Transcriptome of the SigB overexpression strain of cyanobacterium *Synechocystis* sp. PCC6803

Tayyab Saleem, MSc. Otso Turunen, Doc. Taina Tyystjärvi
Department of Life Technologies, University of Turku
MOLECULAR SYSTEMS BIOLOGY

novo nordisk fonden

Introduction

For acclimation to abiotic stresses, *Synechocystis* sp. PCC6803 adjusts its gene expression. Depending on the type of stress, different sigma (σ) factors bind to RNA polymerase to modulate gene expression. One of these stress responsive σ factors is SigB, activated under heat, oxidative stress, and osmotic stress. Adding an extra copy of the *sigB* gene, under the strong *psbA2* promoter, to the *Synechocystis* genome (Fig. 2) increases its expression 4-fold in the SigB overexpression (SigB-oe) mutant compared to the control strain (CS) [1]. Under different environmental stresses, SigB-oe performs better and produces more heterologous proteins than CS [1]. I have studied the SigB-oe transcriptome in an optimal growth environment.

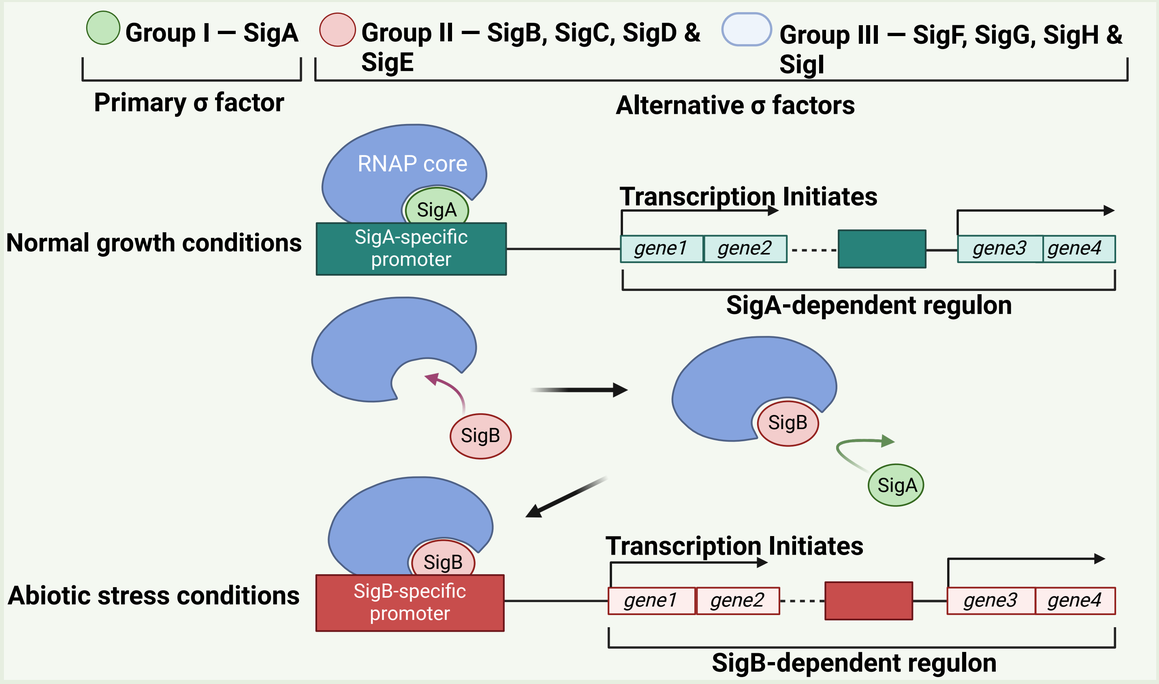

Figure 1. Roles of SigB sigma factor in *Synechocystis.*

SigB-oe and CS strains

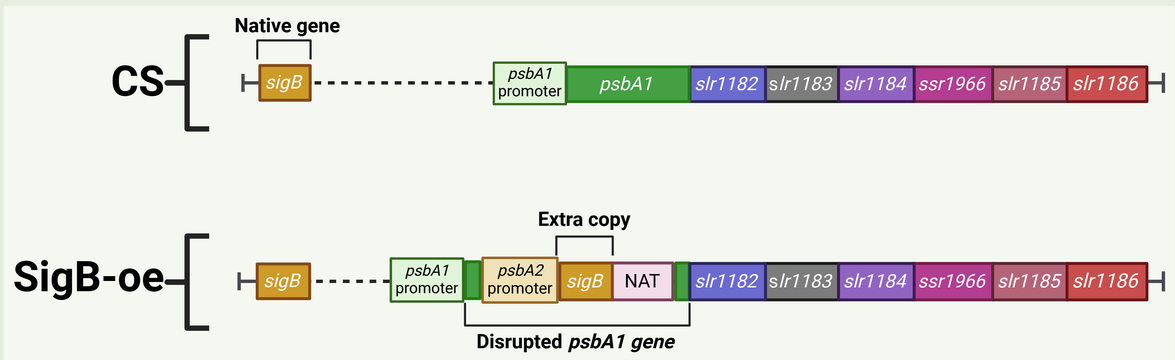

Figure 2. Construct of the SigB-oe mutant, containing the nourseothricin resistance cassette (NAT) [1].

Main Aims

Compare the transcriptomes of the SigB-oe mutant and the control strain (CS) in standard growth conditions.

Studying the content of sigma factors in the RNAP holoenzyme in the SigB-oe strain.

Main Findings

- No substantial differences found between the transcriptomes of SigB-oe and control strains of *Synechocystis*
- RNAP-SigB holoenzyme content was low in both SigB-oe and control strains
- Similar RNAP holoenzyme contents of SigB-oe and control strains explain why the transcriptomes of these strains are similar in standard conditions
- A possible post-transcriptional mechanism limits SigB abundance in the RNAP holoenzyme, in standard conditions

