Single promoters as regulatory network motifs

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***Short Abstract* — We investigate the influence of promoter chromatin architecture on the kinetics of gene activation using a previously described set of promoter variants based on the phosphate-regulated *PHO5* promoter in *S. cerevisiae*. We find the particular architecture of these promoters can result in significant delay in activation, and a memory of previous activation -- dynamical behavior reminiscent of a feed-forward loop but only requiring a single promoter. We suggest the delay and memory is a consequence of chromatin transactions at the promoter. Finally, we show our experimental setup can be generalized as a "gene oscilloscope" to probe the kinetics of heterologous promoter architectures.**

***Keywords* — feed-forward loop, chromatin, promoter, transcription regulation, network motif.**

I. Introduction

TRANSCRIPTIONAL regulatory motifs have been identified as common building blocks in regulatory network structure in both prokaryotes [1] and eukaryotes [2]. At eukaryotic promoters, chromatin can influence the relationship between a gene's expression and TF's activity. This additional complexity might allow single promoters to exhibit dynamical behavior commonly attributed to regulatory motifs involving multiple genes.

Genes in the phosphate starvation pathway of budding yeast contain multiple binding sites for the master TF, Pho4 that have varied affinity location relative to positioned nucleosomes. Using a small library of promoters based on the natural *PHO5*, it was shown that the affinity of nucleosome-free binding sites for Pho4 dictate the activation threshold whereas the affinity of nucleosome-occluded sites dictate the steady-state expression [3]. This [3-4] and other [6] work suggest that activation requires initial binding of Pho4, followed by a chromatin remodeling step to reveal occluded Pho4 binding sites before gene activation. Chromatin remodeling [5] is likely is rate-limiting [6] for activation. We set out to determine if this promoter architecture behaves like a coherent feed-forward loop, with delays in activation due to slow remodeling steps in *cis* and memory of previous activation stored in long-lived "primed" promoter states. To this end, we have probed the kinetics of activation of *PHO5* variants [3] in single cells.

II. Results

Accurate measurement of gene activation kinetics is facilitated by a controllable and observable TF input to a promoter of interest leading to an observable expression output in single cells. Because Pho4p is regulated at the level of nuclear localization, a Pho4p-YFP fusion allows an observable input. We have eliminated various feedbacks [7] in the PHO pathway that enables rapid and precise control of Pho4-YFP localization. Output is monitored via expression of a CFP reporter from a single target promoter of interest. A customized graphical user interface in MATLAB enables cell tracking and visual curation, and a simple deterministic model is used to calculate the instantaneous transcription rate in single cells from the corresponding CFP time series as a function of the nuclear Pho4-YFP localization.

The *PHO5* promoter variants used contained one nucleosome-free and one occluded Pho4p binding site of different affinity. When "OFF" cells were turned "ON" we observed a significant delay in transcription for variants with a lower affinity nucleosome-free Pho4p binding site. The occluded binding site affinity also negatively correlates with activation delay but has less effect. This is consistent with the observation that the occluded site is not necessary for remodeling, though it enhances remodeling when bound by Pho4p [8]. Distinct delay profiles for promoter variants also lead to filtering of short-lived pulses of localized TF. When supplied with a pulse train of nuclear TF, promoter variants are able to differentially filter or detect the persistent signal fluctuations, as in a coherent feed-forward loop. The memory may be provided by an "intermediate" promoter state.

III. Conclusion

Complex interactions at eukaryotic promoters can result in dynamical behavior typically attributed to gene regulatory networks. Single cell kinetic measurements help reveal these dynamics, and we are working to generalize our system to be a "gene oscilloscope" able to test arbitrary promoter architectures unrelated to phosphate-signaling.

References

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