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DISSERTATION SUMMARY

Higher order chromatin structure and gene regulation in *Drosophila melanogaster*

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Segmental identity in Drosophila is determined by two clusters of homeotic genes, the *Antennapedia*- (ANT-C; Kaufman et al. 1990) and the *bithorax*- (BX-C; Lewis 1978) complexes. The complex expression pattern of the BX-C genes is due to the action of nine parasegment-specific *cis*-regulatory domains (Duncan 1987). The activity patterns of these *cis*-regulatory regions are set early in development by protein products of segmentation genes (Shimell et al. 1994). By midembryogenesis, when the products of the segmentation genes disappear, the regulation of the homeotic genes switches to a maintenance mode that preserves the initial pattern of activity through the remainder of development (Paro 1993). Maintenance of the inactive state requires the action of the Polycomb-group (PcG) of proteins. By contrast, the *trithorax*-group (trxG) of genes is responsible for maintaining the active state of homeotic genes. PcG proteins function cooperatively and form multimeric repressor complexes (Shao et al. 1999), which are tethered to the DNA at sequences called Polycomb Response Elements (PRE). The antagonistic activities of trxG and PcG proteins involve modulation of chromatin structure. A deletion that removes the *Frontabdominal-7 cis*-regulatory region (*Fab-7'*) removes a domain boundary element (transforming parasegment 11 into parasegment 12; Gyurkovics et al. 1990) and a silencer element, the *iab-7* PRE (Hagstrom et al. 1997). Transgenic lines containing *iab-7* PRE fragments show pairing-sensitive silencing of the *miniwhite* reporter gene: the eye color of transgenic flies is lighter in homozygous than in heterozygous conditions. This silencing effect is weakend by introducing a PcG mutation, while it is strengthened in a trxG mutant background.

We used two transgenic constructs containing two different *iab-7* PRE fragments for large scale screens to identify previously unknown PcG and trxG genes. Lighteners of eye color were supposed to be caused by mutations in trxG-, while darkeners in PcG genes. We found several mutations in previously characterized, and yet uncharacterized PcG and trxG genes, indicating that our screening method is sensitive enough to identify new members of these groups of genes. After establishing the complementation groups we tested the identified mutations in different *in situ* systems, and in transgenic systems. We have proved that not all members of the PcG are necessary for silencing on a given PRE. We have also shown that the effect of some PcG genes is specific to a given PRE. The fact, we could also identify lighteners of the eye color using a PRE containing transgene indicating, that a TRE-like (Trithorax Response Element) is overlapping or located very closed to the *iab-7* PRE. We could also demonstrate a regulatory network between members of the PcG and trxG genes. Some mutants (*gpp* and *bon*) showed controversial phenotypes: Polycomb-type homeotic phenotype while lightening of the eye color, or trithorax-type homeotic phenotype while darkening the eye color of the transgenic construct. These gain-of-funtion mutants were reverted using X-rays. The revertants were cytologically analysed for identifying the locus, and used for genetic experiments to compare the gain-of-function and loss-of-function phenotypes to further characterize the function of the given genes. Recently we designed four new transgenic constructs carrying different fragments of the *iab-7* PRE cloned between Flp recombinase target sites. FRTs are used to select lines showing pairing-sensitive silencing without the transgenic PRE being involved in any interactions of endogenic PRE sequences. These constructs were injected to embryos. The establishing of transgenic lines is in progress.

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