taq DNA polymerase (Fermentas). The amplicons obtained were inserted into plasmids, pUC57 or pTZ57R/T (Fermentas), using appropriate reagent sets and following the manufacturer's recommendations. Table 1 presents the characteristics of cloned gene-specific fragments applied as hybridization probes. To analyze the *psbA*, *psbD*, *psaA,* and *rrn16* genes, we used fragments of spinach genes. These fragments were also amplified using standard primers M13 and inserted into the pUC57 plasmid.

Preparation of membranes with gene-specific probes; membrane hybridization with ^{32}P-RNA. Using plasmids with inserted gene fragments and standard primers M13, we amplified each DNA fragment to obtain its required quantity. Before loading on the membrane, DNA was denatured by boiling in the presence of 0.5 N NaOH (10 min). Using a special Bio-Dot apparatus (Bio-Rad, United States), amplicons were applied to the nylon membrane, Hybond N^+ (Amersham Pharmacia Biotech, Great Britain) (1 μg/dot, two dots per each gene tested). Hybridization with ^{32}P-RNA molecules produced during in vitro transcription was performed in buffer containing 250 mM Na_2HPO_4, 7% SDS, and 2.5 mM EDTA at 58°C. After hybridization, membranes were washed with buffer 1 (0.5X SSC and 0.1% SDS) first at room temperature and then at 58°C, thereafter twice with buffer 2 (0.2X SSC and 0.1% SDS) at 58°C (each washing lasted for 10 min). Washed membranes were exposed to the Roentgen film (Amersham); after development, the film was scanned using HP ScanJet 5300C. Radioauthograms were analyzed using the program Quantity One (Bio Rad) and Microsoft Excel. Differences in two and more times (the threshold level) were considered significant.

RESULTS

Table 2 contains data about the effects of heat shock on the rate of tested plastid gene transcription. For some genes with most pronounced changes or those with unchanged transcription, radioauthograms reflecting their relative transcriptional activity in control and experimental plants are presented in Fig. 1.

As for photosynthetic genes, most interesting is a coordinated response of genes encoding apoproteins of PSI and PSII reaction centers (*psaA, psaB, psbA*, and *psbD*): their transcription was enhanced markedly in 1.5 h after the start of heating and reduced during following 1.5 h, returning to the initial level. It should be noted that *psaA* and *psaB* are the member of a single operon and are co-transcribed simultaneously, whereas

Table 1. Characteristics of DNA probes synthesized with barley ctDNA and used for hybridization with ^{32}P-RNA produced in the reaction of run-on transcription

Gene name	Primers	Temperature of primer annealing, °C	Amplicon length, bp
psbH	cacaaaccgttgaagatag aattccatccagtaaaatgg	54	190**
psbK	ttgagagtgcgaatacaagc gcaaaaatagggttaggtgg	60	587
psaB	ttccatcgaacgtactctcc tctcactttgggcaattagc	62	485
ycf4	ctaggattcttagcggttgg tcatcagtacgagtcaaggg	60	380
*petL–petG**	tcggttttctatggctgct attgcaaatacgacgtcacg	59	331**
ndhA	agcgggcaagaataaaggat cacagttcccaccgctattt	60	763**
ndhC	tttggacatttctaataatagcaagcc cttttcgccatgcataaactaaac	61	315
ndhF	tggaccagaagcaagcaaga attccttggtgcagttgcga	60	551**
rrn23	taggttagccgaaagatggttataggttt cgagacagtgcccagatcgttac	64	621
*rpl23–rpl2**	gtcataagcgcctataccgt cacttgtccgactgttgcta	60	1079
rpl16	gtgtcattgctcttcgtcct gcttcgtattgtcgagatcc	60	1111
rps16	ccttctcttcgagatcgaac atgctcttggctcgacatag	60	793
rps18	acaacctttttcgtaggcgtc tcgattggaattgtacttttgcgtt	58	285
rpoB	aattcggattggctcttggtc caacaatgcacttctcggac	60	747
clpP1	tcagtatcgcagtgcttgtc gtggctaatctcaggaatgg	60	399**

Notes: For some genes, we prepared primers on the basis of sequences of homologous genes of wheat and rice; therefore, some nucleotides in primers are not complementary to corresponding sequences of barley ctDNA. Noncomplementary sequences are shaded.