Methodology

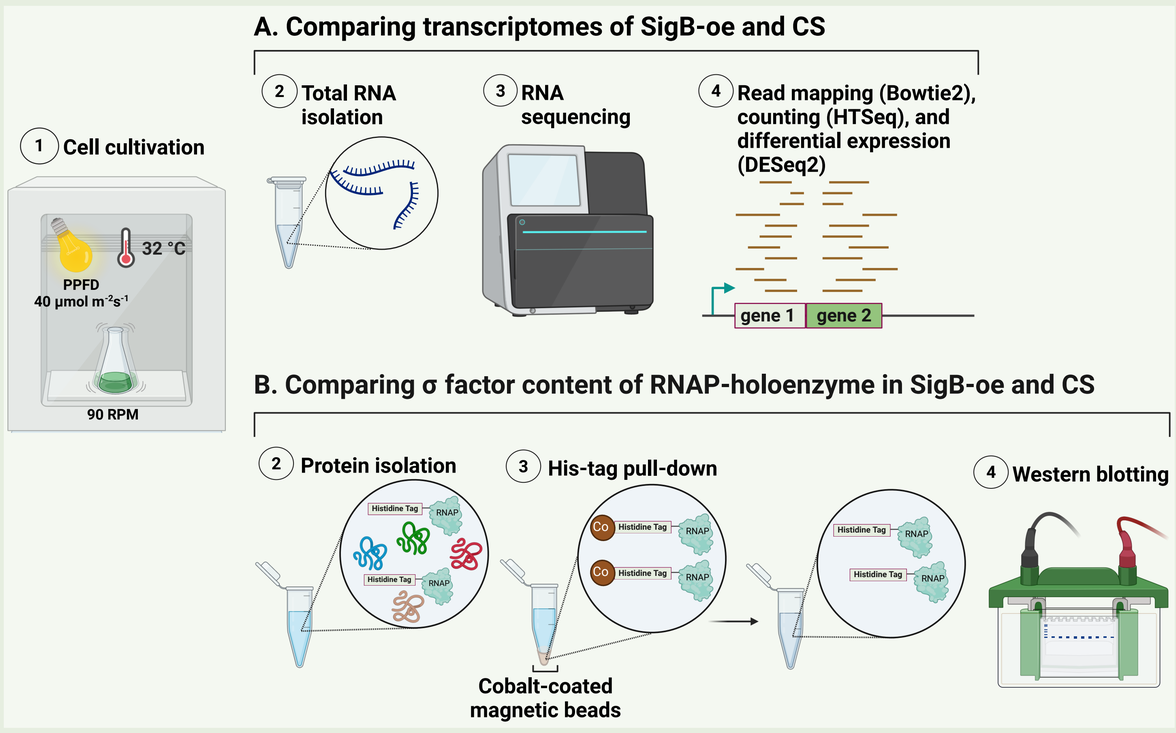

Figure 3. Thesis workflow. A) For comparing transcriptomes of SigB-oe and CS, three biological replicate cultures were grown in standard conditions (photosynthetic photon flux density 40 µmol $m^{-2}s^{-1}$, 32 °C, ambient air, shaken at 90 RPM). Total RNA was isolated using the hot phenol method and sent for commercial sequencing. Sequencing reads were mapped to *Synechocystis* genome using Bowtie2 and counted with HTSeq, and differential expression was analyzed with DESeq2. B) For comparing σ factor content in SigB-oe and CS, a histidine tag was added to the γ subunit of RNAP in both strains. Three biological replicate cultures of SigB-oe+RNAP-His and CS+RNAP-His strains were grown in standard conditions. After isolating soluble proteins, RNAP complexes were pulled down using cobalt-coated magnetic beads. The separation of 0.44 mg of pulled-down proteins was performed using SDS-PAGE, and primary antibodies specific to SigA, SigB, SigC, SigD, and alpha subunit were used for protein detection in Western blotting.

