The role of *Synechocystis* RNA-polymerase ω subunit when acclimating to nitrogen deprivation

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MOLECULAR PLANT BIOLOGY

Introduction

Cyanobacteria are photosynthetic prokaryotes. They use light energy to produce ATP and NADPH to fuel biosynthesis of sugars through carbon fixation (Fig. 1).

Cyanobacterial RNA polymerase (RNAP) core consists of 2 α subunits, β subunit, β' subunit, γ subunit and ω subunit (Fig 2.). ω is the only non-essential RNAP subunit in standard growth conditions [3], but ω-less *Synechocystis* mutant (ΔrpoZ) has high CO_2 lethal phenotype [4]. Lacking ω subunit influence on affinity of different σ factors on the RNAP core. Depending of which of the nine σ factors forms a transcription initiation competent RNAP holoenzyme (Fig 2.) different genes are transcribed.

Cyanobacteria need a nitrogen source to grow. If they are starved of nitrogen cells go into chlorosis and break down proteins to gain nitrogen [1]. Most notably cells break light harvesting phycobilisome proteins (Fig.1). Nitrogen metabolism is tightly coupled with CO_2 acclimation [2] and the ΔrpoZ strain has low expression of inorganic nitrogen transporters [5]. Therefore, it is important to study behaviour of ΔrpoZ cells in nitrogen deprivation.

Aims

This study aims to find out what roles the ω subunit of RNAP play in nitrogen deprivation.

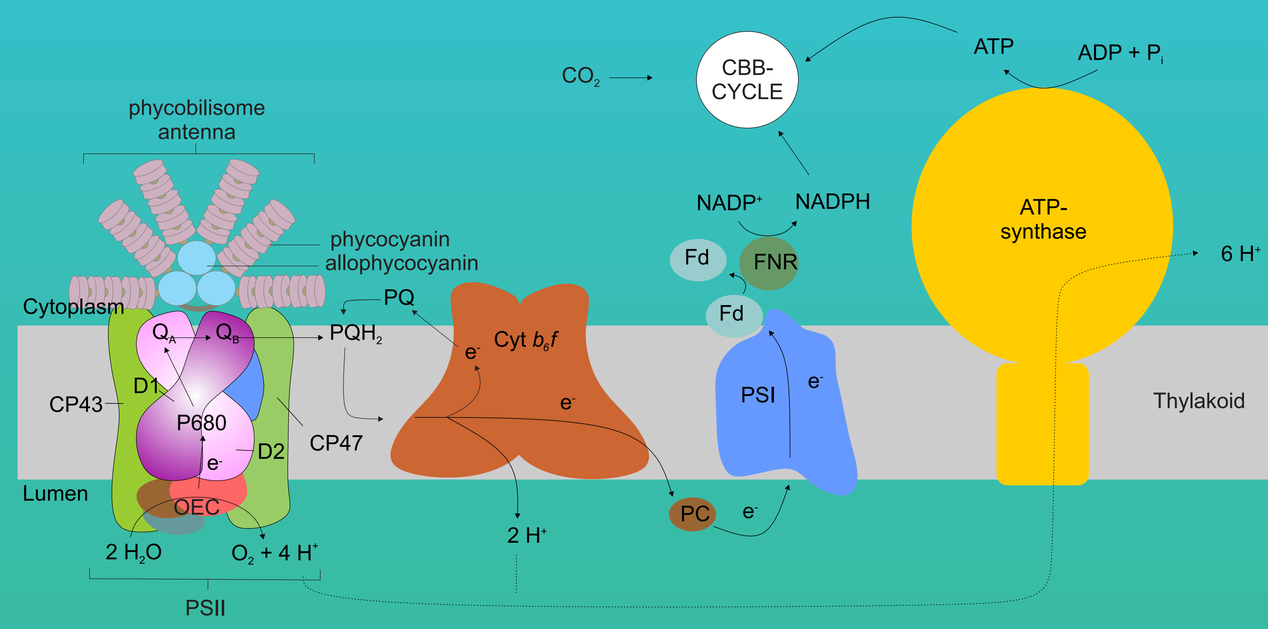

Figure 1. Photosynthetic electron transfer chain and the main photosynthetic complexes in the thylakoid membrane. The phycobilisome antenna consists of phycocyanin and allophycocyanin. It harvests photons to Photosystem II (PSII). Oxygen evolving complex (OEC) in PSII splits water into oxygen, protons and electrons. Electrons are transferred through PSII to plastoquinone (PQ) which in turn gives electrons to cytocrome b_6f (Cyt b_6f). Cyt b_6f *gives electrons to plastocyanin (PC) and releases protons. Photosystem I (PSI) accepts electrons from PC and gives them to ferredoxin (Fd). Ferredoxin-NADP⁺-reductase (FNR) takes electrons from ferredoxin and produces NADPH. Protons produced by OEC and* cyt b_6f *are used to produce ATP. ATP and NADPH are then used in Calvin-Benson-Bassham (CBB)-cycle.* [6]

