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What makes cyanobacteria tick: Understanding promoter regulation in *Synechococcus* sp. PCC 7002

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Background

Biofuel and chemical production in cyanobacterial hosts has recently expanded due to the search for renewable alternatives for conventional products of the petroleum industry. *Synechococcus* sp. PCC 7002 is an ideal cyanobacterial chassis for these proposed industrial applications. It has a rapid doubling time (2.6 – 4 h);[1] can tolerate up to 1.5 M NaCl – 2.5 x seawater, allowing for cultivation with non-freshwater sources and the ability to adapt to changing salinities due to evaporation;[2] has high light tolerance (survives up to 2 x peak sunlight, 4.5 mE m^{-2} s^{-1}),[3] and can grow under a wide range of temperatures which may be experienced in photobioreactor systems (22-40°C).[2]

A recent study generated promoter mutant libraries for controlled expression in *Synechococcus* sp. PCC 7002.[4] However, this study utilized ideal laboratory conditions (38°C and 60 µmol photons m^{-2} s^{-1}) with continuous light, while most real-world applications will rely on natural sunlight and environmental temperatures. Additionally, very little information is available regarding natural expression levels and regulatory mechanisms in *Synechococcus* sp. PCC 7002. In order to advance *Synechococcus* sp. PCC 7002 as a cyanobacterial chassis, we must have an in-depth understanding of native promoter regulation to allow for genetic manipulations that have minimal impact on the natural metabolism of this host and also utilize the natural regulatory mechanisms to optimize pathways for temporal and light regulation that accompanies the diurnal cycle.

Objectives

- Identify and characterize native promoters in *Synechococcus* sp. PCC 7002 with various expression levels (strong, moderate, low, and weak) and regulatory patterns (constitutive, linear phase, and stationary phase expression).
- Determine the effect of continuous vs diurnal light conditions on expression from these native promoters.
- Identify sequence motifs corresponding to promoter expression level and regulation in *Synechococcus* sp. PCC 7002.

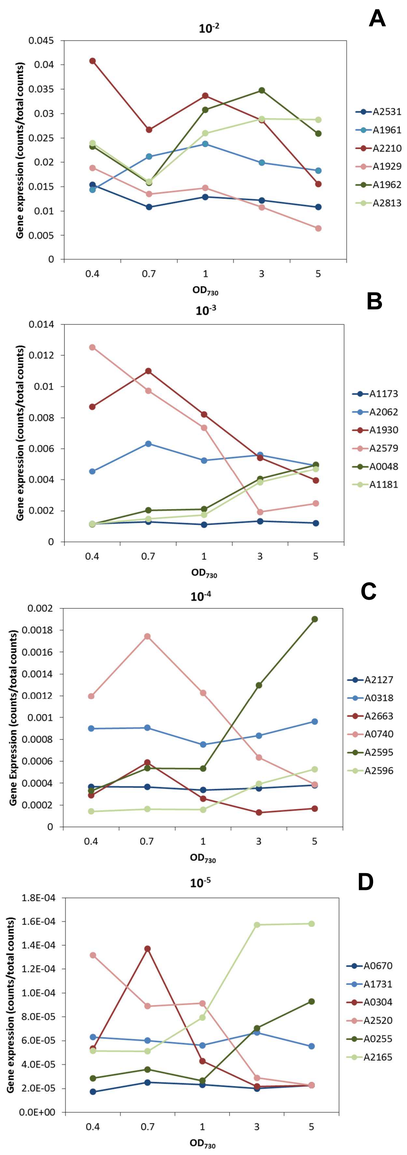
Technical Approach and Design

Native Promoter Selection

Table 1. Select promoters with varying expression levels (10^{-5} – 10^{-2}) and regulation (constitutive, linear phase, and stationary phase) along with their respective gene products.

