The ω Subunit of RNA Polymerase Is Essential for Thermal Acclimation of the Cyanobacterium Synechocystis Sp. PCC 6803

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Abstract
The rpoZ gene encodes the small ω subunit of RNA polymerase. A ΔrpoZ strain of the cyanobacterium Synechocystis sp. PCC 6803 grew well in standard conditions (constant illumination at 40 μmol photons m⁻² s⁻¹; 32 °C; ambient CO₂) but was heat sensitive and died at 40 °C. In the control strain, 71 genes were at least two-fold up-regulated and 91 genes down-regulated after a 24-h treatment at 40 °C, while in ΔrpoZ 394 genes responded to heat. Only 62 of these heat-responsive genes were similarly regulated in both strains, and 80% of heat-responsive genes were unique for ΔrpoZ. The RNA polymerase core and the primary σ factor SigA were down-regulated in the control strain at 40 °C but not in ΔrpoZ. In accordance with reduced RNA polymerase content, the total RNA content of mild-heat-stress-treated cells was lower in the control strain than in ΔrpoZ. Light-saturated photosynthetic activity decreased more in ΔrpoZ than in the control strain upon mild heat stress. The amounts of photosystem II and rubisco decreased at 40 °C in both strains while PSI and the phycobilisome antenna protein allophycocyanin remained at the same level as in standard conditions. The phycobilisome rod proteins, phycoerythins, diminished during the heat treatment in ΔrpoZ but not in the control strain, and the nblA1 and nblA2 genes (encode NblA proteins required for phycobilisome degradation) were up-regulated only in ΔrpoZ. Our results show that the ω subunit of RNAP is essential in heat stress because it is required for heat acclimation of diverse cellular processes.

Introduction
DNA-dependent RNA polymerases (RNAPs) catalyze the transcription of genetic information from DNA to RNA. The core of the multi-subunit RNAP is conserved throughout all cellular life forms [1]. The RNAP core of the majority of eubacteria, contains a catalytic center consisting of β and β′ subunits [2], two identical α subunits that enhance transcription efficiency and participate in promoter recognition [3], and a small ω subunit. In cyanobacteria, however, the RNAP core consists of six subunits because β′ has been split into two parts, an N-terminal γ subunit and a C-terminal β′ subunit [4]. For promoter recognition and transcription initiation, the bacterial RNAP core recruits a σ factor. Bacteria encode one essential primary σ factor and varying number non-essential σ factors [5]. Different σ factors favor different promoters thus orchestrating the transcriptional efficiencies of different genes.

The ω subunit of the RNAP core is encoded by the rpoZ gene. Knock out strains of the ω subunit have been constructed in the proteobacterium Escherichia coli [6], the actinobacteria Mycobacterium smegmatis [7], Streptomyces coelicolor [8] and Streptomyces kasugaensis [9], and in the cyanobacterium Synechocystis sp. PCC 6803 [10], indicating that rpoZ is not an essential gene. Studies in E. coli have revealed that the ω subunit acts as a molecular chaperone for the β′ subunit [11], suggesting that the ω subunit has a similar role as the essential eukaryotic RPB6 subunit of RNAP [12]. We have recently shown that in the ΔrpoZ strain of Synechocystis, recruitment of the primary σ factor, SigA, by the RNAP core occurs less frequently than in the control strain, and as a consequence, many highly expressed genes are down-regulated in ΔrpoZ [10].

The optimum temperature for Synechocystis is 30–32 °C but cells grow for a few days even at 43 °C [13–15]. Pretreatment of Synechocystis cells in mild heat stress leads to acquired thermotolerance allowing survival in otherwise lethal temperatures up to 50 °C [16–18]. Photosynthesis is a heat-sensitive process [19], and photosystem II (PSII) is the most vulnerable component, for which it takes hours to fully acclimate to an elevated temperature [20]. Transcriptomics and proteomics studies have revealed that heat treatment induces expression of many heat shock genes and numerous genes with unknown functions [20,21].

