Design and application of stationary phase combinatorial promoters

SEED2016 Technical Report

Victoria Hsiao[1,*], Aileen Cheng[1], Richard M. Murray[1]

March 29, 2016

1. Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125.

* Corresponding Author: Victoria Hsiao, vhsiao at caltech dot edu.

Keywords: Stationary phase, combinatorial promoters, biofilms

Abstract

Current bacterial synthetic circuits rely on the fast dilution and high protein expression that occurs during exponential phase. However, constant exponential phase is both difficult to ensure in a lab environment and almost certainly impractical in any natural setting. Here, we characterize the performance of 13 *E. coli* native σ38 promoters, as well as a previously identified σ38 consensus promoter. We then make tetO combinatorial versions of the three strongest promoters to allow for inducible delayed expression. The design of these combinatorial promoters allows for design of circuits with inducible stationary phase activity that can be used for phase-dependent delays in dynamic circuits or spatial partitioning of biofilms.

Introduction

We envision the future of synthetic biology to involve the deployment of engineered bacteria into potentially harsh and minimal nutrient environments for long periods of time. Although current synthetic circuit testing is done almost exclusively in exponential growth phase, this is neither a realistic environment for natural bacteria nor a reasonable expectation for engineered strains outside of the laboratory. The common constitutive and inducible promoters reduce their gene expression activity considerably when the population reaches stationary phase, and there are not yet well-defined parts libraries for stationary phase active promoters. Stationary phase gene expression in E. coli has been widely studied [1, 2, 3, 4, 5] but we have not yet been able to take advantage of stationary phase stage promoters for synthetic circuits.

Here, we present a small step towards finding, characterizing, and engineering a stationary-phase active promoter library by mining the existing literature for known stationary phase sigma-factor promoters, testing their activity, and engineering them into inducible combinatorial promoters. The creation of this library would have a number of significant advantages. Most importantly, cell functionality could be partitioned into exponential phase tasks and stationary phase tasks. Cells could be programmed to conserve resources until some quorum had been reached, and experimental strains would not have to be constantly diluted to maintain exponential growth. Furthermore, dynamic circuits that rely on delayed stationary phase activity would be repressed until the start of the experiment and presence of the inducer. We also examine the use of these promoters for visualization and spatial partitioning of bacterial biofilms.

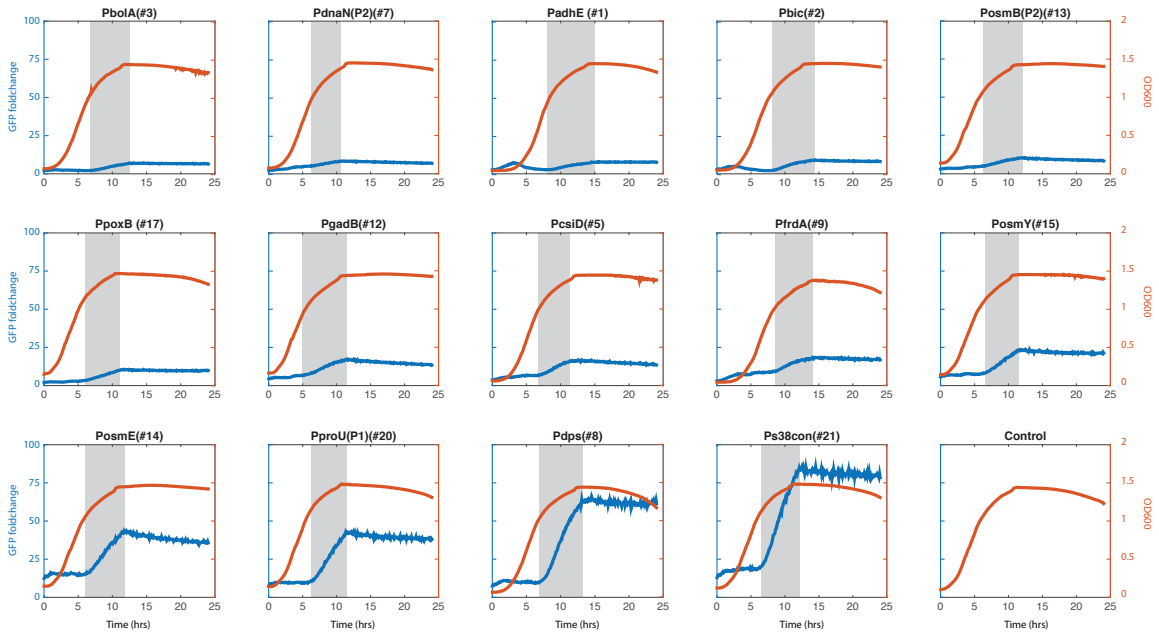

Figure 1: Panel of stationary phase promoters. Native promoter sequences in DH5a-z1 E. coli as previously identified by Lee *et. al* [2], ordered by increasing strength of GFP expression. Orange traces represent OD600 and blue traces represent GFP fold change as normalized by the DH5a-Z1 control strain. Time of GFP increase is highlighted with gray boxes. All of the promoters turned on in late-log phase. Cells were grown at 37C in LB media.