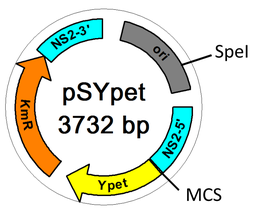
Locus Tag	Regulation	Expression Level	Gene Product
A0670	Constitutive	10^{-5}	conserved hypothetical protein
A1731	Constitutive	10^{-5}	hypothetical protein
A2127	Constitutive	10^{-4}	*accC*, acetyl-CoA carboxylase, biotin carboxylase
A0318	Constitutive	10^{-4}	outer membrane protein, OMP85 family, UDP-3-0-acyl N-acetylglucosamine deacetylase
A1173	Constitutive	10^{-3}	polyketide synthase
A2062	Constitutive	10^{-3}	*fusA*, ribosomal protein S10, translation elongation factor Tu
A2531	Constitutive	10^{-2}	conserved hypothetical protein
A1961	Constitutive	10^{-2}	*psaA*, photosystem I P700 chlorophyll A apoprotein A1
A2520	Linear phase	10^{-5}	conserved hypothetical membrane protein
A0304	Linear phase	10^{-5}	conserved hypothetical proteins
A2663	Linear phase	10^{-4}	*bfr*, bacterioferritin
A0740	Linear phase	10^{-4}	ATP synthase subunit I
A1930	Linear phase	10^{-3}	*apcA*, allophycocyanin α subunit
A2579	Linear phase	10^{-3}	hypothetical protein
A2210	Linear phase	10^{-2}	*cpcA*, phycocyanin α subunit
A1929	Linear phase	10^{-2}	*apcB*, allophycocyanin β subunit
A0255	Stationary phase	10^{-5}	glycosyl transferase, WecB/TagA/CpsF family
A2165	Stationary phase	10^{-5}	conserved hypothetical proteins
A2595	Stationary phase	10^{-4}	conserved hypothetical protein
A2596	Stationary phase	10^{-4}	conserved hypothetical protein
A0047	Stationary phase	10^{-3}	conserved hypothetical protein, CheW-like domain; methyl-accepting chemotaxis protein
A1181	Stationary phase	10^{-3}	ATPase, AAA family domain protein
A1962	Stationary phase	10^{-2}	*psaB*, photosystem I protein A2
A2813	Stationary phase	10^{-2}	S-layer like protein; probable porin

- *Synechococcus* sp. PCC 7002 promoter selected based on previous RNA-seq results.[5]

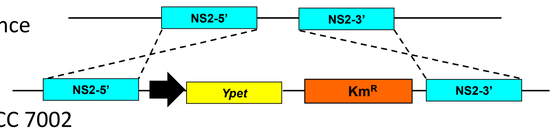


Figures 1A-D (right): Gene expression of select *Synechococcus* sp. PCC 7002 promoters from previous RNA-seq study.[5] (A) Strong expression (10^{-2}), (B) moderate expression (10^{-3}), (C) low expression (10^{-4}), and (D) weak expression (10^{-5}). Blue = constitutive, Red = linear phase, Green = stationary phase.

Promoter Construct Design



- Promoter = 500 bp upstream sequence
- Insert promoter into MCS
- Linearize with SpeI digestion
- Transform into *Synechococcus* sp. PCC 7002
- Construct integrates into putative neutral site 2 (NS2)
- Kanamycin selection for multiple rounds to obtain genetically identical segregants
- PCR based confirmation



Results

Promoter Characterization

- Some correlation of promoter strength with RNA-seq data (10^{-2} and 10^{-3} > 10^{-4} and 10^{-5})
- However, the strongest promoters (A2579 and A2520) were from 10^{-3} and 10^{-5} expression levels from RNA-seq
- RNA-seq constitutive promoters have either constitutive (A2531, A1173, A2062, A2127, A0318, A0670, A1731) or stationary phase expression (A1961). However, many constitutive promoters are not significantly different from wild type.
- RNA-seq linear phase expression promoters show either constitutive (A2210, A1929, A1930, A2663, A0740, A0304) or stationary phase expression (A2579, A2520). The apparent constitutive expression may be due to a long half-life of Ypet (GFP stable for > 1 day).[6]
- Some promoters had different expression under diurnal light
  - A2531 was expressed constitutively under continuous light but had linear phase expression under diurnal light conditions.
  - A0670 showed increased expression under continuous light conditions compared to diurnal.
- Evaluation of stationary phase promoters under diurnal light conditions is ongoing.
- Relatively low dynamic range for most *Synechococcus* sp. PCC 7002 promoters.