Previous studies have shown that group 2 σ factors play roles in acclimation to elevated temperatures. The group 2 σ factor gene sigB is rapidly up-regulated upon a heat shock [22,23] and the SigB factor, in turn, up-regulates especially the expression of the small heat shock protein Hsps [14] and some other heat shock proteins [24]. Although SigC does not regulate heat shock genes, it
Heat Acclimation of S. 6803 Requires the $\delta$ Subunit of RNA Polymerase

Results and Discussion

The $\Delta$poZ strain has difficulties in acclimation to elevated temperature

In our standard growth conditions, continuous light at the photosynthetic photon flux density (PPFD) of 40 $\mu$mol m$^{-2}$ s$^{-1}$, and 32°C, the $\Delta$poZ strain grows like CS [10]. At 40°C, CS grows essentially like it grows at 32°C (Fig. 1A), the doubling times during the first day being 11.6±0.2 h (Fig. 1) and 11.4±0.3 h [10] at 40°C and 32°C, respectively. The $\Delta$poZ strain grew more slowly than CS during the first day at 40°C (Fig. 1A), with a doubling time of 18.5±2.0 h. A survival test indicated that the $\Delta$poZ strain contained only 3.5×10$^7$±0.4×10$^7$ colony forming units (CFUs) after 24-h growth at 40°C while CS contained almost a hundred thousand times more CFUs, 3.4×10$^9$±0.1×10$^9$. Transfer of cells back to the standard conditions did not rescue $\Delta$poZ cells after two days of incubation at 40°C, but cells died. The initial growth of $\Delta$poZ was slow at 38°C, with the doubling times for the first day of 12.1±0.3 h and 15.2±0.8 h for CS and $\Delta$poZ, respectively (Fig. 1B). At 38°C, however, the $\Delta$poZ cells were able to acclimate, and similar doubling times, 25.4±0.5 h for CS and 25.2±1.6 h for $\Delta$poZ, were measured after the second day (Fig. 1B). The $\Delta$poZ+$\delta$poZ strain, in which the $\delta$poZ gene has been re-introduced to the genome under the strong $psbA2$ promoter [10], grew similarly as CS at 40°C (Fig. 1A). This indicates that the heat-sensitive phenotype of $\Delta$poZ is due to the lack of the $\delta$ subunit.

A DNA microarray analysis in standard conditions revealed that many genes involved in carbon concentrating mechanisms (CCM) and carbon fixation are down-regulated in the $\Delta$poZ strain compared to CS [10]. Because temperature rise decreases the availability of inorganic carbon (the equilibrium concentration of dissolved CO$_2$ at 40°C is only 82% of that at 32°C), we tested if growth can be rescued by improving the availability of soluble inorganic carbon by increasing the pH of the growth medium to 8.3. Alkaline conditions have been previously shown to rescue many mutants with deficiencies in carbon metabolism. The growth of the heat-sensitive $\sigma$ factor mutant $\Delta$sigC can be rescued by improving the availability of soluble inorganic carbon at 45°C by rising the pH of the growth medium from 7.5 to 8.3 [15,25]. Furthermore, Synechocystis strains ANdhB, lacking a functional NAD[P]H dehydrogenase complex, and ANdhD3/NdhD4, with an inactivated CO$_2$ uptake system, are able to grow at pH 8.3, but not at pH 7.5 [28], and even a mutant deficient of the main carboxysome operon can be grown in alkaline conditions [29]. In contrast to mutants with deficiencies in carbon concentrating mechanisms, the growth of $\Delta$poZ cells at 40°C was not rescued at pH 8.3 (Fig. 1C), indicating that the heat-lethal phenotype of $\Delta$poZ is probably not only caused by deficiencies in CCM. The growth rates of CS and $\Delta$poZ were 10.6±0.2 h and 11.2±0.3 h, respectively, when cells were grown in BG-11 medium without added bicarbonate in standard conditions, confirming that $\Delta$poZ cells are able to cope with low carbon conditions. Furthermore, the similarity of the growth rates in the presence and absence of added
bicarbonate suggest that the bicarbonate addition to BG-11 has a negligible effect on the inorganic carbon content of the medium in growth experiments performed under ambient air.

