Disentangling the Sources of Species-Specific Gene Expression Patterns in Drosophila Embryos

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***Short Abstract* — Gene expression patterns can diverge between species due to changes in a gene's regulatory DNA or changes in the proteins, e.g. transcription factors, that regulate the gene. We developed a modeling framework to uncover the sources of expression differences in blastoderm embryos of three Drosophila species. Using this framework and cellular-resolution expression measurements of inputs and outputs of regulatory circuits active in the embryo, we found the function of some circuits is conserved between species, while others diverge. We confirmed our predictions using transgenic *D. melanogaster* lines, which demonstrate differing degrees of conservation in sets of orthologous *cis*-regulatory elements.**

***Keywords* — transcriptional regulatory network, gene expression divergence, *cis*-regulatory elements**

I. Background

CHANGES in gene expression patterns have been linked to phenotypic differences between individuals and species [1-4]. These studies identified dramatic gene expression changes, such as gain or loss of an expression pattern, that underlie morphological phenotypes. Though more difficult to detect, small quantitative changes in gene expression may also lead to variation between individuals and the evolution of new phenotypes between species. A challenge is to contextualize this expression variation: what are its sources and phenotypic consequences?

We completed a survey of gene expression in blastoderm embryos of 2 *Drosophila* species [5], *D. yakuba* and *D. pseudoobscura*, complementing the existing dataset for *D. melanogaster* [6]. Our high-resolution imaging methods result in a gene expression atlas, in which the relative expression levels for 12 key transcription factors (TFs) in the anterior-posterior patterning network are mapped onto each cell in an average 3D embryo. Our global analysis of these 3 datasets showed that while individual genes differ quantitatively in their spatiotemporal gene expression patterns, cellular gene expression profiles for the whole set of genes are largely similar. This finding suggests that the overall function of this transcriptional network is conserved, but the function of individual regulatory circuits in this network may have diverged between species.

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II. Approach

To disentangle contributions of different sources to expression divergence, we define each circuit in this network based on the inputs and output of individual *cis*-regulatory elements (CREs), stretches of regulatory DNA comprised of TF binding sites. We then create a cell-by-cell model to relate the levels of the input TFs to the endogenous expression pattern directed by each CRE. To assess the degree of regulatory function conservation of orthologous CREs, we fit the model's parameters in one species and apply them to the other species. To investigate the contribution of sequence changes in CREs to expression divergence, we made transgenic lines with orthologous CREs driving a reporter in *D. melanogaster*.

III. Conclusion

In this network, we find differing degrees of conservation in CRE circuit function. The function of some sets of CREs is strongly conserved between species, and differences in CRE output are due to differences in the spatiotemporal expression patterns of input TFs. In other cases, orthologous CREs diverge in function.

The results of the transgenic experiments show that we can use a simple measure of sequence function to explain the quantitative differences in the function of orthologous CREs. Together these results allow us to comprehensively understand the divergence in gene expression between species. The general modeling and experimental framework can be easily extended to encompass other types of regulatory DNA, e.g. promoters and untranslated regions.

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