Variation in Expression of Even-skipped at Precisely Timed Stages in *Drosophila melanogaster*

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Variation in gene expression has been observed in a wide collection of organisms. Studies using microarray have identified 7% genes expressed differently among all four cultured yeast isolates (Townsend et al., 2003), 18% between two natural population of *Fundulus sp* (Oleksiak et al., 2002), and 27% between at least two of four isolates of *Drosophila melanogaster* (Baker et al., 2007). Nevertheless, given the heterogeneous nature of the subjects due to failure to control physical stages, one cannot attribute the observed differences solely to intrinsic differentiation in expression. Thus, in our study, we hypothesized that the expression level of crucial developmental gene during a narrow window of embryogenesis would be highly conserved among genetic isolates.

We investigated the expression variation of even-skipped (eve) among four D. melanogaster strains, South Africa (A), Hawaii (H), Australia (A), and Malawi (M). Eve codes for a transcriptional repressor protein and contributes to segmentation, polarization, and neuron formation in *Drosophila* embryos. Eve is highly expressed during the blastoderm stage, remarked by an active division and migration of cells to form blastula. The developmental significance of blastoderm stage and eve indicated a highly conserved expression mechanism.

We divided the blastoderm stage into three substages based on obvious morphological differences: the syncytial, the mid-blastoderm transition (MBT), and the cellular stage. In syncytial stage, the nuclei division is unaccompanied by division of cell membrane. During MBT, the cells start to invaginate, where the cell membrane indents to encapsulate each nucleus. The completion of MBT leads to cellular blastoderm stage, where all the cells are enclosed by its own cell membrane. To ascertain that the embryos laid in comparable physical stages, we collected embryos in a 1.5 h window, 2.5 to 4 h post fertilization. Visual confirmation under microscope agreed that among four strains the distribution of embryos in each substage remained similar.

We grinded the embryos to extract mRNA under the manufacturer protocol provided with RNeasy kit. The mRNA product was converted into cDNA, whose quality was examined with PCR followed by electrophoresis. Samples exhibited bright bands on the alga gel under UV light contained an appropriate amount of qualitied cDNA, thus chosen for further analysis using qPCR (real-time PCR). Each strain had three biological replicas, each generated three technical replicas when we prepared the plates for qPCR.

The amount of eve mRNA in each sample was inferred from the qPCR results. In contrast to our hypothesis, eve expression varied dramatically among four strains. In short, Strain A and H had significantly higher eve expression than strain M and S ($p < 0.05$). In fact, the expression level in A was 16-fold as high as the expression level in strain S. We concluded that despite the precisely timed, narrow window of development, and despite the critical role of eve in embryonic patterning, significant expression variations still exist among *Drosophila* isolates. Meanwhile, embryogenesis seemed to be extremely robust to variation in gene expression level.

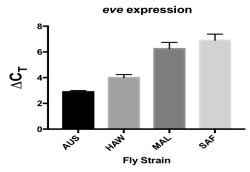

Figure 1. Expression fo eve differed significantly among four Drosophila isolates (ANOVA, p value = 0.008); eve is expressed significantly higher in A than in M and S; eve is expressed significantly higher in H than in M and S

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Founded by the INBRE, IDeA Networks of Biomedical Research Excellence and NIH, National Institute of Health

Reference

Baker, D. A., Meadows, L. A., Wang, J., Dow, J. A., & Russell, S. (2007). Variable sexually dimorphic gene expression in laboratory strains of Drosophila melanogaster. Bmc Genomics, 8(1), 454.

Oleksiak, M. F., Churchill, G. A., & Crawford, D. L. (2002). Variation in gene expression within and among natural populations. Nature genetics, 32(2), 261.

Townsend, J. P., Cavalieri, D., & Hartl, D. L. (2003). Population genetic variation in genome-wide gene expression. Molecular Biology and Evolution, 20(6), 955-963.