Transcriptomes of SigB-oe and CS

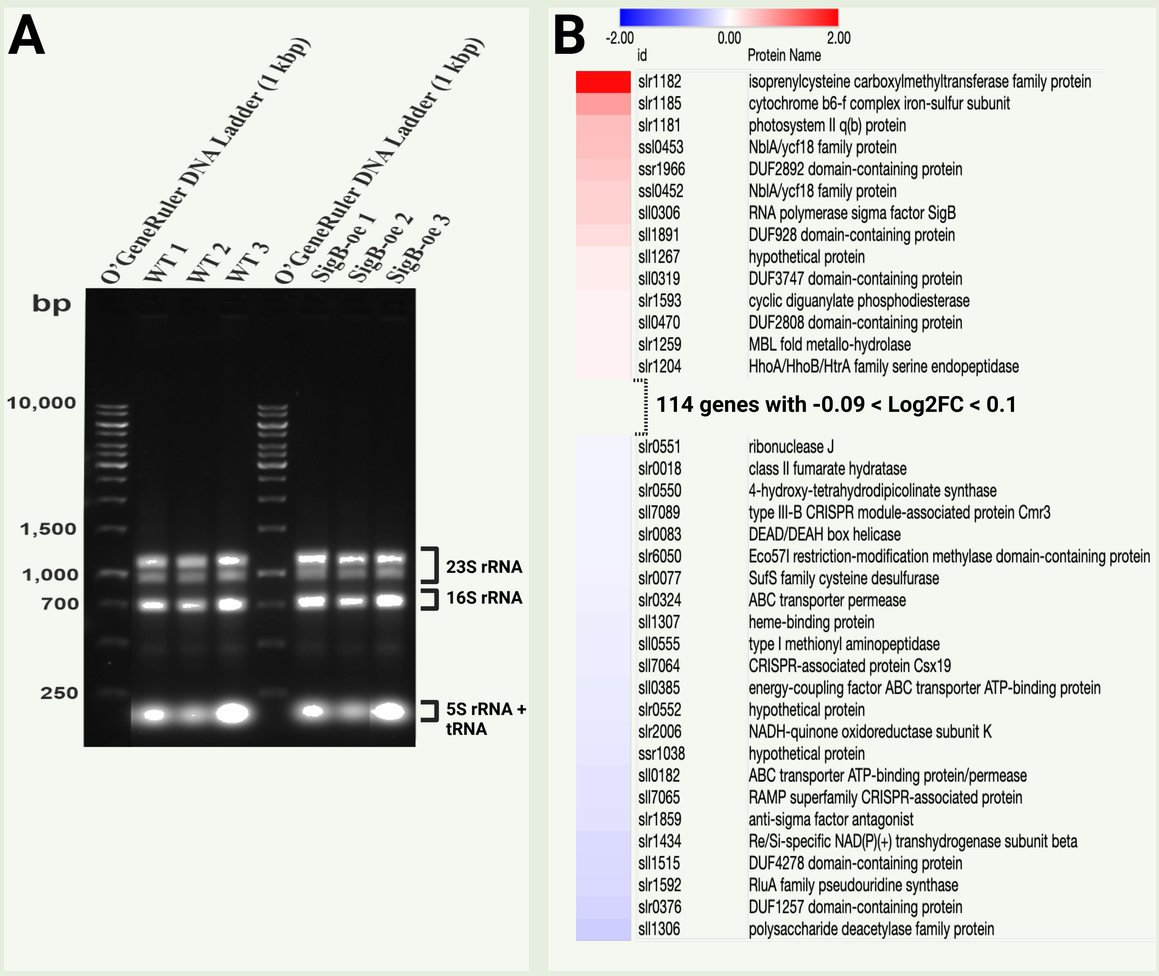

Figure 4. A) Total RNA isolated from SigB-oe and CS cells. B) The heat map showing transcripts with statistically significant ($p<0.05$) differences between the SigB-oe and control strains.

RNAP holoenzymes of SigB-oe and CS