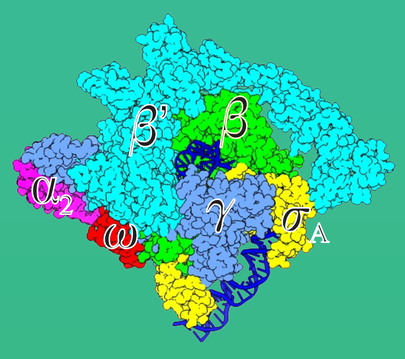

Figure 2.Synechocystis sp. PCC 6803 RNA-polymerase holoenzyme PDB id: 8GZG [7]

Conclusions

The ω subunit of cyanobacterial RNA polymerase has only a minor role in adaptation to nitrogen deprivation.

Reason for low respiration in ω-less mutant during nitrogen deprivation is under study.

Results

Amounts of phycobilisome, PSII and PSI

Breakdown of phycocyanin can be seen in both strains as nitrogen deprivation progresses (Fig. 5a). The amount of photosystem II subunit CP43 decreases in both strains (Fig. 5b), but the photosystem I subunit PsaB (Fig 5c) shows lower amount in control strain than in ΔrpoZ in nitrogen deprivation.

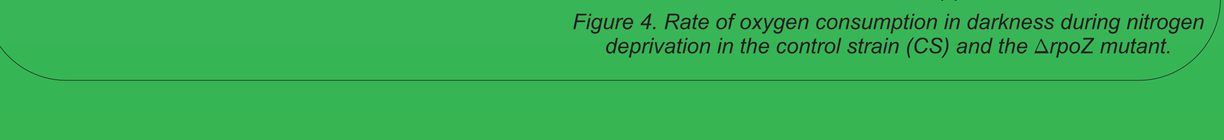

Figure 5. Amount of a) phycocyanin, b) photosystem II subunit CP43 and c) photosystem I subunit PsaB in the control strain (CS) and the ΔrpoZ mutant after 4, 7 and 24 hours in nitrogen deprivation. Amounts of proteins related to photosynthesis were measured by using western blot. Specific antibodies were used to detect phycocyanin (Phycobilisome), CP43 (PSII) and PsaB (PSI), from total protein extracts separated with SDS-PAGE.

Respiration

Cyanobacteria can also use oxygen consuming cellular respiration (Fig. 3).
Rate of respiration increases in early nitrogen starvation control and ΔrpoZ strains (Fig. 4). Trend is that control strain has higher respiration activity.

Figure 3. Simplified cyanobacterial respiration. NDH-1 takes electrons from NADPH and reduces PQ to PQH_2, which can then donate electrons to terminal oxidases which consume oxygen to produce water.

Figure 4. Rate of oxygen consumption in darkness during nitrogen deprivation in the control strain (CS) and the ΔrpoZ mutant.

Results

Photosynthetic activity

Oxygen evolution rate decreases >60 % after 8 hours of nitrogen deprivation, but maintains plateau phase between 8 hour and 24 hour timepoints (Fig. 5). After 48 hours of nitrogen depletion no photosynthetic activity was detected. No major differences between the strains.