Since \( \Delta rpoZ \) survived only for a limited time at 40\(^\circ\)C, all subsequent experiments were done by growing cells first in standard conditions to OD\(_{730}\)=1, and then transferring the cells to 40\(^\circ\)C for 24 h. The 24-h heat treatment was selected because drastic difference between growth of mutant and CS was obvious after the first 24-h (Fig. 1A). Both strains grew during the 24-h treatment at 40\(^\circ\)C (OD\(_{730}\) increased from 1.0 to 1.5 in CS and to 1.4 in \( \Delta rpoZ \), respectively), suggesting that dense \( \Delta rpoZ \) cultures might tolerate high temperature better than dilute cultures.

**Comparison of gene expression of the control and \( \Delta rpoZ \) strains at 40 \(^\circ\)C**

To get a more comprehensive picture on why \( \Delta rpoZ \) is not able to acclimate to mild heat stress, gene expression changes were studied by DNA microarray analysis. For DNA microarray analysis, CS and \( \Delta rpoZ \) were grown in standard conditions and then treated at 40\(^\circ\)C for 24 h before RNA was isolated. In addition, the results from standard growth conditions [10] were used as controls. All microarray data are available in GEO (accessions GSE59451). In the control strain, 71 genes were at least two-fold up-regulated upon heat treatment and 91 genes were down-regulated to one half or less (Fig. 2A). Complete lists of up- and down-regulated (Table S1) and down-regulated (Table S2) genes in CS are included as supplemental material. In \( \Delta rpoZ \), the heat treatment induced up-regulation of 200 genes (Fig. 2A, Table S3) and down-regulation of 194 genes (Fig. 2A, Table S4). Thus, 2.4 times more genes responded to mild heat treatment in the mutant strain than in CS (Fig. 2A).

The differentially expressed genes were assigned to functional categories according to Cyanobase (Fig. 2B), and a heat map was constructed to further facilitate comparison between strains (Fig. 3). The heat map includes genes that were up or down regulated upon mild heat stress in \( \Delta rpoZ \), in CS or both, and in addition transcript levels of these heat-responsive genes were compared in \( \Delta rpoZ \) and CS in standard growth conditions. All results included in Fig. 3 are collected in Table S5.

Only 33 genes were down-regulated upon mild heat-treatment in both strains (Fig. 2A, Table S6). Nearly 40% of them encode hypothetical or unknown proteins (Fig. 2B, Table S6); genes with an assigned name are included in Fig. 2A. For the genes with known function, the decrease in the expression of the \( desB \) gene, encoding an acyl-lipid desaturase, is most probably an acclimation response compensating for temperature-induced increase in membrane fluidity. Up-regulation of the \( desB \) gene in low temperatures and adjustment of lipid saturation are well known responses to low and high temperature [30,31]. The heat shock genes have been shown to be rapidly but only transiently up-regulated upon heat shock [21]. Up-regulation of heat shock genes typically occurs within minutes and transcripts disappear during the first hours of heat treatment. Accordingly, none of the heat shock genes was up-regulated after a 24-h treatment at 40\(^\circ\)C. The \( hspA \) gene was up-regulated in \( \Delta rpoZ \) in standard conditions [10] but this difference between the strains disappeared after the mild heat treatment. The \( groES \) heat shock gene was down-regulated in both strains and in addition the \( htpG \) heat shock gene was down-regulated in CS (Table S2) and the \( dnaJ \) heat shock gene was down-regulated in \( \Delta rpoZ \) (Table S4). In addition to heat shock proteins, some other proteins have been suggested to affect heat responses. The \( clpB1 \) gene encoding a protease, and \( slr1674 \) (a hypothetical protein) have shown to affect rapid heat responses, whereas \( hik34 \) (encoding a histidine kinase) and \( htpA1 \) (encoding a hydrogenase formation protein) affect sustained thermotolerance of PSI\(\mathrm{II} \), and \( cpcC2 \) (encoding a phycobilisome rod linker polypeptide) is essential for both responses [32].