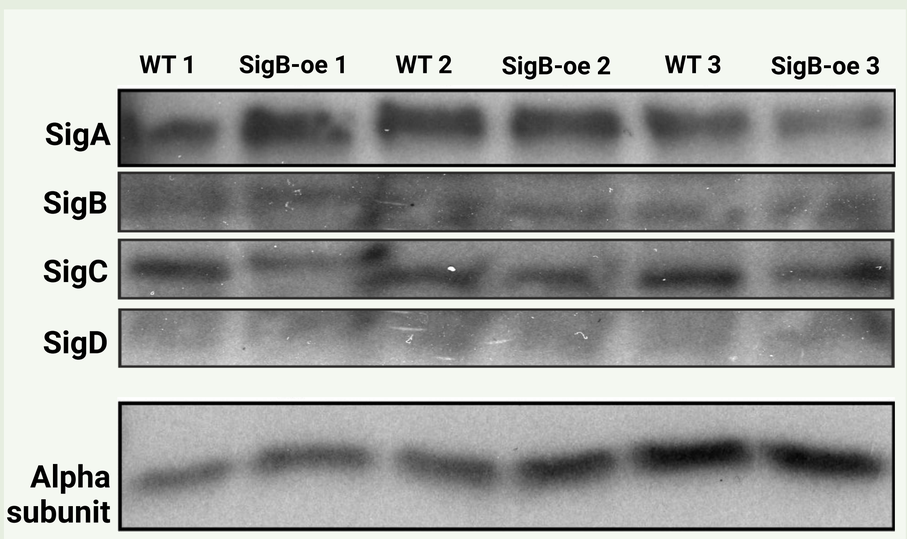

Figure 5. Content of sigma factors in the RNAP holoenzyme and the alpha subunit of RNAP.

References

[1] Turunen, Saleem, Kurkela, Kallio, Tyystjärvi (2024). Engineering RNA polymerase to construct biotechnological host strains of cyanobacteria, Physiologia Plantarum 176:e14263.