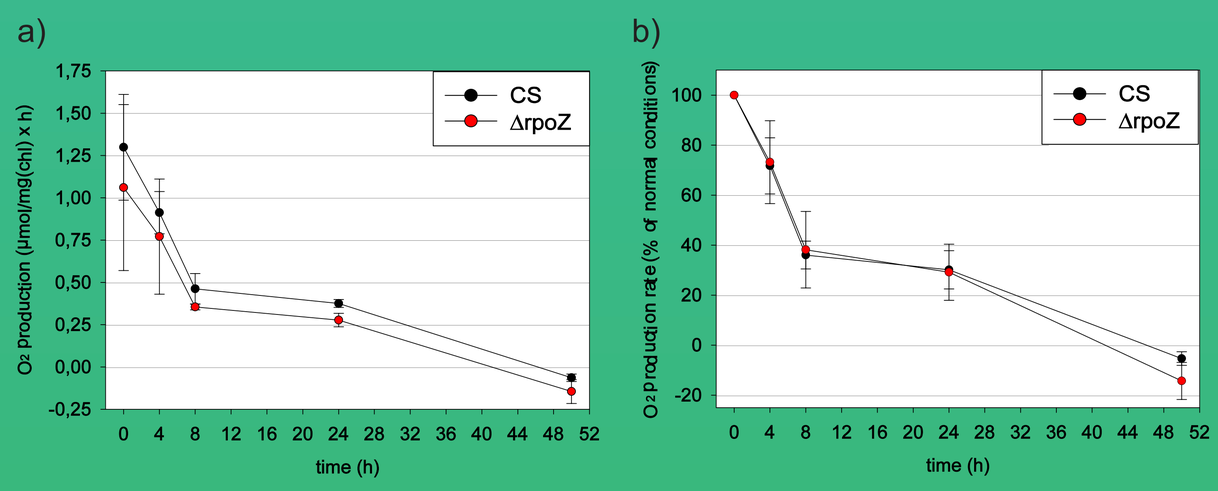

Figure 5. Oxygen evolution rate of the ΔrpoZ mutant and the control strain (CS) in nitrogen deprivation. a) Absolute values and b) percentage change from normal conditions. Cyanobacterial cultures were grown in BG-11, after which cells were washed with and put into nitrogenless BG-11 to induce starvation. Photosynthetic activity was studied by measuring oxygen evolution with oxygen electrode. Rate of oxygen evolution was measured by following oxygen evolution for 3-4 min with 10 mM $NaHCO_3$ under saturating light (PPDF ~2100 µmol/m^{-2}/s^{-1}).

PSII activity

PSII activity drops to 50 % in only 4 hours of nitrogen deprivation and after 48 hours no PSII activity is left (Fig. 6). However in the ΔrpoZ strain there is some activity left after 48 hours of nitrogen depletion.

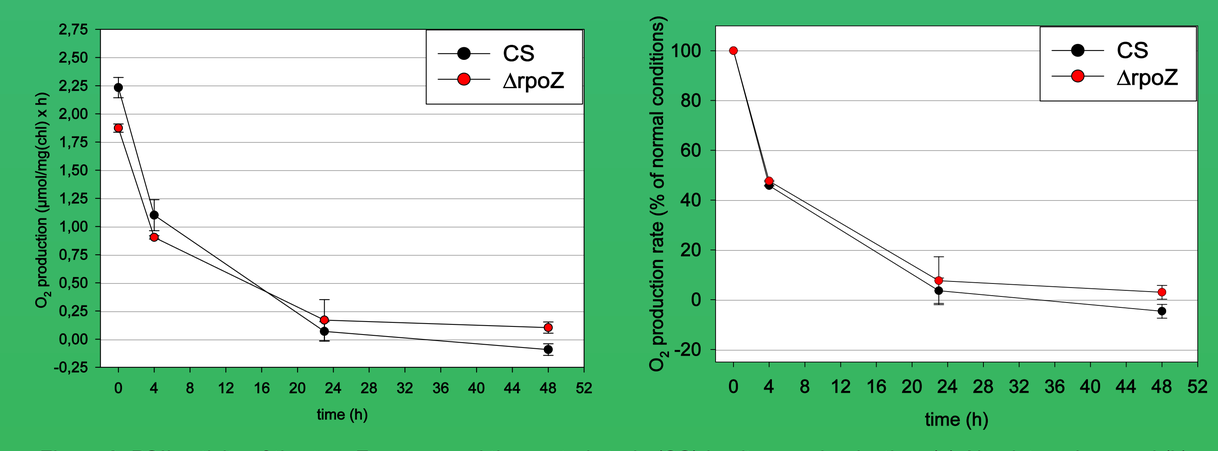

Figure 6. PSII activity of the ΔrpoZ mutant and the control strain (CS) in nitrogen deprivation. (a) Absolute values and (b) a percentage change from normal conditions. Cyanobacterial cultures were grown in BG-11, after which cells were washed with and put into nitrogenless BG-11 to induce starvation. PSII activity was measured by adding 0.5 mM DCBQ into the sample which takes the electrons from PSII and prevents the electron flow to PQ. Oxygen evolution was also measured in darkness during PSII activity measurement and rate of consumption was subtracted from production results.

Future experiments

Biological replicates of photosynthesis proteins as well as proteins related to oxygen consumption (NDH-1 & flavodiirons) are going to be studied with western blotting.

In addition, the transcript levels of those proteins studied with Western blotting will be measured with qPCR.

References

[1] Forchhammer & Schwartz (2019) Nitrogen chlorosis in unicellular cyanobacteria – a developmental program for surviving nitrogen deprivation. *Environmental Microbiology* **21(4)**: 1173–1184
[2] Forchammer & Selim (2020) Carbon/nitrogen homeostasis control in cyanobacteria. *FEMS Microbiology Reviews* **44(1)**: 33-53
[3] Gunnelius et al. (2014a) The omega subunit of the RNA polymerase core directs transcription efficiency in cyanobacteria. *Nucleic Acids Research* **42**: 4606-4614
[4] Kurkela et al. (2017) Acclimation to High CO2 Requires the ω Subunit of the RNA Polymerase in Synechocystis. *Plant Physiology* **174**: 172-184
[5] Kurkela et al. (2024) A novel signaling cascade regulates growth of the cyanobacterium Synechocystis sp. PCC 6803 according to CO2. Manuscript
[6] Sarvikas et al. (2017) Fotosynteesi.
[7] Shen et al. (2023) An SI3-σ arch stabilizes cyanobacteria transcription initiation complex. *PNAS* **120(16)**: e2219290120

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