9 SUMMARY

9.1 PIPSQUEAK AND GAGA-FACTOR

The Drosophila GAGA-factor (GAF) has been implicated with diverse and partially contrary functions. GAF has initially been identified as a classical transcription factor because it stimulates transcription of many known genes. But soon GAF was suspected to function in organizing higher order chromatin structure. There are reports that GAF can activate transcription, that it can silence genes, that it can block enhancers and that it may play a role in chromosome condensation and segregation during mitosis. GAF plays a role in maintaining an active and a repressed state of homeotic gene expression. This points to a role of GAF in both trxG and PcG mediated gene regulation. It is suspected that GAF acts through a chromatin remodeling mechanism mediating its diverse functions.

GAF binds to GA-rich elements mediating the functions GAF has been implicated with. It has recently been observed that these GAGA DNA elements can be bound by a second Drosophila protein, encoded by the pipsqueak (psq) gene. The data presented here suggest that both proteins, Psq and GAF, act in concert as partners.

The binding patterns of Psq and GAF on polytene chromosomes are identical. Psq and GAF are associated in a protein complex and they directly bind to one another through their BTB domains. Taken together, these data are consistent with a new model presented here (Fig. 27). This model explains how GAF and Psq might cooperate as obligatory partners in the transcriptional control of many genes. Genetic interaction studies demonstrate that psq and Trl have similar functions. Psq and GAF act together in the transcriptional activation and silencing of homeotic genes. Thus, GAF and Psq have properties of trxG- and PcG-proteins.

As on polytene chromosomes, the binding patterns of Psq and GAF on mitotic chromosomes also show a complete overlap. These results suggest that Psq and GAF share common functions not only in the control of target genes at euchromatic sites, but also in heterochromatin organization and mitosis. This hypothesis was further supported by the finding that psq acts, like Trl, as an enhancer of position effect variegation suggesting a role of Psq in establishing heterochromatin structures.

The genetic, biochemical, and cytogenetic data presented here strongly suggest that Psq and GAF act together as partners in the control of homeotic and many other genes. Future studies on GAF function will therefore have to include this partner, and may thus provide novel insights into the mechanism of action of this important chromatin factor.

9.2 PIEFKE

Here, we have set out to characterize a new member of the Psq family. By searching the Drosophila genome we have identified a new gene, piefke (pfk), encoding a Psq-domain protein. Additionally, Piekfe (Pfk) contains a BTB/POZ domain. The gene has been cloned and a recombinant protein was expressed in bacteria. The protein was purified and used to generate polyclonal antibodies.

Using these antibodies we analyzed the expression of Pfk. Pfk is a nuclear protein present in larval salivary glands and in ovaries.

Immunostaining of polytene chromosomes shows that Pfk binds to chromosomal loci. Pfk is indeed a chromatin component. It was not tested if the protein binds directly to the DNA via the Psq-domain or if it is just chromatin associated. Pfk shows only limited colocalization with Psq/GAF-complexes on polytene chromosomes. A partial colocalization of Pfk and HP1 has been observed. This colocalization includes not only heterochromatic regions but also euchromatic loci.

The localization studies presented here give a first hint on possible functions of Pfk in organizing chromatin structures. Future experiments are directed towards the identification of interacting proteins of Pfk. It will also be important to identify target sequences and genes of Pfk. Future experiments will especially have to include the determination of the biological functions of Pfk by identifying and characterizing pfk mutants.