* DNA probe embraces the regions of two genes and hybridizes with transcripts of both genes.

** Amplicon inserted into the plasmid is verified by sequencing and corresponds to the ctDNA zone limited by a corresponding primer pair.

the genes *psbA* and *psbD* are transcribed independently of these genes and each other. The genes encoding other PSII proteins behaved otherwise: transcription of the *psbH* displayed a tendency to activation but did not achieve a threshold level; transcription of the *psbK* gene was essentially unchanged. Transcription of the *ycf4* gene, involved in the assembly of the PSI [24], and also transcription of the *petL* and *petG* genes, encoding small subunits of cytochrome b_6lf complex, was also almost unchanged.

In 3 h after temperature elevation, we observed transcription activation of the *ndhA* and *ndhF* genes encoding subunits of NADPH plastoquinone oxidoreductase, whereas transcription of the *ndhC* gene changed insubstantially. Transcription of the genes for ribosomal proteins was activated: transcription of the *rps16* and *rps18* genes was enhanced after 3-h exposure, whereas transcription of the *rpl16* and *rpl23–rpl2* genes was significantly increased in 3 h in the first experiment and in 1.5 h, in the second one. Transcription of the genes encoding subunits of PEP (multisubunit RNA polymerase) was enhanced after 3 h of high temperature action. Table 2 presents data only for the *rpoB* gene, but other plastome *rpo* genes (A, C1, and C2) behaved similarly (data not shown). The *rpoB, rpoC1*, and *rpoC2* genes are the member of a single operon and are cotranscribed, whereas the *rpoA* gene is transcribed independently of them. After 3-h heating, we observed a

Table 2. Changes in the transcription rates of plastome genes in 7-day-old barley seedlings under high temperature

Gene	Exp. 1		Exp. 2	
	1.5 h	3 h	1.5 h	3 h
psbA	2.20	1.57	2.94	1.66
psbD	2.70	1.09	2.66	1.01
psbH	1.61	1.74	1.70	1.59
psbK	1.10	0.97	1.18	0.98
psaA	2.47	1.31	2.73	1.41
psaB	2.07	1.52	2.84	1.54
ycf4	1.11	1.21	1.33	1.00
petL–petG	1.14	1.40	1.08	1.49
ndhA	1.14	3.33	1.43	2.90
ndhC	0.96	1.40	1.00	1.03
ndhF	0.96	3.21	1.72	1.99
rrn16	0.71	1.48	0.75	0.82
rrn23	0.62	1.52	0.99	0.74
rpl16	1.53	3.26	2.00	1.79
rpl23–rpl2	1.63	2.38	2.38	1.25
rps16	1.21	2.66	1.32	2.11
rps18	1.15	2.39	1.10	2.53
rpoB	1.03	3.12	1.58	1.97
clpP	1.09	1.61	1.50	1.80

Note: The ratios of R_{40} to R_{21} are presented, where R_{40} is the transcription rate in plants exposed to 40°C for 1.5 or 3 h and R_{21} is the transcription rate of the same gene in nonheated plants kept at a growing temperature of 21°C. The results of two independent experiments are presented. The changes in the transcription rate in two and more times are considered significant; the values two and more indicate transcription activation.

tendency for activation of the *clpP1* gene, the only plastome gene encoding protease, but this activation was below the threshold level.