Native promoter panel

We began with a set of 21 sigma38 promoters previously identified in the literature [2]. $\sigma 38$, also known as σS or RpoS, is a RNA polymerase co-factor that is selectively expressed during stationary phase from the *rpoS* gene. First identified by Lange and Hengge-Aronis in 1991[6], $\sigma 38$ is the main stationary phase and stress response promoter and controls more than 500 genes [7]. After identifying the section of putative promoter sequences, we designed a test plasmid with the promoter sequence, a standardized bicistronic ribosomal binding sequence (BCD2-RBS), and a fast folding green fluorescent protein (sfGFP). Figure 1 shows the full panel of $\sigma 38$ promoters tested, in which all the promoters have minimal expression until late-log phase and then express GFP with a range of characteristic strengths. The full panel includes 13 native promoters and one sigma38 consensus promoter created from the consensus motifs of the other promoters [2]. The control was a non-fluorescent DH5a-Z1 strain. All of the promoters turned on in late-log phase – we are currently investigating whether that is due to late-log expression of $\sigma 38$ or individual cells reaching stationary phase at different times.

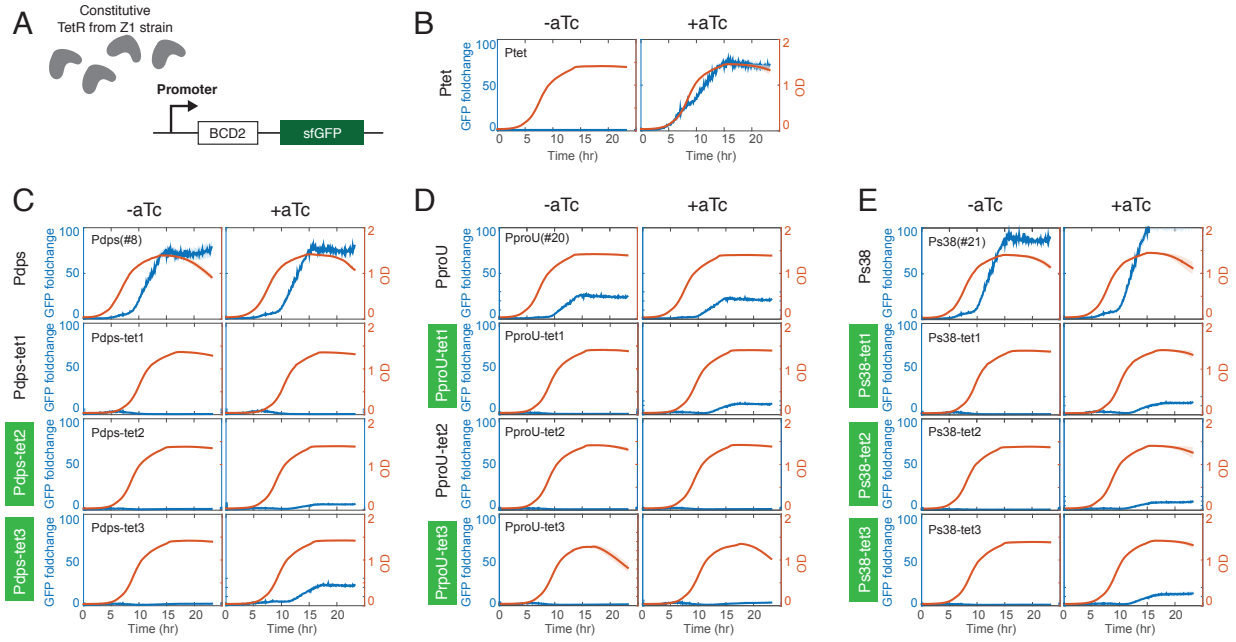

Figure 2: Panel of combinatorial $\sigma 38$ promoters. Combinatorial versions of the strong three native $\sigma 38$ promoters were designed and tested. Orange lines represent OD600 traces, blue lines show GFP fold change over control. A) Design of the test system consisted of cloning each promoter variation in front of BCD2-sfGFP and then transforming the plasmid into a Z1 strain that constitutively makes TetR repressor. B) Control plasmid with Ptet-GFP that only expresses GFP when aTc is present in the media (Added at time t=0h). C) Pdps native promoter and variations. Working combinatorial promoters are highlighted in green. D) PproU promoter and variations. E) Ps38 consensus and variations.

