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DISSERTATION SUMMARY

Study of two overlapping genes in *Drosophila melanogaster*

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From an independent screen we isolated two *Drosophila melanogaster* genes. Based on homology searches we named one of the genes *dada2a/drpb4*. The other gene got the *dtat* name from its ability to bind the HIV TAR RNA. The *dada2a/drpb4* gene codes for two different proteins through alternative splicing. The dRPB4 protein is the 4th largest subunit of the RNA polymerase II, which attaches to the holoenzyme only in stress conditions in budding yeast. In the fission yeast the protein is a stable member of the RNA polymerase II, and probably this is the docking site for the C-terminal Domain phosphatase of the RPB1 protein. The *dada2a/drpb4* gene codes for another protein, the dADA2a. This is an adaptor protein, which has its yeast homology too. In the yeast this protein is a member of two adaptor complexes, the ADA and the SAGA complexes. They act like a bridge between the transcription factor and the RNA polymerase II, mediating the effect of the transcription factor. In the fruitfly, there are two ADA2 proteins, the dADA2a and the dADA2b. The first was isolated in our laboratory, the second in a cooperating French laboratory. The dtat gene codes for a protein of unknown function, although there are similar proteins in many species found in the database. My goal was to investigate the arrangement of the two genes in the Drosophila melanogaster genome, to examine their transcription regulation and to find out something from the function of the dTAT protein.

As I mentioned the *dada2a/drpb4* gene codes for two types of mRNAs, the dada2a and the drpb4 mRNA. The first exons of these mRNAs are common and the further exons of the dada2a mRNA are located in the first intron of the drpb4 mRNA. The dada2a mRNA itself has two splice variants, they differ in the length of the 2nd exon, but this difference is not shifting the open reading frame. The importance of these two dADA2a variants is not known yet.

The two genes are transcribed in opposite orientation, and they are in head-to-head arrangement. Further studies, including primer extensions, 5'RACE and RNase protection assays, revealed that the genes are overlapping with each other.

The tight arrangement of these genes might suggest a common transcriptional regulation. The observed overlapping transcription prompted us to investigate the possible involvement of a novel gene-silencing phenomenon, the RNA interference (RNAi) in the transcriptional regulation of these genes. RNAi was described in many species including *Drosophila*, and if it is triggered by the introduction of a synthetic dsRNA, it can evoke the degradation of the corresponding mRNA.

To test RNAi, ^{32}P-labelled ssRNAs from different regions of the overlapping genes were synthesised and treated with *Drosophila* embryonic extract. The specific degradation of the labelled ssRNA might indicate the induction of RNAi due to the in vivo appearance of the dsRNA. We observed degradation of ssRNAs corresponding to the overlapping parts of these genes. Control RNA from a closely spaced adjacent gene without overlapping transcription was not degraded.

Our results suggest that RNAi can also be triggered by endogenous dsRNA, and it can regulate endogenous genes by post-transcriptional gene silencing.

To find the function of the dTAT protein we identified its domain structure. It showed that it has an S-adenosine methionine binding domain, but we could not prove that it binds to S-adenosine methionine. From the screen in which we identified the protein we knew that it could bind RNA. We also verified this observation with RNA binding assay. We made yeast-two-hybrid screen to identify the interacting partners. We found interaction between the mouse homologue protein and the human Embryonic Ectoderm Development protein. This protein is a polycomb-like protein, which plays a key role in the embryonic development. It has a homologue in the *Drosophila melanogaster*, the extra sex combs (esc), and genetic studies in the fruitfly underlined the interaction between the dTAT and the esc.

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