The results obtained showed that, under high temperature, expression of some plastome genes is enhanced, at least at the transcription level. How can this occur? We can suppose two main modes of transcription regulation in chloroplasts. First, all known chloroplast transcription factors are coded in the nucleus [11]. Therefore, changes observed could result from changes in the expression of nuclear genes encoding chloroplast transcription factors. Transcription of the *psbD* gene is enhanced just in such a mode under the effect of changed illumination or some other stressors [20, 25–27]. Second, temperature elevation could induce some changes in the chloroplast transcription apparatus per se, for example, affect properties of transcription factors. The examples of such regulation are not known so far; however, some mechanisms potentially capable of regulating transcription factor activity operate in chloroplasts [28].

To distinguish between these two possibilities, we subjected to heat shock isolated chloroplasts; in this case, newly synthesized nuclear transcription factors could not penetrate into chloroplasts from the cytoplasm. Since 3-h exposure to 40°C could result in complete degradation of the transcription apparatus, we examine only the effects of 1.5-h chloroplast heating. Chloroplasts were isolated from plants not subjected to heat shock and then were incubated at 21°C (control) or 40°C (heat shock) for 1.5 h. Thereafter, transcription reaction was performed under similar conditions. The results are presented in Table 3 and Fig. 2.

The analysis of data presented in Table 3 shows that, under conditions of hyperthermia, transcription in isolated chloroplasts changed stronger than in chloroplasts subjected to the action of high temperature within the cells of the living leaf. When we compare the results obtained after heating intact plants (Table 2) and isolated chloroplasts (Table 3), we note that, after 1.5-h exposure to 40°C of isolated chloroplasts, of the genes encoding apoproteins of the photosystem reaction centers was not activated, as distinct from their transcription in intact plants. Moreover, suppression of these genes and also the genes of the cytochrome b_6/f complex (*petL–petG*) was observed. And vice versa, in isolated chloroplasts, transcription of the *ndhC* gene was enhanced, whereas it was not changed in intact plants. However, in some cases, the consequences of 1.5-h heating of isolated chloroplasts and 3-h heating of intact plants were similar. In both cases, transcription of the *rpl23–rpl2* and *rps16* genes was activated. This similarity was only partial: other genes activated by 3-h heating of intact plants demonstrated suppressed transcription after 1.5-h heating of isolated chloroplasts. Some other similarities of heated chloroplasts and intact plants were a tendency to activation of transcription of the *psbH* gene and unchanged transcription of the *psbK* gene (Tables 2, 3; Figs. 1, 2).

DISCUSSION

In this work, we studied the effects of high temperature on the rate of plastome gene transcription in 7-day-old barley seedlings and found that transcription of many plastome genes was enhanced by treatment with 40°C. Two types of responses to a temperature rise from 21 to 40°C could be distinguished. After 1.5-h plant heating, chloroplast transcription of the genes encoding the apoproteins of PSI and PSII reaction centers (*psaA, psaB, psbA*, and *psbD*) was enhanced; after 3-h exposure to 40°C, transcription activation of these genes was small or even absent (Table 2). Transcription of the *psbD* gene changed similarly in response to changes in illumination: transcription activation was observed after 0.5 h, attained the highest level after 1–2 h, and then reduced [20]. As a result of this activation, the quantity of *psbD* mRNA synthesized from the

