Identification and characterization of epigenetic regulatory factors in *Drosophila melanogaster*

Summary of the Ph.D. thesis

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INTRODUCTION AND OBJECTIVES

Each cell of the higher order eukaryotes contains the same genetic information, however this information is utilised differently by different cell types. According to their program of differentiation, different cells express or turn off different genes by epigenetic regulatory factors. If this gene expression pattern is not properly established in time and space, the morphological development becomes distorted. Developmental biology, and especially developmental genetics aims to map the genetic regulatory pathways which establish and stabilize the gene expression patterns of different cell lineages in time and space, thus allowing the differentiation and specialization of cells.

The gradient of early morphogenetic factors determine the ‘on’ and ‘off’ states of genes epigenetically, however, this is not enough for the correct biochemical and morphological differentiation; the early expression pattern of genes has to be maintained throughout development.

The fruitfly (*Drosophila melanogaster*) proved to be a very useful model organism for understanding the early determination of cells and the maintenance mechanisms of gene expression patterns. It also turned out that the knowledge gained by studying the development of the fruitfly efficiently contributes to genetic investigations in mammals, since not only the maintenance mechanism of the gene expression pattern is conserved, but the epigenetic factors which stabilize this pattern show a high degree of homology as well.

Homeotic genes are conserved master genes (transcription factors), which become active early in embryogenesis, and stay active throughout development. Their expression is segment specific, and their role is to ensure the identity of a certain segment by regulating different effector genes.

In *Drosophila melanogaster*, homeotic genes localize in two homeotic complexes. The *Antennapedia*-complex (ANT-C) regulates segmental identity from the head to the second thoracic segment, while the *bithorax*-complex (BX-C) is responsible for that of the more
posterior segments. The BX-C encodes three homeotic genes (Ultrabithorax, abdominal-A, Abdominal-B), which regulate the identity of the segments by large segment specific cis-regulators. In each segment, a new cis-regulator is turned on (its chromatin structure 'opens up'), resulting in the activation of a new homeotic gene, or raising the expression level of a homeotic gene which is also active in more anteriorly located segments.

The expression levels of homeotic genes set early in development are maintained by the products of two groups of genes throughout development. Proteins belonging to the POLYCOMB-group (PCG) function cooperatively and form multimeric repressor complexes, thus maintaining the inactive state ('closed' chromatin structure) of cis-regulators, while the members of the TRITHORAX-group (TRXG) are responsible for stabilizing the active state of cis-regulators ('open' chromatin conformation). The DNA targets of the POLYCOMB- and TRITHORAX-group proteins in the cis-regulators of homeotic genes are the PRE (Polycomb Response Element) and the TRE (Trithorax Response Element) sequences.

The use of transgenic constructs containing isolated PRE sequences helps the genetic investigation of the members and the function of the protein complexes formed on a certain PRE sequence. The expression of the reporter gene cloned in the construct is sensitive to the dosage of the PcG and trxG genes in a pairing-sensitive manner: mutations in the members of the Polycomb-group darken, while mutations in the trithorax-group genes lighten the eye-colour of the flies carrying the homozygous construct. In our experiments we used transgenic constructs which contained two different fragments of the iab-7 PRE (a PRE sequence located in the infraabdominal-7 cis-regulator, close to the Fab-7 boundary) between a reporter gene (miniwhite) and its enhancer. One of the iab-7 PRE fragments covers a wide region of the PRE, while the other one contains only a minimal PRE, which is absolutely necessary for the silencing function.

Our first aim was to identify new members of the Polycomb- and the trithorax-group in mutagenesis screens. With the help of the newly identified alleles, we expected to gain
further information on the structure and function of the \textit{cis}-regulators of the homeotic genes, as well as on function of the protein complexes formed on these \textit{cis}-regulators. Besides, we intended to create a tool which enables the investigation of the \textit{iab-7} PRE and TRE in a high resolution.
METHODS USED IN THE STUDY

Classical genetic methods:
- EMS-induced mutagenesis, reversion screens by the means of X-rays, X-rays-induced mutagenesis
- analysis of mutant lines: lethal phase analysis, complementation analysis, analysis of phenotypic interactions
- recombination, mapping
- P-element mobilization
- analysis of polytene chromosomes

Molecular methods:
- *in situ* hybridization
- cloning of transgenic constructs and their injection into embryos (DNA purification, digestion by restriction enzymes, PCR, gel electrophoresis, isolation of fragments, ligation, transformation of bacteria, preparation of competent cells)
SUMMARY OF THE RESULTS

Using EMS and X-rays, we performed large scale mutagenesis screens in the background of two transgenic constructs containing the iab-7 PRE. One of these constructs is the 18.73.1, which covers a relatively wide fragment of the iab-7 PRE region. The other is the 102.103.2 containing only the minimal PRE sequence of the same region.