The vast majority of genes up-regulated upon a mild heat treatment in the control or \( \Delta rpoZ \) strains belonged to functional categories hypothetical or unknown (Figs. 2B and 3, Table S6). The other large group of up-regulated genes was transport and binding proteins comprising 20 and 21 genes in CS and \( \Delta rpoZ \), respectively (Figs. 2B and 3). Many of them, including ammonium/methylammonium permeases, ABC-type basic amino acid and glutamine transporter, a permease protein for urea transporter and a manganese transporter (Table S6), were up-regulated in both strains. However, some transporters were up-regulated in one strain only, like nitrate/nitrite transporter genes, which were among the most highly up-regulated in \( \Delta rpoZ \), but were not up-regulated in CS. Some other differences in central nitrogen metabolism genes were detected in addition. The \( nblA1 \) and \( nblA2 \) genes encoding phycobilisome degradation proteins were up-regulated only in \( \Delta rpoZ \), while \( glnB \), encoding the nitrogen metabolism regulator protein PII [33], was up-regulated only in CS. Interestingly, \( Rce37 \), controlling some sugar catabolism genes in parallel with SigE mainly during nitrogen starvation [36], was up-regulated upon heat stress in both strains, but up-regulation of its target genes \( glgP \) and \( glgX \) was only detected in \( \Delta rpoZ \). Differential regulation of several genes involved in nitrogen metabolism may suggest that acclimation of nitrogen metabolism to elevated temperature fails to occur normally in \( \Delta rpoZ \).

Seven genes showed opposite expression change in \( \Delta rpoZ \) and CS upon mild heat stress. Five genes were down-regulated in \( \Delta rpoZ \) and up-regulated in CS, but only one of these genes, \( trnD \) encoding tRNA (guanine-N1)-methyltransferase, has an assigned function (Fig. 2A). On the other hand, two genes were up-regulated in \( \Delta rpoZ \) and down-regulated CS; these genes were \( gcvP \) encoding glycin dehydrogenase and \( ccmR \), which encodes a repressor protein regulating many genes involved in carbon concentrating mechanisms [37]. In standard growth conditions, the \( ccmR \) gene is down-regulated simultaneously with the down-regulation of its target genes and operons [10] indicating complex regulation of carbon concentrating mechanisms in \( \Delta rpoZ \).

According to DNA microarray results, more than 80% that showed up or down regulation in \( \Delta rpoZ \) did not respond similarly to a mild heat treatment in CS (Fig. 2A). Up-regulation of photosynthetic and respiratory genes was more common in \( \Delta rpoZ \) than in CS (Figs. 2B and 3). Furthermore, many genes for biosynthesis of amino acids and cofactors, prosthetic groups and carriers were up-regulated upon heat stress in \( \Delta rpoZ \) strain but only few in CS (Fig. 2B).

Although \( \Delta rpoZ \) grew well in standard conditions, the DNA microarray analysis revealed that 187 genes were at least two-fold up-regulated and 212 genes down-regulated in \( \Delta rpoZ \) cells compared to CS in standard growth conditions [10]. Our next question was whether the genes showing different response to mild-heat treatment in \( \Delta rpoZ \) and CS were similarly or differentially expressed in the standard conditions. The heat map reveals that numerous genes up-regulated upon heat stress in \( \Delta rpoZ \) were actually down-regulated in \( \Delta rpoZ \) compared to CS in standard conditions (Fig. 3). For example, genes encoding NADH dehydrogenase subunits that were shown to be down-regulated in \( \Delta rpoZ \) in standard conditions [10] were up-regulated in \( \Delta rpoZ \) upon heat treatment but not in CS (Fig. 3). Furthermore, many genes that were down-regulated upon mild heat stress in \( \Delta rpoZ \)
were found to be up-regulated in ΔrpoZ compared to CS in standard conditions (Fig. 3). In standard conditions we showed that recruitment of the primary σ factor SigA occurs less frequently in ΔrpoZ than in CS, which leads to down-regulation of many highly expressed genes in ΔrpoZ [10]. The physiological experiments using group 2 σ factor mutant strains have revealed that SigB and SigC factors are important for high temperature acclimation responses [14,15,25] and thus is tempting to speculate that the α subunit not only affects the recruitment of SigA but also the recruitment of the other σ factors.