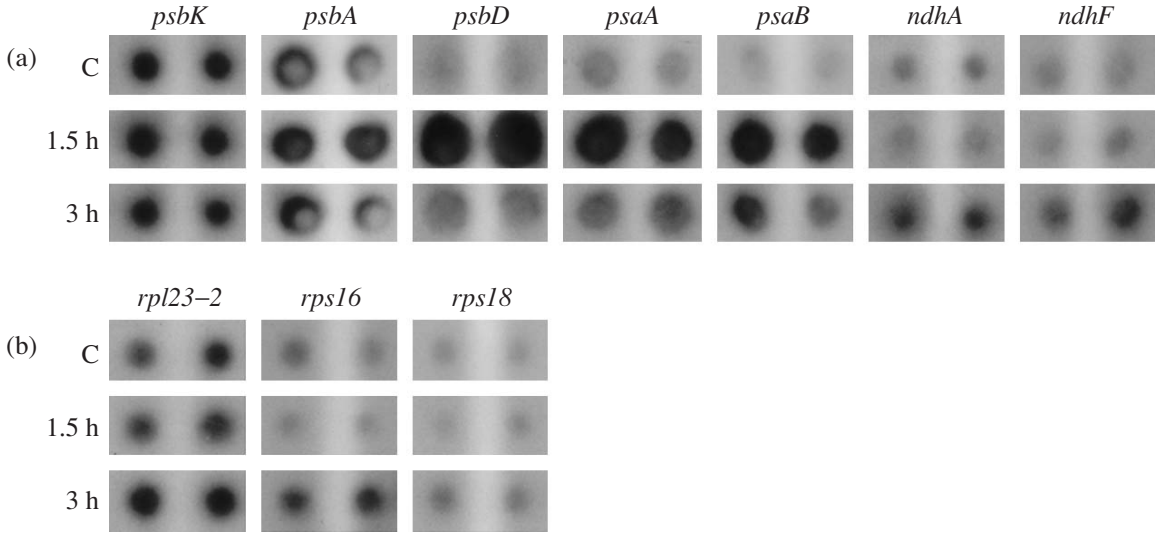

Fig. 1. Effect of high temperature on the transcription rate of plastome genes in 7-day-old barley seedlings.
(a) Genes of the photosynthetic apparatus; (b) genes for proteins of plastid ribosomes. C—control plants grown at 21°C; 1.5 h and 3 h—plants exposed to 40°C for 1.5 and 3 h.

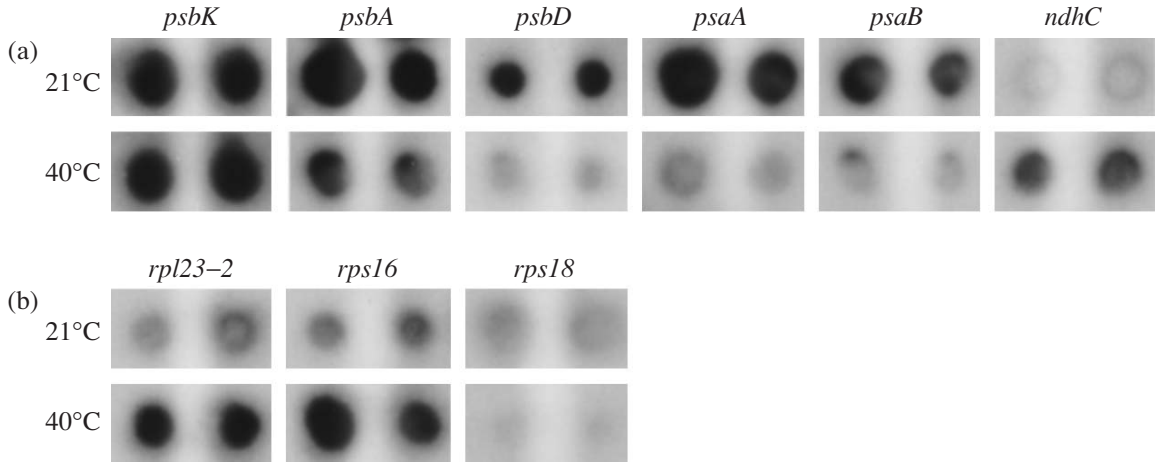

Fig. 2. Effect of high temperature on the transcription rate of plastome genes in isolated barley chloroplasts.
(a) Genes of the photosynthetic apparatus; (b) genes for proteins of plastid ribosomes. Chloroplasts were isolated from 7-day-old barley seedlings and incubated at 21°C (growing temperature) or 40°C for 1.5 h.

light-responsive promoter, which was still unchanged in 1 h after the change in illumination, increased substantially after 2 h and did not change later [20, 25]. Under conditions of heat shock, the levels of *psaA* and *psbD* mRNAs increased also after 3-h treatment [14].

