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

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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 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.

Figure 1. Roles of SigB sigma factor in Synechocystis.

Transcriptomes of SigB-oe and CS



| | 0.00 | 2.00 Protein Name |
|-------|------|--|
| 1182 | | isoprenylcysteine carboxylmethyltransferase family protein |
| 1185 | | cytochrome b6-f complex iron-sulfur subunit |
| 1181 | | photosystem II q(b) protein |
| 10453 | | NbIA/ycf18 family protein |
| r1966 | | DUF2892 domain-containing protein |
| 10452 | | NbIA/ycf18 family protein |
| 0306 | | RNA polymerase sigma factor SigB |
| 1891 | | DUF928 domain-containing protein |
| 1267 | | hypothetical protein |
| 0319 | | DUF3747 domain-containing protein |
| 1593 | | cyclic diguanylate phosphodiesterase |
| 0470 | | DUF2808 domain-containing protein |
| 1259 | | MBL fold metallo-hydrolase |
| 1204 | | HhoA/HhoB/HtrA family serine endopeptidase |
| 114 g | ene | es with -0.09 < Log2FC < 0.1 |
| 0551 | | ribonuclease J |
| 0010 | | alago II fungayata budyataga |

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

| sll7089 | type III-B CRISPR module-associated protein Cmr3 |
|---------|---|
| slr0083 | DEAD/DEAH box helicase |
| slr6050 | Eco57I restriction-modification methylase domain-containing protein |
| slr0077 | SufS family cysteine desulfurase |
| slr0324 | ABC transporter permease |
| sll1307 | heme-binding protein |
| sll0555 | type I methionyl aminopeptidase |
| sll7064 | CRISPR-associated protein Csx19 |
| sll0385 | energy-coupling factor ABC transporter ATP-binding protein |
| slr0552 | hypothetical protein |
| slr2006 | NADH-quinone oxidoreductase subunit K |
| ssr1038 | hypothetical protein |
| sll0182 | ABC transporter ATP-binding protein/permease |
| sll7065 | RAMP superfamily CRISPR-associated protein |
| slr1859 | anti-sigma factor antagonist |
| slr1434 | Re/Si-specific NAD(P)(+) transhydrogenase subunit beta |
| sll1515 | DUF4278 domain-containing protein |
| slr1592 | RluA family pseudouridine synthase |
| slr0376 | DUF1257 domain-containing protein |
| sll1306 | polysaccharide deacetylase family protein |
| | |

Methodology

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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 holoenyzme and the alpha subunit of RNAP.

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⁻²s⁻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.

References

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