RNA polymerase and total RNA contents decrease in mild heat stress more in CS than in ΔrpoZ

Next we analyzed the RNAP content of cells in mild heat stress. The cells were grown under standard conditions and then transferred to 40°C for 2, 6 or 24 h. Western blots showed a clear decrease of RNAP during the high temperature treatment in CS; after one day treatment, cells had lost 45% of the RNAP core subunits α and β (Fig. 4AB). On the contrary, the ΔrpoZ strain lost less than 10% of RNAP core subunits α and β (Fig. 4AB). In
addition, the amount of the primary σ factor, SigA, decreased in heat stress; after 24-h heat treatment 45% and 17% of SigA was lost in CS and D<sub>rpoZ</sub>, respectively (Fig. 4C). The v subunit decreased similarly in CS as the other RNAP core subunits (Fig. 4D). In accordance with decrease of RNAP in CS, the total RNA content of CS cells decreased from 1.2 μg/ml in cultures with OD<sub>730</sub> = 1 [10] to 0.8 μg/ml after a 24-h treatment at 40°C (Fig. 4E). In the D<sub>rpoZ</sub> strain, the RNA content was similar as in CS in standard conditions [10]. The RNA content of D<sub>rpoZ</sub> decreased only 17% during the 24-h heat treatment (Fig. 4E) suggesting that the higher RNAP content of D<sub>rpoZ</sub> keeps transcription in D<sub>rpoZ</sub> more active than in CS in mild heat stress. However, the stability of transcripts is known to vary according to environmental cues [38] and we cannot rule out the possibility that the RNA contents of CS and D<sub>rpoZ</sub> are affected by RNA stability at high temperatures.

More than 90% of the total RNA in cells consists of rRNA, and analysis of total RNA by agarose gel electrophoresis revealed that the rRNA content of CS was lower than that of the D<sub>rpoZ</sub> strain (Fig. 4E). In E. coli, severe heat stress has shown to disturb ribosome assembly [39] and on the other hand, ribosomes form inactive 100S dimers when cells enter a non-growth mode in stationary phase [40,41]. In our mild heat stress conditions, CS grew as well as in standard conditions, indicating that translation remained fully active although the rRNA content of the cells decreased. Increase in temperature speeds up enzyme reactions and a lower amount of ribosomes might provide fully active translation in a slightly elevated temperature. In the case of D<sub>rpoZ</sub>, further experiment are required to find out whether a high rRNA content directly affects ribosome content and whether all ribosomes are translationally active or not.

We used total RNA samples in DNA microarray analysis, and the decrease in the RNA content of the cells during mild heat stress might affect the DNA microarray results, as we do not know whether the mRNA/rRNA ratio remained similar in all samples. However, overall signal intensities in the DNA microarray raw data did not reveal any systematic differences between the treatments or the strains, suggesting that the mRNA/rRNA ratio was not drastically different between samples. The method used for data normalization was found to be important when time series samples were analyzed [42]; in pairwise comparisons, performed in the present study, the quantile method is regularly used.

**Photosynthetic capacity of D<sub>rpoZ</sub> decreased in mild heat stress**

Photosynthesis is known to be a heat sensitive process. A 60-min heat treatment at 42°C was shown to reduce photosynthetic activity by 15% [43], and many parts of photosynthetic reactions, including carbon fixation by Rubisco and photosynthetic light
Figure 4. Contents of RNA polymerase and RNA in mild heat stress in the control and ΔrpoZ strains. Total proteins were isolated after 0, 2, 4 and 24 h treatments at 40°C, samples containing 50 µg of protein were separated with SDS-PAGE, and the amounts of the α(A), β(B), SigA(C) and ω(D) subunits of RNAP were determined by western blotting. (E) Total RNA content of cells after 24-h heat treatment. Total RNA content in 1-mL sample (OD730 = 1) of CS (black bars) and ΔrpoZ (white bars) cell cultures incubated for 24 h at 40°C. Each result represents the mean of three biological replicates and the error bars denote SEM. A 5-µl sample of isolated RNA was separated in 1.2% agarose gel and stained with ethidium bromide to visualize rRNA.

