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Driving Gene Expression in the Heterochromatic Environment of the Fourth Chromosome of D. melanogaster

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Driving Gene Expression in the Heterochromatic Environment of the Fourth Chromosome of *D. melanogaster*

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Genomes of higher eukaryotes can be divided into two fundamental and dynamic subtypes: euchromatin and heterochromatin. In general, genes that are active in a euchromatic environment are silenced when transposed to heterochromatin. However, heterochromatin is not devoid of actively functioning genes. The main goal of our project is to identify regulatory elements that drive transcription of heterochromatic genes. The fourth chromosome of *Drosophila melanogaster* represents an excellent model for this study: ~80 genes within this heterochromatic domain are expressed.

Insertion of an *hsp70-white* transgene, which results in a uniform red eye phenotype when present in euchromatin, into a heterochromatic region on the fourth chromosome results in sporadic silencing, or Position Effect Variegation (PEV). We replaced the *hsp70* promoter of *hsp70-white* with a 5' genomic fragment of a highly-expressed fourth chromosome gene, *Rad23*. Insertion of the *Rad23-white* transgene into the same location switched the *hsp70-white* PEV phenotype to a uniform full red eye, suggesting that the *Rad23* fragment is sufficient to drive strong expression of the euchromatic white reporter. A series of experiments with reporter constructs containing fragments of varying lengths of the *Rad23* promoter region is helping us identify the minimal length of the *Rad23* promoter fragment to drive white expression. The removal of a 250 bp *Rad23* promoter fragment bringing an upstream 1360 transposon closer to the TSS did not result in silencing of the white reporter. An additional construct where a 100 bp *Rad23* promoter fragment replaces the corresponding portion of the *hsp70* promoter in the *hsp70-white* transgene resulted in the loss of PEV, but low level expression (light orange eyes). Additional experiments to identify the essential elements of the 5'noncoding regulatory region of the *Rad23* gene are underway.