ABSTRACT

IN VIVO ANALYSIS OF THE MOLECULAR MECHANISM OF REPRESSION OF THE *DROSOPHILA* LONG-RANGE REPRESSOR HAIRY

By

Carlos Alberto Martinez

The *Drosophila* Hairy protein belongs to a widely conserved class of transcriptional repressors called Hairy and Enhancer-of-Split (HES) proteins that have important roles in embryogenesis and cell fate determination. HES family members are characterized by a conserved basic helix-loop-helix DNA binding domain, an Orange domain, and a C-terminal WRPW motif that binds the TLE/Groucho corepressor. While enormous advances have been made in elucidating the biological functions of HES proteins, there is very little molecular understanding of the mechanism by which they repress transcription. To address this issue, Hairy repression was analyzed in the context of a highly defined *Drosophila* embryo system. The system consisted of embryos carrying a *lacZ* reporter transgene activated ubiquitously by the yeast Gal4 protein, the latter of which was expressed under control of the *daughterless* promoter. Uniform repression of the system was achieved by the heat shock induction of a LexA-Hairy fusion protein that can bind to and repress the reporter. Using embryo chromatin immunoprecipitation, I have analyzed the recruitment of activators, coactivators, repressors, corepressors, and chromatin modifications associated with Hairy repression. The results show that during repression activators and coactivators remain bound to the promoter, suggesting that Hairy does not work by blocking activator and coactivator recruitment. Chromatin immunoprecipitation analysis showed that during repression Groucho was recruited to the entire four kilobase coding region of the *lacZ* reporter. Groucho binding was accompanied by recruitment of

the histone deacetylase Rpd3, a known Groucho interacting protein, and a subsequent loss of H3 and H4 acetylation levels at the promoter and one kilobase downstream. Concomitant with the loss of histone acetylation levels, a two-fold increase in total histone H3 occupancy was seen at the promoter region, suggesting that Hairy repression is associated with chromatin remodeling. Given that previous studies had characterized Hairy as a long-range repressor capable of dominantly inhibiting distal elements, the results suggest a model wherein Groucho spreading and subsequent histone deacetylation can lead to long-range gene silencing. However, unlike previously characterized long-range silencing mechanisms that involve corepressor spreading, the repression observed in this system was transient.