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**DISSERTATION SUMMARY**

# Identification of "anchoring genes" in the promoter-upstream region of the *Drosophila Abd-B* domain

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The *Abd-B* gene determines the segmental identity of four different abdominal segments (from the 5th to the 8th) through the action of extensive segment-specific *cis*-regulatory regions (*iab-5, iab-6, iab-7, iab-8*). Although these regulators are located many kilobases away the promoter, physical contact occurs between them through the looping out of the intervening DNA sequences.

Genetic studies gave evidence that regulatory interactions between promoter and enhancer elements can be formed not only in *cis* but in *trans*, as well, between non-continuous DNA molecules. These *trans*-interactions depend on the physical proximity of homologous chromosomes. Taking advantage of this phenomenon, called "transvection", we have tried to identify genes that help to anchor distant enhancers to the *Abd-B* gene.

Our previous results (Sipos et al. 1998) strongly suggest the existence of a novel mechanism that tethers *cis*-regulatory regions to the promoter upstream region of the *Abd-B* gene of the bithorax-complex. The strength of *cis*-interaction is inversely proportional to the size of the deletion in the upstream region suggesting that this region consists of numerous discrete elements that cooperate in locking individual *cis*-regulators to the *Abd-B* gene.

The very sensitive relationship between the size of the sixth tergite of Drosophila males and the expression level of the *Abd-B* gene enables the identification of "anchoring genes" required for tethering of *iab-7* regulatory region to the promoter-upstream region. Most likely, mutation of "anchoring genes" would eliminate or weaken the *trans*-suppression of the *Fab-7* phenotype in *Fab-7/Abd-B* males, resembling to the phenotype observed in the presence of chromosomal rearrangements.

Screening for this phenotype gives also the opportunity to identify new genes involved in the pairing of homologous chromosomes, known or yet unknown repressors of the *Abd-B* homeotic gene, or mutants, which simply carry chromosomal rearrangements.

We performed a large-scale F1 mutageneric screen using EMS and ENU, as they more often induce point mutations than chromosomal rearrangements. From more than 90000 males we selected 500 modifiers. Due to the high degree of sterility only 63 of them carried reproducible phenotype. 22 of them were mapped to the 2nd chromosome and 41 on the 3rd chromosome.

To restrict the number of total hits we made complementation analysis. On the 2nd chromosome we isolated 2 single hits and one big complementation group (20 members). On the 3rd chromosome we found 3 different complementation groups and 25 single hits.

To filter out those hits that generated chromosomal rearrangements we performed specificity test by using pairing-sensitive transvection-systems characteristic for the 2nd ($bw^V$) and for the 3rd ($Cbx^1Ubx^1$) chromosome. Chromosomal rearrangements disturb the communication between the two homologs and therefore suppress the mutant phenotypes toward wild type. The $bw^V$ phenotype was suppressed in 2 lines. Suppression of the $Cbx^1Ubx^1$ phenotype occurred in 8 cases. The $Cbx^1Ubx^1$ system also enabled the identification of new putative repressors belonging to the Polycomb-group.

The phenotype of complementation groups was "roughly mapped" between recessive markers on the mapping chromosomes. In further steps, deletion analysis and allelic complementation enabled the identification of mutated genes. We identified on the 2nd chromosome *grainyhead* (single hit) – a negative regulator of the *Abd-B* gene and *ebi* (big complementation group) – a previously unknown interactor of the *Abd-B* homeotic gene. The identification of the mutated genes on the 3rd chromosome is still under investigation. Using immunohistochemistry, we want to find out the regulatory hierarchy between the Abd-B and the mutated genes.

## References

Sipos L, Mihaly J, Karch F, Schedl P, Gausz J, Gyurkovics H (1998) Transvection in the *Drosophila* Abd-B domain: extensive upstream sequences are involved in anchoring distant cis-regulatory regions to the promoter. Genetics 149(2):1031-1050.

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