A Screen Designed to Identify Proteins that Potentially Bind the *BhC4-1* Ring Gland Enhancer

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The DNA puff *BhC4-1* gene is amplified and transcribed in *Bradysia hygida* salivary glands at the end of the fourth larval instar. The functional characterization of the DNA puff *BhC4-1* promoter revealed that the proximal promoter contains both a 129 bp salivary gland enhancer and a 67 bp ring gland enhancer that drive tissue specific patterns of developmentally regulated gene expression. The *Drosophila* ring gland is a specialized larval endocrine organ and the mechanisms that control gene expression in this organ are largely unknown. The *BhC4-1* 67 bp ring gland enhancer is to our knowledge the only identified enhancer that is able to drive developmentally regulated gene expression exclusively in the ring gland. Here we have employed the yeast one-hybrid system to identify transcriptional activators that are able to bind the *BhC4-1* 67 bp ring gland enhancer. Our initial screen, performed in the presence of 45mM 3-AT, led to the identification of 97 positive clones that are able to activate the HIS reporter gene in the one-hybrid system. In order to further eliminate false-positive clones we used the *lacZ* gene as a second reporter gene which reduced the number of initial 97 positive clones to 74 clones. The positive clones that are able to activate both reporter genes are being sequenced. 39% of the positive clones were sequenced and a preliminary analysis reveal that the identified clones can be classified into the following gene ontology categories: cytoskeleton (21%), translation (17,5%), nucleic acid binding (10%), electron carrier (10%), transporter (7%), cell cycle (3%), unknown functions (14%) and others (17,5%).

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