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DISSERTATION SUMMARY

In situ dissection of the bxd PRE in *Drosophila melanogaster*

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We investigate the regulation of eukaryotic gene expression by the modification of higher order chromatin structure. Our studies focus on the most extensively studied silencer region in the homeotic bithorax complex of *Drosophila melanogaster*, called bithoraxoid Polycomb Response Element (bxd PRE). However, all the previous experiments have used mobile element constructs with various reporter genes; no *in situ* dissection of this region has been performed. In different tests, PREs often proved to be of varying size; moreover, results were sometimes even contradictory. PREs are also known to function cooperatively, and to behave differently in different chromosomal contexts. Therefore, to analyze how PREs function in their natural chromosomal context, and to determine any possible similarities in their structure, we have generated *in situ* deletions within a 3-kb region of the bxd PRE, and examined their effect.

To carry out the deletional analyses of the bxd PRE, we have devised a novel strategy for gene conversion in *Drosophila*. We designed two different types of conversion constructs, which allow us to generate small deletions of either pre-determined or random size. Templates for conversion include FRT-sites flanking small genomic sequences or restriction sites for the yeast I-SceI enzyme, which permit generating small deficiencies by flp/FRT recombination or by the I-SceI induced double strand DNA breaks, respectively. The FRT sites can also be used to merge different deletions generated by us.

Using chromatin immuno-precipitation, 3 sub-elements with potential PRE-activities were identified in the examined 3-kb region of the bxd PRE. However, we have found that only the removal of the central one (670 bp) decreases bxd PRE function to a detectable degree. Flies carrying this deletion showed partial posteriorly directed transformations (wing into haltere, 3rd thoracic segment into 1st abdominal segment) with a penetrance of ~8% when heterozygous. Consistent with our findings, no increase in the penetrance was observed when we extended the size of the deleted sequence to the whole 3-kb region. Considering that bxd and iab-7 PREs share significant similarity in the pattern of GAGA and PHO protein binding sites, we have identified the shortest fragment (184 bp), containing a minimum set of the two protein binding sites, whose removal still lessens bxd PRE function to a detectable degree.

Besides observing adult phenotypes, we have also examined the expression pattern of the Ultrabithorax (Ubx) gene controlled by the bxd region, using antibody staining against the UBX protein in larval CNS. The Gal4VP16 marker gene present in our constructs enabled us to study indirectly the local chromatin structure of the bxd regulatory region through the UAS-GFP system. In agreement with the adult phenotypes, the Ubx gene shows only a modest ectopic expression when the significant part of the bxd PRE is deleted. In contrast to Ubx, GFP shows an extreme ectopic expression pattern even in the head segments. We have also found a striking difference in the gene expression level of these two genes: the intensity of GFP decreased, while it increased in case of Ubx in deletion-bearing heterozygotes. Very likely, these differences reflect the differences between the regulation of the two genes: Gal4VP16 is most likely regulated by a single neighboring enhancer, while Ubx falls under a complex regulation that modifies the effect of bxd PRE deletion. Our data also imply that the bxd PRE binds to at least one Ubx-enhancer and exclusively regulates its activity.

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