In the background of the 18.73.1 transgenic construct we identified 35 new complementation groups, while with the use of the 102.103.2 we isolated 50 mutations, which complement the mutations in all the known genes of the Polycomb-group and trithorax-group. In our two screens, we identified alleles of the following previously known genes: Suppressor of Polycomb at 37D (1 allele), wingless (3 alleles), Polycomblike (13 alleles), Suppressor of zeste 12 (1 allele), Posterior sex combs (3 alleles), Sex comb on midleg (15 alleles), verthandi (1 allele), Suppressor of zeste 2 (1 allele) and male-specific lethal-type alleles (2 alleles). We have not identified any alleles of the Polycomb, Enhancer of zeste, and trithorax genes. However, we isolated 3 alleles of kohtalo2 (kto2), an unknown gene located in the Df(3L)kto2 deletion.

We isolated two previously unknown complementation groups, which we named Fehér szem (Fes; 3 alleles), meaning white eye and Piros szem (Pis; 8 alleles), meaning red eye according to their effect on the expression of the reporter gene.

The effect of the Fes alleles on the iab-7 PRE is direct, since these alleles do not influence the eye-colour of flies that carry transgenic constructs containing the Mcp PRE. We mapped Fes to the left arm of the second chromosome, between the black and the purple marker genes. The fact that we could isolate Fes alleles using the minimal PRE containing 102.103.2 transgenic construct suggests that a TRE-like sequence is overlapping or located very close to the iab-7 PRE. This FRE (Fes Response Element) sequence, although not bound by the TRITHORAX protein is similar to a genuine TRE in many aspects. The genetic interactions of Fes suggest that the FES protein is a new member of the TRITHORAX-group.
*Pis* proved to be allelic to the gene encoding DSFMBT, the histone-binding protein of the PHO Repressive Complex (PHORC), one of the complexes formed by POLYCOMB-group proteins. Biochemical data are also supported by our observations that *Pis* shows characteristic genetic interactions with the members of the *Polycomb*-group.

Some of the newly isolated mutations showed controversial phenotypes (trithorax-type homeotic phenotype, while darkening the eye-colour). We attempted to revert these potential gain-of-function alleles by the means of X-rays. The reversion screens were successful in several cases. The identification of common chromosomal breakpoints of the revertants, and the following complementation analysis revealed that 94A and 117A are the alleles of the *grappa* and the *bonus* genes, respectively. The reversion of the *bon*\textsuperscript{117A} mutation showed that the complementation group of 4 members identified on the third chromosome is identical to *bonus*. In our reversion screens, we identified several lightener mutations. Three of these lighteners are *Fes* alleles, while 3 other mutations belong to a previously unknown complementation group.

The first allele of the *grappa* gene was isolated in our laboratory, and the revertants of the *grappa*\textsuperscript{94A} allele helped in cloning the gene. According to our findings and the results of our collaborating partner, *GRAPPA* is the homolog of the DOT1 protein of the yeast, and thus the H3 K79 methyltransferase of the *Drosophila*.

Both the revertants of the *bonus*\textsuperscript{117A} allele and the P-element excision alleles are lighteners of the transgenic eye-colour, suggesting that *bonus* is a member of the *trithorax*-group. This suggestion was confirmed by the finding that *bonus* alleles interact with the deletion of the *trithorax* gene.

In our mutagens screens, apart from successfully identifying new members of the *PcG* and the *trxG*, we gained information on the specific features of the silencing mechanism established on the iab-7 PRE sequence. Our findings imply that not all the *PcG* members are necessary for the silencing activity. We suggest that a TRE-like sequence (an FRE), bound
specifically by the Fes protein is located close to the \textit{iab-7 PRE}. The loss-of-function alleles of several genes ($Psc$, $Su(Pc)37D$, $vtd$) changed the transgenic eye-colour contrary to our expectations, implying that PCG and TRXG proteins form a complex gene regulatory network, regulating both their own genes, and other $PcG$ and $trxG$ genes.

Since our previous findings suggest that a more distant TRE is located inside the Mval-HindIII fragment (~1200 bp) of the \textit{Fab-7-iab-7 PRE} region, we designed a set of new transgenic constructs. With our 4 new overlapping constructs we would like to map the position of this potential TRE. We cloned the PRE fragments between FRT (Flip Re\-\check{c}minase Target) sequences, so that we are able to select those lines in which the transgenic PRE functions independently of endogenous PRE sequences.

We hope that our newly isolated mutants and our new PRE-containing transgenic constructs will help us in fine-mapping the POLYCOMB- and TRITHORAX-complex tethering sequences of the \textit{Fab-7-iab-7 PRE} region.
PUBLICATIONS

Publications directly related to the thesis


Publications not related directly to the thesis


