Systems biology analysis of the *cis*-regulatory control of the expression of *Drosophila even-skipped* stripes 2 and 3

Ah-Ram Kim[1], Shuling Hou[2], David Sharp[3] and John Reinitz[1]

***Short Abstract* — Here we present a quantitative and predictive model of the the transcriptional readout of two enhancer fusions of the -3.8 to -3.3 kb (stripe 3 enhancer) and the -1.6 to -1.1 kb (stripe 2 enhancer) regions of the gene *eve* in either orientation without spacer sequence between them. The model is based on a set of transcription factor binding sites and quantitative, time-resolved expression data at celluar resolution. We utilized the ØC31-mediated site-specific transgenesis system to obtain quantitative gene expression maps of different reporter genes.**

***Keywords* — Transcription, *even-skipped* gene, ØC31-mediated site-specific transgenesis, quantitative model.**

THE classical picture of the regulatory regions of metazoan genes is that complete promoters are collections of independent enhancers, and each enhancer is composed of a cluster of binding sites. The foundations of the above description, as well as clear evidence of its incompleteness are seen in the biology of the DNA that regulates the expression of stripes 2 and 3 of the *Drosophila* pair-rule gene *even-skipped*(*eve*) [1-4].

Eve directs the formation of seven transverse stripes of expression during the blastoderm stage of embryogenesis [5]. The transcriptional regulation of these stripes is thought to be controlled by a series of separate enhancers in the *eve* promoter [2, 6, 7]. The *eve* stripe 2 enhancer is located between -1.6 and -1.1 kb upstream of the transcriptional start site and the stripe 3 enhancer is located between -3.8 and -3.3 kb. The two enhancers are separated by about 1.5 kb of DNA. Each of those fragments is the smallest fragment that directs the expression of *lacZ* in a pattern coextensive with the native stripe 2 and 3, and hence they are called "minimal stripe element 2 and 3" (MSE2 and MSE3)[34]. If the two enhancers are directly attached without being separated by a spacer sequence, the fused construct generates a novel pattern, in which either stripe 2 or stripe 3 expression is repressed, depending on the relative position of the two enhancers [3]. However, an essentially normal pattern is obtained when as little as 160 bp sequence is inserted between the enhancers [3].

There are no *in vitro* assays for transcription by polymerase II which use only known substances and are faithful to the *in vivo* regulation of transcription. Thus, computational modeling with quantitative input data is required to gain an increased understanding of the enhancer function. We developed a mathematical model that involves four mechanisms: activation, repression by competition, repression by quenching, and repression by direct repression. We utilized the ØC31-mediated site-specific transgenesis system to obtain quantitative gene expression maps of different reporter genes [8]. We showed that site-specific integration allows for direct comparison of gene expression data in the reporter genes. Our model suggests that interactions between enhancers are important for generating novel patterns of gene expression during development.

[1]Department of Applied Mathematics and Statistics,and Center for Developmental Genetics, Stony Brook University, Stony Brook, NY 11794-3600, USA. E-mail: arkim@ic.sunysb.edu, reinitz@odd.bio.sunysb.edu

[2]Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA.

[3]Chief Science Office, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. E-mail: dcso@lanl.gov

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