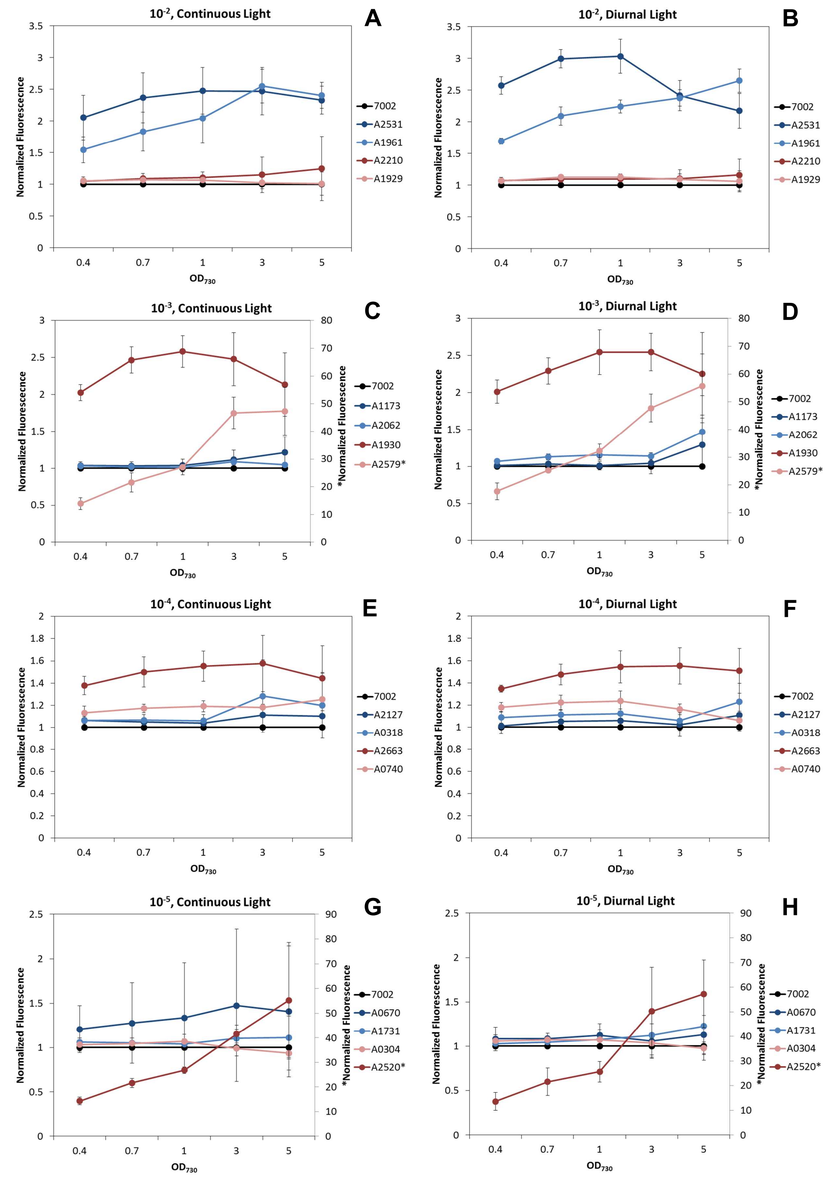
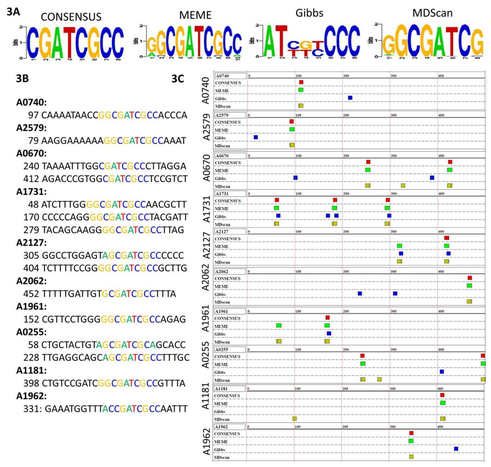
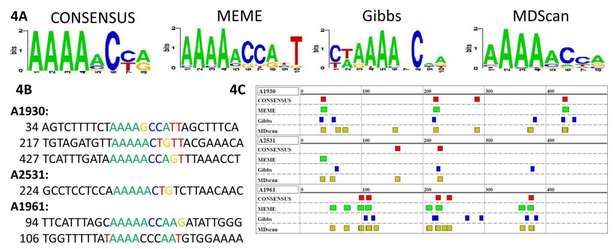


Figure 2. Normalized fluorescence (fluorescence of promoter mutant / fluorescence of 7002) for 485 nm excitation and 528 nm emission across the growth profile of *Synechococcus* sp. PCC 7002, as measured by OD_{730} under continuous light and 12/12 diurnal light conditions (60 µmol photons m-2 s-1). Data are averages of at least 3 biological replicates for two transformants with error bars representing the standard deviation.

Promoter Motifs





Figures 3 and 4. Promoter motifs predicted by using Melina II with CONSENSUS, MEME, Gibbs, and MDScan motif finders. (3) All promoters. (4) Moderate expression promoters.(A) Consensus motifs; (B) Motif sequences; (C) Motif location in 500 bp upstream sequence.

- Promoter motif GGCGATCG identified in 10 out of 30 promoter regions (Figure 3), yet there is no observable trend in the expression of Ypet from these promoters.
- Promoter motif AAAAACCA is consistent among promoters showing moderate expression levels (2- to 3-fold higher Ypet fluorescence compared to wild type at the same optical density).

Conclusions

- Poor correlation between RNA-seq results and Ypet promoter expression, which may be due to strain genetic drift, different environmental conditions, or regulatory regions outside of the 500 bp upstream sequence.
- Verification of moderate and low constitutive promoters and high stationary phase promoters.
- Only a few promoters (A2531 and A0670) showed different expression under diurnal light conditions.
- Two conserved promoter motifs were identified, but their regulatory roles remain to be determined.

References

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