Engineered combinatorial promoters

From this panel of working sigma38 late-log phase promoters, we chose the strongest three promoters, PproU(#20), Pdps(#8), and Ps38consensus (#21) to create combinatorial promoters. For each of the three s38 promoters, we designed three variations of a tetR combinatorial promoter. In the tet1 variant, a single tetO DNA binding motif was placed directly upstream of the promoter sequence 3 of the -10 region. In the tet2 variant, the tetO motif was placed between the -10 and the -35 regions, with all of the original spacing bases

deleted. In the tet3 variant, the tetO motif was again placed between the -10 and -35 regions with 2 original spacing bases on either side.

All nine combinatorial promoter variants, plus the original promoters, and a control Ptet promoter were tested in media with and without aTc in a DH5a-Z1 strain which constitutively produces tetR (Figure 2A). The control Ptet-GFP plasmid turns on only when aTc is present in the media (added at time = 0h) (Figure 2B). The original stationary phase promoters turn on in late log phase independently of aTc, and the successful combinatorial promoters only turn on in late log phase when aTc is present (Figure 2CDE). The working combinatorial promoters are highlighted with green rectangles, with seven out of nine working on the first attempt.

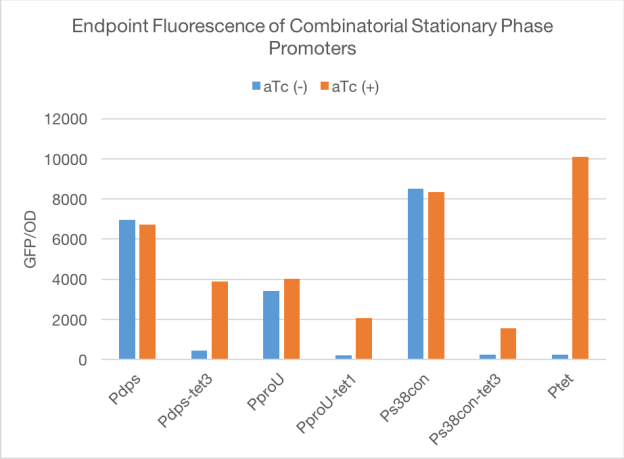

Figure 3: Endpoint fluorescence of combinatorial versus native stationary phase promoters.

We can compare the performance of the top three combinatorial promoters (dps-tet3, proU-tet1, s38con-tet3) with the native promoters, and we see at least a 50% reduction in overall expression strength (Figure 3). This indicates that replacing the bases between the -10 and -35 regions either decreases overall $\sigma 38$ affinity, or the aTc induction was not high enough to full relieve tetR repression.

Spatial patterning of biofilms

E. coli naturally form biofilms that are comprised of multiple layers of cells in different growth phases [7, 8]. The availability of stationary phase active promoters in the context of biofilms means that cells could be programmed to express different functions based on their location within the naturally occuring biofilm. Within a natural bioflim, the cells on the edges are in exponential phase and dividing to expand the biofilm, the cells in the bottom central layer are post-exponential and no longer dividing, and the cells in the upper central layer, furthest from the nutrients in the agar, are in stationary phase (Figure 4A) [8]. In preliminary experiments, when we spot strains with the native $\sigma 38$ promoters onto LB agar, we see spatially dependent expression of GFP compared to a constitutive strain (Figure 4B). Notably, we see differential expression of the each of the three different stationary phase promoters, with Pdps expression primarily around the outer ring, PproU expression going out in tendrils from the center, and s38consensus expression evenly spread out (Figure 4B).

When the combinatorial promoters Pdps-tet3, PproU-tet1, and Ps38-tet3 are tested on plates with and without aTc, we can observe GFP expression turning on based on aTc availability. Because the overall output of these promoters is much weaker than the endogenous promoters, it is more difficult to see the same phenotypic differences observed in Figure 4B, thus far.