Activation of transcription was observed not for all genes of the electron transport chain of photosynthetic membranes. The genes encoding PSII peripheral proteins did not display such a response: transcription of the *psbH* gene manifested a slight tendency to activation; transcription of the *psbK* gene was essentially unchanged. Transcription of the *petL* and *petG* genes encoding small subunits of cytochrome b_6/f complex and the *ycf4* gene encoding the PSI assembly factor was almost unchanged.

Proteins of the photosystem reaction centers are evidently most sensitive to stresses [29]. In any case, proteins D1 and D2 of the PSII reaction center degraded under high light more rapidly than CP43 protein of PSII, large subunit of Rubisco, and subunit β of ATP synthase [30]. Under heat shock, proteins of photosystem reaction centers are also very vulnerable [5, 10]. It seems likely that transcription activation of the genes for apoproteins of photosystem reaction centers is one of the earliest plant responses aimed at to the maintenance of the photosynthetic apparatus activity under

Table 3. Changes in the transcription rates of plastome genes in barley isolated chloroplasts under high temperature

Gene	Exp. 1	Exp. 2
psbA	0.53	0.55
psbD	0.27	0.67
psbH	1.54	1.56
psbK	1.02	1.14
psaA	0.26	0.28
psaB	0.24	0.59
ycf4	0.33	0.86
petL–petG	0.40	0.45
ndhA	0.62	0.83
ndhC	2.93	2.05
ndhF	1.72	1.27
rrn16	3.29	0.78
rrn23	1.48	0.51
rpl16	1.56	1.03
rpl23–rpl2	2.15	2.69
rps16	2.61	2.66
rps18	0.30	0.48
rpoB	0.40	0.53
clpP	0.41	0.75

Note: Chloroplasts isolated from 7-day-old barley seedlings were incubated at 21°C (temperature of seedling growing) or 40°C in darkness for 1.5 h. The ratios of R_{40} to R_{21} are presented, where R is the transcription rate in chloroplasts exposed to corresponding temperature. The values two and more indicate transcription activation, whereas 0.5 and less, its suppression.

hyperthermia. Our data permit a supposition that hyperthermia-induced transcription activation is referred to only the genes for proteins of photosystem reaction centers and possibly a small number of other genes, which products are strongly damaged during heat shock.

In the connection with our results, the experiment with *Arabidopsis thaliana* protoplasts performed under constant conditions in darkness could be mentioned: an enhanced expression of the sigma-factor 5 (Sig5) resulted in the transcription activation of the *psaA, psbA, psbB*, and *psbD* genes, whereas an enhanced expression of the sigma-factor 2 activated transcription of the *psbA* and *trnEYD* genes [27]. Let us note that, in barley seedlings subjected to heat shock, transcription of the *trnEYD* operon occurred independently of that of *psbA* and other genes of the photosystem reaction centers (data not shown). Sigma subunits are the basal transcription factors controlling functioning of plastid multisubunit RNA polymerase (PEP) [28]. The genes for sigma-factors 1, 2, and 5 evidently originated before subdivision of land plants into mosses and vascular plants [31]; therefore, it seems quite possible that they control transcription in chloroplasts of barley and *A. thaliana* in similar mode. In *A. thaliana*, Sig5 is a transcription factor regulated by various stressors using different pathways: light-dependent and light-independent [26]. It might be that, in barley seedlings under heat shock, Sig5 is involved in transcription activation of the genes for apoproteins of the photosystem reaction centers.