decrease of PSII (measured using an antibody against the PSII core protein CP43) and 10 to 15% decrease of Rubisco (measured using an antibody against RbcL) occurred in both strains (Figs. 5A and 5B), while the PSI content (antibody against PSI reaction center protein PsA) remained at the same level as in standard growth conditions (Fig. 5C). In CS, the phycobilisome antenna proteins phycocyanin and allophycocyanin remained constant during the 24-h heat treatment at 40°C (Figs. 5D and 5E). However, in ΔrpoZ the phycocyanin content of the cells decreased (Fig. 5D) although allophycocyanin (Fig. 5E) remained at the same level as in the control conditions. Interestingly, heat treatment induced up-regulation of the nblA1 and nblA2 genes (encoding the phycobilisome degradation proteins NblA1 and NblA2, respectively) in ΔrpoZ but not in CS (Fig. 3). NblA1 and NblA2 proteins form a heterodimer [33] that acts as an adaptor guiding the Clp protease to phycobilisomes [43]. These findings suggest that up-regulation of NblA proteins in ΔrpoZ upon heat stress induces degradation of phycobilisome rods that consist of phycocyanin.

After the 24-h treatment at 40°C, the light-saturated photosynthetic activity of CS, measured by oxygen evolution, was 92% of that measured in standard conditions (Fig. 6). In standard conditions, light-saturated photosynthetic activity of ΔrpoZ was circa 20% lower than in CS (Fig. 6A) and it further decreased in mild heat stress being only 68% of that measured in CS after 24-h treatment at 40°C (Fig. 6). The light-saturated PSII activities of the cells grown in mild heat stress, measured using a quinone electron acceptor, were 2.02±0.08 and 1.39±0.16 µmol O2/OD730/h in the control and ΔrpoZ strains, respectively, indicating that PSII of the ΔrpoZ strain was vulnerable to heat-treatment.

Conclusions

The heat-lethal phenotype of ΔrpoZ strain emphasizes the view that the small ω subunit of RNAP is an important core polypeptide although cells can survive without it in optimal laboratory conditions. The total RNA content of the cells remains higher in ΔrpoZ than in CS during heat stress, and therefore the heat-lethal phenotype of ΔrpoZ is probably not caused by a decrease in active RNAP due to the proposed chaperone-like activity of the ω subunit. Instead, our data suggest that numerous heat acclimation processes malfunction in ΔrpoZ. As summarized in Fig. 7, these acclimation processes include adjustment of transcription, photosynthesis and nitrogen metabolism. Gene expression respond differently in ΔrpoZ and CS, and the data indicate that the small ω subunit affects expression of specific genes not only in standard growth conditions but also during heat stress.

Materials and Methods

Strains, growth conditions and growth measurements

The glucose tolerant control strain of Synechocystis sp. PCC 6803 [46], the ω subunit inactivation strain ΔrpoZ and the complementation strain ΔrpoZ+ρpoZ [10] were grown in BG-11 medium supplemented with 20 mM Hepes pH 7.5. The OD730 of liquid cultures was set to 0.1 (0.35 µg of chlorophyll (Chl)/µl), and the cells were grown (30 ml of cell culture in a 100-ml Erlenmeyer flask) at 32°C, 38°C or 40°C in air level CO2 under constant illumination at the PPFD of 40 µmol m⁻² s⁻¹ and shaking at 90 rpm. In some experiments, as indicated, BG-11 medium was supplemented with 20 mM Hepes, pH 8.3. Samples of dense cultures were diluted with BG-11 before the optical density was measured, so that OD730 did not exceed 0.4, and the dilutions were taken into account when the final results were
calculated. All measurements were conducted on at least three independent biological replicates.

Survival rates at 40 °C

OD730 was set to 0.1, and cells were grown at 40 °C for 24 h. The OD730 was measured, cells were diluted with fresh BG-11 medium to OD730 = 0.1. Then culture was serially diluted to 1:10, 1:100, 1:1000 and 1:10 000; and twenty drops containing 10 μl of the dilutions were spotted onto BG-11 plates. Plates were grown in standard conditions for one week, the colonies were counted and CFUs were calculated as CFUs/1-ml cell culture with OD730 = 0.1.