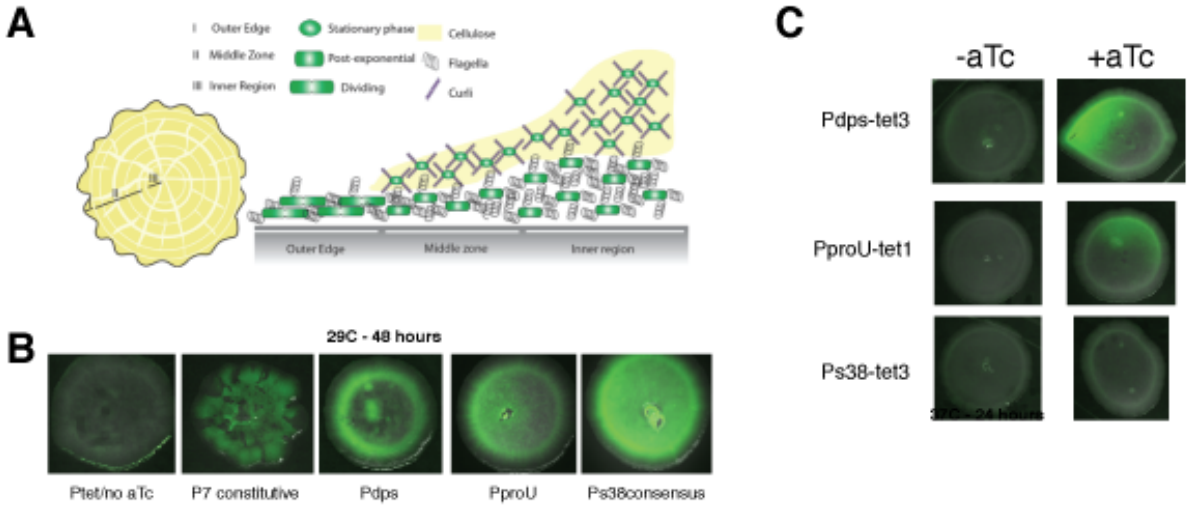

Figure 4: Use of stationary phase promoters for understanding biofilms. A) Diagram copied from Hobley *et. al* [8]. *E. coli* biofilms are comprised of multiple layers of cells in different growth phases. B) Imaging of native $\sigma 38$ promoters within an *E. coli* biofilm. Ptet-GFP with no aTc and the constitutive P7-GFP strains are presented as controls. C) Biofims of colonies with combinatorial $\sigma 38$ promoters with and without aTc.

Conclusion

In future work, we would like to further understand the biological mechanisms behind these promoters - are they active in late log phase, or are different cells reaching stationary phase at different times? What is the protein production rate after the population reaches full stationary phase? We would also like to explore interesting new genetic circuit designs that can be achieved with delays implemented using these promoters.

In order to fully take advantage of spatially-dependent expression, we need to first understand promoter expression in the context of the *E. coli* biofilm. In addition to taking top down images, we are planning to take cross-sections of the biofilm to see if layers can be visualized.

$\sigma 38$ is not only the main stationary phase promoter, it's also the general stress response promoter. It would be advantageous to find the promoter sequences of less promiscuous growth-phase dependent transcription factors.

Materials and Methods

All cells were grown in LB media at 37C in DH5a-z1 E. coli. Biofilms were spotted from 5-10ul of liquid cultures and grown at 29C or 37C for 24-48 hours.

Acknowledgments

VH is supported by the Department of Defense (DoD) through the National Defense Science & Engineering Graduate Fellowship (NDSEG) Program. Research supported in part by the Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

References

[1] G Miksch and P Dobrowolski. Growth phase-dependent induction of stationary-phase promoters of Escherichia coli in different gram-negative bacteria. *Journal of Bacteriology*, 177(18):5374–5378, September 1995.

[2] S J Lee and J D Gralla. Sigma38 (rpoS) RNA polymerase promoter engagement via -10 region nucleotides. *The Journal of Biological Chemistry*, 276(32):30064–30071, August 2001.

[3] Stephan Lacour and Paolo Landini. SigmaS-dependent gene expression at the onset of stationary phase in Escherichia coli: function of sigmaS-dependent genes and identification of their promoter sequences. *Journal of Bacteriology*, 186(21):7186–7195, November 2004.

[4] Tomohiro Shimada, Hideki Makinoshima, Yoshito Ogawa, Takeyoshi Miki, Michihisa Maeda, and Akira Ishihama. Classification and strength measurement of stationary-phase promoters by use of a newly developed promoter cloning vector. *Journal of Bacteriology*, 186(21):7112–7122, November 2004.

[5] Thomas E Gorochowski, Eric van den Berg, Richard Kerkman, Johannes A Roubos, and Roel A L Bovenberg. Using synthetic biological parts and microbioreactors to explore the protein expression characteristics of Escherichia coli. *ACS Synthetic Biology*, 3(3):129–139, March 2014.

[6] R Lange and R Hengge-Aronis. Identification of a central regulator of stationary-phase gene expression in Escherichia coli. *Molecular Microbiology*, 5(1):49–59, January 1991.

[7] Diego O Serra, Anja M Richter, Gisela Klauck, Franziska Mika, and Regine Hengge. Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. *mBio*, 4(2):e00103–13, 2013.

[8] Laura Hobley, Catriona Harkins, Cait E MacPhee, Nicola R Stanley-Wall, and Sonja-Verena Albers. Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS microbiology reviews*, 39(5):649–669, September 2015.