Synthetic Cis Regulatory Modules

Roee Amit[1,2], Hernan Garcia[3], Rob Phillips[4], Frances Arnold[2], and Scott Fraser[1]

***Short Abstract* — Recent reports have highlighted the importance in development of an alternative mechanism for transcription called stalled or "poised" transcription, in which the polymerase is stalled at or near the transcriptional start site awaiting an upstream activation signal(1, 2). In this work we study in a synthetic setting the kinetics and regulatory characteristics of a bacterial class of stalled polymerase – σ^{54}. We find that the kinetics and computational characteristics are fundamentally different than the standard recruitment model. The expression kinetics is described via the highly non-linear J-function for looping (3), which when combined with DNA binding proteins as regulators yields a regulatory region that has arithmetic computational characteristics.**

I. Purpose

TRANSCRIPTION is traditionally described as a poissonian process by which an RNA polymerase is recruited to a promoter by an activator to form an open complex. However, there is a second mechanism for transcription initiation, which is ubiquitous in nature: "stalled" or poised transcription. In stalled transcription the polymerase binds its promoter, but remains stalled unable to form an open complex. Initiation occurs when an activator or 'driver' bound upstream in an enhancer region makes contact with the stalled polymerase via DNA looping, and subsequently hydrolizes ATP to generate an OC to release the polymerase.

In bacteria, the σ^{54}-NtrC system is the best characterized stalled transcriptional system from both functional and structural perspectives(4, 5). In metazoans recent work has shown that a substantial percentage of developmentally important genes in drosophila are transcribed through a stalled mechanism(1, 2). Interestingly, the mechanism for activation of PolII has been likened in several studies to σ^{54}, suggesting that stalled transcription may be more fundamental to biology than the recruitment type as represented by most bacterial σ^{70} promoters (6).

Why would stalled polymerization be a preferred choice of many developmentally associated genes? In order to answer that question, we studied the kinetic and regulatory potential of a σ^{54} system in a synthetic setting, by constructing a library of artificial regulatory regions termed synthetic cis regulatory modules (SCRM). SCRM strains were engineered with regulatory regions of varying DNA length, each containing a cassette of TF binding sites.

[1]Division of Biology, California Institute of Technology, E-mail: roee.amit@caltech.edu
[2]Division of Chemistry and Chemical Engineering, California Institute of Technology
[3]Department of Physics, California Institute of Technology
[4]Division of Engineering and Applied Science, California Institute of Technology

II. Summary of Results

Our findings suggest that the kinetics and regulatory characteristics of stalled transcription are fundamentally different than the standard recruitment (σ^{70}) model.

A. Kinetics given by looping J-function

σ^{54} transcriptional kinetics is best described via an equilibrium measure called the 'J-function' typically used to model looping. Thus, rather than being a poissonian process, transcription in this case is described by a steady-state model that is dependent on the DNA length and phase.

B. Bimodal repression

Regulatory regions containing a single repressor binding site exhibit a switch-like bimodal behavior of either complete repression or only percentage inhibition. The bimodality is reminiscent of FET transistor analog switch, with large gain differentiating between the 2 states. Thus, the non-linear properties of the J-function endow this system with non-trivial regulatory potential.

C. Continuous gradient converted to step function

Regulatory regions containing cassettes of several binding sites for a particular type of TF (either the repressor TetR or activator TraR) exhibit output functions that convert a continuous chemical gradient into a discrete step function. In addition, the output functions exhibit an emergent cooperativity signature that is identical to the number of binding sites in the cassette.

III. Conclusion

Together, our results indicate that the kinetics of stalled transcription combined with ability to integrate several signals at the SCRM endow the whole system with complex, arithmetic computational characteristics that can encode for a precise and discrete set of output functions.

References

[1] Muse, G. W., Gilchrist, D. A., Nechaev, S., Shah, R., Parker, J. S., Grissom, S. F., Zeitlinger, J. & Adelman, K. (2007) *Nat Genet* **39,** 1507-11.
[2] Zeitlinger, J., Stark, A., Kellis, M., Hong, J. W., Nechaev, S., Adelman, K., Levine, M. & Young, R. A. (2007) *Nat Genet* **39,** 1512-6.
[3] Shimada, J. & Yamakawa, H. (1984) *Macromolecules* **17,** 689-698.
[4] Atkinson, M. R. & Ninfa, A. J. (2005) *Curr. Op. Microbiol.* **8,** 168-173.
[5] Rappas, M., Bose, D. & Zhang, X. (2007) *Curr. Op. Struct. Biol* **17,** 110-116.
[6] Lin, Y. C., Choi, W. S. & Gralla, J. D. (2005) *Nat Struct Mol Biol* **12,** 603-7Big MR, Rocky Mountain Town, Santa Fe, NM, private communication.