Physicochemical Modeling of Antagonistic Transcriptional Regulation by Yan and Pnt

Thomas G. W. Graham[1], Aaron R. Dinner[2], and Ilaria Rebay[3]

Transcriptional networks play a central role in cellular information processing, yet it remains a significant challenge to model transcriptional regulation at even the simplest eukaryotic promoters. The goal of this project is to develop and test a statistical thermodynamic model of transcriptional regulation by the transcription factors Yan and Pointed (Pnt), two antagonistic effectors of receptor tyrosine kinase (RTK) signaling in *Drosophila*. This combined experimental and theoretical approach should provide useful information for future network-level models of the Yan/Pnt module and may yield more general insights for the quantitative modeling of eukaryotic transcriptional regulation.

***Keywords* — Transcriptional regulation, *Drosophila*, statistical thermodynamic models**

I. Introduction

THE Ets-family transcriptional activator Pointed (Pnt) and the Ets-family transcriptional repressor Yan are central components of a regulatory module in *Drosophila* that controls diverse cell-fate determination events in response to signaling from the receptor tyrosine kinase (RTK) pathway [1-2]. Phosphorylation of both Yan and Pnt by activated MAP kinase (MAPK) inactivates Yan and activates Pnt, thereby derepressing and activating their mutual target genes [3-4]. This core Yan/Pnt motif is embedded in a more complicated network of positively and negatively-acting feedback loops that operate at transcriptional, translational, and posttranslational levels [4-7]. As a first step towards understanding the dynamics of the "Yan/Pnt module" in response to RTK signaling, this project seeks to model transcriptional regulation by activated forms of Yan and Pnt.

II. Theory

We will attempt to model transcriptional regulation by Yan and Pnt using a statistical thermodynamic approach similar to those previously used for modeling gene regulation in prokaryotes [8-10]. The model assumes that each configuration of transcription factors and RNA polymerase bound to a promoter/enhancer is associated with some average level of transcription activity. In addition, it is assumed that the "configuration space" of factors bound to the promoter is sampled within the cell on a timescale that is short compared to the lifetime of the transcriptional reporter being measured. Given these assumptions and an appropriate model for the free energies of different configurations, the probability of each configuration and the ensemble average activation can be calculated based on Boltzmann statistics. Performing these calculations over a range of different transcription factor concentrations provides a theoretical prediction for the cis-regulatory input function (CRIF) [11]. CRIFs can similarly be calculated for different enhancer variants and the predictions compared with experiment.

III. Proposed Experiments

We are generating transgenic flies with Yan/Pnt-responsive destabilized ECFP (dECFP) reporters integrated at defined genomic loci using recombination-mediated cassette exchange (RMCE) [12]. These fly lines will be crossed to other lines that express constitutively active versions of Yan and Pnt tagged with mEGFP and mCherry, respectively. The GAL4-UAS system and/or induction by heat-shock will be used to drive the expression of the activator/repressor at variable levels. Levels of the reporter and of the two transcription factors will be quantified either by flow cytometry of cells from dissociated tissue or by fluorescence microscopy followed by high-throughput image analysis. Initial experiments will involve simplified artificial Yan/Pnt-responsive reporter constructs containing tandem repeats of identical Ets binding sites [3]. These will be followed by experiments with actual regulatory sequences from confirmed Yan/Pnt target genes. CRIFs will be compared for the P1 and P2 isoforms of Pnt and for the wild-type and monomeric mutant versions of Yan [6], and the results analyzed and interpreted in the framework of the model.

Acknowledgements: T.G. is supported by the NIH undergraduate Program in Physical and Chemical Biology (PCBio)

[1]The College, [2]Department of Chemistry, and [3]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637

References

[1] Lai ZC, Rubin GM (1992) *Cell* **70,** 609-620.
[2] Rebay I, Rubin GM (1995) *Cell* **81,** 857-866.
[3] O'Neill EM, Rebay I, Tjian R, Rubin GM (1994) *Cell* **78,** 137-147.
[4] Gabay L et al (1996) Development **122,** 3355-3362.
[5] Vivekanand P, Tootle TL, Rebay I (2004) *Mech. Dev.***121,** 1469-1479.
[6] Qiao F et al (2004) *Cell,* **118,** 163-173.
[7] Li X and Carthew RW (2005) *Cell* 123, 1267-1277.
[8] Bintu et al (2005) *Curr. Opin. Gen. Dev.* **15,** 116-135. (2 papers)
[9] Ackers GK, Johnson AD, Shea MA (1982) *PNAS,* **79,** 1129-33.
[10] Shea MA, Ackers GK (1984) *J. Mol. Biol.,* **181,** 211-230.
[11] Setty Y, Alon U (2003) *PNAS,* **100,** 7702-7707.
[12] Bateman JR, Lee AM, Wu CT (2006) *Genetics* **173,** 769-777.