The second plant response to temperature elevation we observed was an enhanced transcription of the genes for NADPH plastoquinone oxidoreductase, ribosome proteins, and PEP after 3-h incubation at 40°C (Table 2). At this stage, some disturbances in the functioning of the photosynthetic apparatus were observed in 7-day-old barley seedlings [18, 19]. Therefore, in this case, we can assume plant response to heat shock. It seems likely that this is a defense response of the second level, the response not only to high temperature but also to some disturbances in biological processes. NADPH plastoquinone oxidoreductase is involved in the protection of the photosynthetic apparatus against hyperthermia by suppressing the generation of reactive oxygen species [9]. Like in the first reaction, not all genes of this complex were activated: transcription of the *ndhA* and *ndhF* genes was enhanced, but transcription of the *ndhC* gene was not changed relative to control value (Table 2). The *ndhF* gene encodes the largest subunit of this complex, whereas the *ndhC* gene, one of the smallest one. It might be that subunits encoded by the *ndhA* and *ndhF* genes degrade more rapidly; therefore, their synthesis is activated. Transcriptional activation of genes for ribosomal proteins and RNA polymerase evidently occurred because, under heat shock conditions, the synthesis of not only the electron transport chain components but also the machinery performing this synthesis is required.

Summing up, we can describe changes observed as follows. At temperature elevation, probably before heat shock, transcription of the genes for most vulnerable proteins, the apoproteins of PSI and PSII reaction centers, is activated. By the moment of the impairment of the photosynthetic apparatus functioning, activation of these genes ceases and other genes, which encode proteins protecting the photosynthetic apparatus (*ndh* and slightly activated *clpPI*), and those providing for the synthesis of its components (ribosome proteins and RNA polymerase) are activated. It seems quite possible that these defense responses are not only separated in time but also activated through different pathways.

It was aforementioned that observed changes in the transcription rate could result from changes in the expression of nuclear genes encoding plastid transcription factors (Sig5, for example) [20, 25–27] and/or from changes of transcription factor properties within chloroplasts [28]. To distinguish between these possibilities, we examined chloroplast transcription after heating isolated organelles, i.e., under conditions when the penetration of de novo synthesized transcription factors into chloroplasts is impossible (plastome does

not contain genes encoding transcription-controlling factors; all of them are encoded in the nucleus [11]). It turned out that, after 1.5-h chloroplast heating within plant tissues and in tubes, changes in transcription differ substantially (Tables 2, 3). We can suppose at least three reasons for this phenomenon: (1) isolated chloroplasts are more vulnerable to stressors than chloroplasts inside the living cell; (2) transcription factors cannot be transported into isolated chloroplasts; and (3) intact plants were subjected to heat shock under illumination, whereas isolated chloroplasts were kept in darkness (in both cases, control and experimental samples were kept under similar conditions of illumination). So far, we cannot attruibute the differences observed to a single factor.

However, activation of the *rpl23–rpl2* and *rps16* genes occurs during hyperthermia in vivo and in vitro as compared with the transcription of these genes in control plants. We observed this effect after 3-h heating of intact plants (Table 2, Fig. 1), i.e., at time when first signs of the photosynthetic apparatus injury were observed [18, 19]. In the second experiment, the *rpl23–rpl2* genes were activated already after 1.5-h heating (Table 2). In isolated chloroplasts, transcription of the *rpl23–rpl2* and *rps16* genes was enhanced after 1.5-h heating in both experiments (Table 3, Fig. 2). We have supposed earlier that transcriptional changes occurring after 3 h of heating could immediately result from disturbances in biological processes. It seems quite expected that, in isolated chloroplasts, heat shock develops much more rapidly. Therefore, in isolated chloroplasts heated for 1.5 h, we observed changes similar to those occurring in intact plants after 3 h (and may be later). When it is true, we could not be certain that transcription of the genes for apoproteins of the photosystem reaction centers is not activated in isolated chloroplasts: it might be that such activation occurs earlier.

Nevertheless, activation of the *rpl23–rpl2* and *rps16* gene transcription we observed in intact plants and isolated chloroplasts permits a supposition that, under conditions of heat shock, the properties of transcription factors change inside the chloroplasts, and this suggests that the mechanism controlling the activity of the transcription machinery and/or transcription factors operate in chloroplasts.

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