No Compromise: Single molecule imaging reveal mechanisms for speed and precision in developmental gene expression.

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***Short Abstract* — It has been shown that strong gene induction in eukaryotic cells leads to large bursts of gene expression – increasing total mRNA synthesis at the cost of substantially elevating cell-cell variation in expression levels. We investigate how metazoan organisms handle this tradeoff of speed and noise. Using a newly developed method to identify single mRNA transcripts in intact Drosophila embryos, we show that progressive up-regulation of developmentally critical genes occurs without loss of precision between individual cells. We show that this increased precision is achieved by a combination of mechanisms, each which effect promoter priming instead of pol II recruitment.**

***Keywords* — Drosophila, hunchback, mRNA counting, gene regulation.**

I. Purpose

QUANTITATIVE measurements of eukaryotic transcription in single cells have demonstrated a high degree of stochastic variation among clonal cells in copy number of mRNA and protein, resulting from the bursts of molecule synthesis [1,2,3]. Whole-genome expression analysis in yeast concluded that this expression noise increases generally with expression level [4,5], and that regulated genes, which respond to external signals, are especially subject to high degrees of variation upon strong induction [5,6]. A similar tradeoff between expression rate and noise has been reported in mammalian cell culture lines [1,6]. We investigate the molecular mechanisms of gene regulation in the developing Drosophila embryo, to see how this challenge is addressed in a system where reduction of expression noise is generally important for proper development [7].

II. Methods

We have developed methods to allow single mRNA counting in whole-mount embryos and apply this approach to investigate the nature of expression noise for the key developmental patterning genes *hunchback* and *snail*. We examine cell populations that are naturally exposed to different concentrations of the activators for the genes (respectively Bcd and Dorsal/Twist) and characterize the distribution of total mRNA across these cells. Additionally, we measure the fraction of time an induced cells spends actively transcribing the gene. Using BAC-transgenesis, we change the number of binding sites available for activator binding and the frequency of enhancer promoter interactions. We test the effects of these modifications on total expression and expression variability.

III. Conclusion

We find, contrary to expectation, that relative noise in *hunchback* expression actually decreases when synthesis rate increases. We show evidence that the Bcd binding induces a transcriptionally competent state to the promoter but does not affect the pol II recruitment and transcriptional release rates substantially. We find separable clusters of Bcd sites, which can act independently to further enhance the rate of promoter priming without affecting pol II recruitment. This mode of regulation provides a high burst frequency with small burst size, leading to reduced variation under strong induction. Dorsal and Twist appear to play a similar role in promoter priming rather than modulating transcription initiation.

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