DNA microarray analysis

For DNA microarray studies, OD730 was set to 0.1, and the cells were grown in standard growth conditions for three days. Then the samples from standard conditions (OD 730 = 1, 40 ml) were harvested by centrifugation at 4500 g for 6 min at 4 °C in pre-cooled centrifuge tubes [10] or cells were treated at 40°C under continuous illumination, PPF 40 μmol m−2 s−1, for 24 h before harvest. The RNA was isolated using the hot-phenol method as described in [47], and further purified with RNeasy Mini Kit (Qiagen) to remove DNA contaminations. A 8x15 K custom Synechocystis sp. PCC 6803 array (Agilent) was used in microarray experiments [48], and hybridizations and data collection were done as described previously [49]. The data were normalized using the quantile method and the t-test was used to identify differentially expressed genes. A gene was considered differentially regulated if log2 of the fold change was $\geq 1$ (at least two-fold up-regulated) or $\leq -1$ (down-regulated to one half or less) and $P < 0.05$. Gene expression data were visualized with a heat map drawn with the open source software Multiple Experiment Viewer [50].

Total RNA content of the cells

Cells were first grown in standard growth conditions and then treated at 40°C under continuous illumination, PPF 40 μmol m−2 s−1, for 24 h before harvest. Total RNA was isolated with the hot-phenol method [47] from 1-ml of cell culture with OD730 = 1, and suspended in 12 μl of water. RNA concentration was measured with NanoDrop spectrophotometer and RNA were visualized by running 5-μl samples on 1.2% agarose gels and staining the gels with ethidium bromide.

Western blotting

Cells (25 ml; OD730 = 1; 3.5 μg Chl a/ml) were harvested from standard growth conditions, or treated at 40°C under continuous illumination (PPFD 40 μmol m−2 s−1) for 2, 6, or 24 h before harvesting. Total proteins were isolated as described previously [51]. Protein samples containing 1.6 μg (allophycocyanin, phyco-cyanin), 5 μg (CP43), 10 μg (PsaB, Rubisco), 20 μg (the ω subunit)
or 50 μg (α, β, and SigA subunits of RNAP) of total proteins were solubilized for 10 min at 75°C and separated by 10% NEXT GEL SDS-PAGE (Amresco) according to the manufacturer’s instructions. Proteins were transferred to Immobilon-P membrane (Millipore). Antibodies against allophycocyanin of *Porphyridium cruentum* (AS08 277), CP43 of *Arabidopsis thaliana* (AS11 1787), PsaB of *Arabidopsis thaliana* (AS10 695), phycocyanin of *Porphyridium cruentum* (AS08 278), and RbcL (AS03 037), and custom polyclonal antibodies recognizing α, β, γ and SigA subunits of *Synechocystis* RNAP [10] were purchased from Agrisera. The Goat Anti-Rabbit IgG (H+L) alkaline phosphatase conjugate (Zymed) and the CDP star chemiluminescence kit (New England Biolabs) were used for detection. Immunoblots were quantified with a FluorChem image analyzer (Alpha Innotech Corp.).

**Photosynthetic activity**

Light-saturated photosynthetic activity in *vivo* was measured (1 ml sample, OD730 = 1) with a Clark type oxygen electrode (Hansatech Ltd.) at 32°C in BG-11 medium supplemented with 10 mM NaHCO3. The light-saturated PSII activity was measured using 0.7 mM 2,6-dichloro-p-benzoquinone as an artificial electron acceptor, and samples were also supplemented with 0.7 mM ferricyanide to keep the electron acceptor in oxidized form.

**Supporting Information**

**Table S1** Genes at least two fold up-regulated in the control strain after a 24-h treatment at 40°C.

(PDF)

**Table S2** Genes down-regulated to half or less in the control strain after a 24-h treatment at 40°C.

(PDF)

**Table S3** Genes that were at least two fold up-regulated in *ArpoZ* after a 24-h treatment at 40°C.

(PDF)

**Table S4** Genes down-regulated to half or less in the *ArpoZ* strain after a 24-h treatment at 40°C.

(PDF)

**Table S5** List of genes included in Fig. 3 and their expression data.

(PDF)

**Table S6** List of genes that were similarly or oppositely regulated in CS and *ArpoZ* after heat treatment.

(PDF)

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**Author Contributions**

Conceived and designed the experiments: LG KH TT. Performed the experiments: LG JK SK MP. Analyzed the data: LG JK KH MP TT. Wrote the paper: LG JK KH SK MP TT.

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