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Functional interplay between factors involved in transcription in *Drosophila melanogaster*

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Regulation of transcription, the synthesis of RNA from a DNA template, is one of the most important steps in control of cell growth and differentiation. Gene regulation occurs in the context of chromatin where recruitment of chromatin remodeling complexes such as ATP-dependent remodeling complexes and histone modifying enzymes (HAT, HDAC, HMT, etc.) represents a crucial step in gene transcription (Svejstrup 2004). We are interested in studying the physical and functional interaction between proteins involved in transcription regulation such as the RNA PolII subunit Rpb4, the transcription factor p53, and adaptor proteins of the chromatin modifier complexes.

The RNA polymerase II is composed of a ten-subunit core and a two-subunit dissociable subcomplex comprising the forth and the seventh largest subunits, Rpb4 and Rpb7. In *Drosophila*, Rpb4 is a product of a bicistronic gene together with the ATAC histone acetyltransferase complex constituent ADA2a (Pankotai et al. 2010). The alignment of Ada2a and Rpb4-related sequences indicated that the two proteins share the same transcription unit. Comparison of the related protein and nucleotide sequences revealed that Ada2a and Rpb4 coding region are present in similar organization in 12 *Drosophila* species, other then *D. melanogaster*; however a similar gene organization in other organisms cannot be identified. We investigated the mechanism by which the two mRNAs are generated. From RT-PCR analysis we concluded that the shift between Ada2a and Rpb4 mRNA formation takes place by splice acceptor site selection. Ada2a protein has another distinct homolog in *Drosophila*, Ada2b which is present in the SAGA complex. Although the two proteins contain the same conserved domains and their interacting partners are rather similar, they are part of two distinct HAT complexes. We questioned what confers the complex specificity of the Ada2 adaptors, more precisely which regions are responsible for their interaction with different transcriptional coactivators. Firstly, different Ada2a/Ada2b or Ada2b/Ada2a hybrids proteins were generated by joining PCR fragments corresponding to functional domains of one and the other ADA2. All the hybrid plasmids were generated using the Gateway system and were successfully tested on western blot for their expression in *Drosophila* S2 cells. Since the chimeric structure of the proteins did not disturb their expression in S2 cells, plasmids suitable for embryo injections were generated. Our goal is to see if their expression will restore one or the other ADA2 function. The coding region of hybrid proteins were inserted into a P-element containing vector allowing site specific insertion and injected into *Drosophila* embryos. Two out of six plasmids are ready for *in vivo* analysis in *Ada2a* and *Ada2b* mutant background.

Previous studies have shown that HATs, beside their ability to relax chromatin, can regulate many other factors through acetylation, including transcription factors (Wang et al. 2001). In mammalian system it was shown that the Gcn5-containing acetyltransferase complex (STAGA) plays a role in p53-dependent gene activation. Ada2b and Gcn5L proteins are identified as direct interacting partners of the p53 transcription factor (Gamper and Roeder 2008). According to previous studies *Drosophila* p53 is a functional homolog of mammalian p53 (Ollmann et al. 2000). We investigated whether the adaptor proteins of the HAT complex have any effect on p53 transcriptional activity. A plasmid containing three copies of *rpr* consensus binding site upstream of the luciferase gene was constructed. The reporter plasmid was cotransfected into S2 cells with different constructs expressing Ada2a, Ada2b or Ada3 proteins and performed luciferase assays. There was no significant change in transcriptional activity of the p53 responsive element. Although it was previously described that hAda3 can increase p53 trancriptional activity (Wang et al. 2001), perhaps in *Drosophila* S2 cells the efficiency of this interaction requires additional factors which can promote a significant response. We also tested plasmid vectors containing *Drosophila melanogaster* p53 (Dmp53) and a point mutant form of it ($Dmp53^{K302R}$) in reporter assays. Expression of either the wild type or the mutant variant increased the activity of the reporter gene 20 fold compared to control. No significant change was observed in the level of transcriptional activation ability of the wild type and the mutant variant of Dmp53. Future *in vivo* studies are planned to understand the mechanism by which p53 is activated.

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