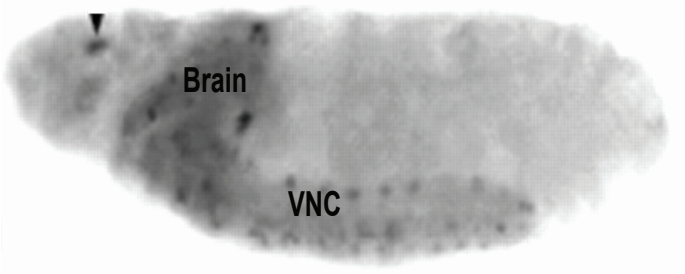
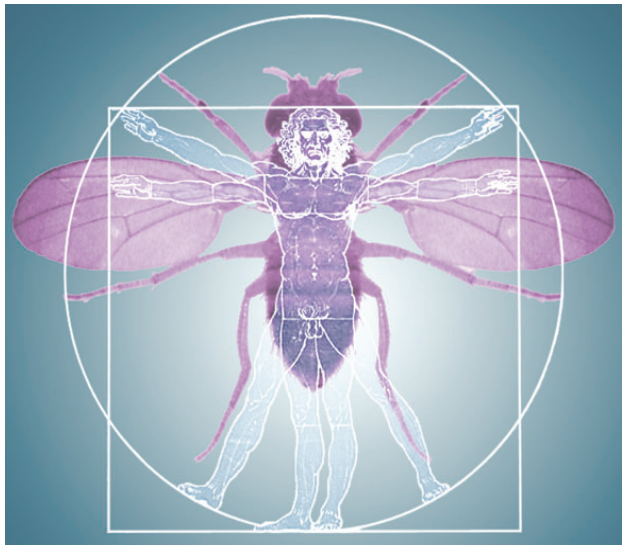
Abstract



Side view of *Drosophila* embryo ST 18 AEL

The *D. melanogaster* embryonic neuroblast NB5-6T generates a set of four neurons expressing the Apterous (Ap) transcription factor. One of these cells is a unique neuropeptide-producing neuron, which expresses the FMRFa neuropeptide. The selective expression of Apterous and FMRFa, combined with a wealth of information regarding this lineage, makes the NB5-6 and the Apterous neurons a powerful model system for understanding neuronal development. To identify novel regulatory genes that control neuronal development, a forward genetic screen scoring for expression of a FMRFa-EGFP transgene, was previously carried out. A subset of these mutants show an increase in the number of FMRFa cells generated, indicating a failure in proliferation control. Using a deletion-based genetic mapping approach I have mapped the genes mutated in these proliferation mutants. This approach revealed several novel genes with regulatory roles in neuronal development i.e., *18wheeler, Pre-mRNA splicing factor (Prp8), GATAe, CG2469* and *CG2034*. Molecular analysis furthermore identified the specific mutations in the *18wheeler* and *Prp8* genes.

''If nature were not beautiful, it would not be worth knowing, and if nature were not worth knowing, life would not be worth living.'' Henri Poincare

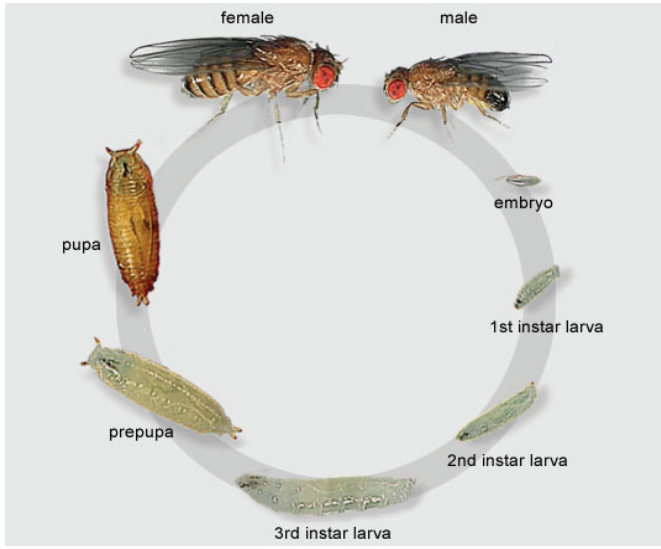


Acknowledgements
I am indebted to my supervisor Stefan Thor, for his support during this work.
I am also grateful to The Bloomington Stock Center for sharing fly lines. It is a also a pleasure to thank all who made this thesis possible.

References:
1- http://flymove.uni-muenster.de/
2- Botas J. (2007) Drosophila researchers focus on human disease. Nature Genetics 39, 589

Contact information:
Cell ph +46-(0)737- 01 61 68
E-mail: shaba768@student.liu.se

Identifying novel regulatory genes controlling cell specification and proliferation in the Apterous cluster of the *Drosophila melanogaster* embryonic central nervous system through a genetic screen



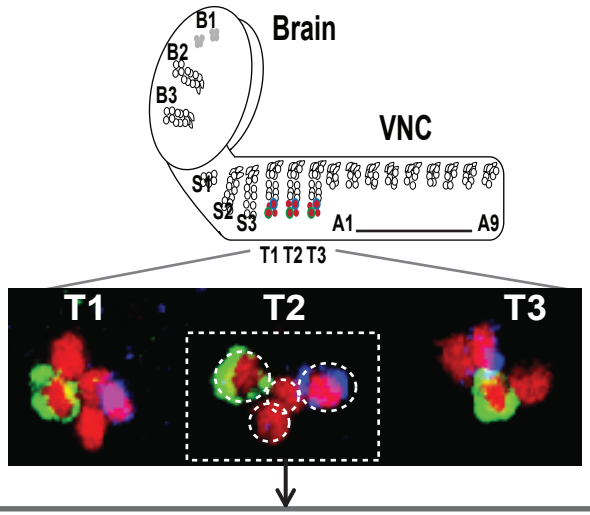
Master thesis 2012

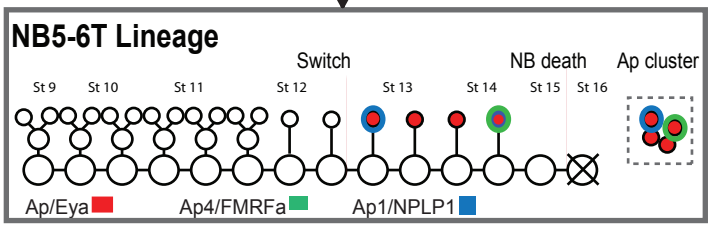
Linköping University

Shahrzad Bahrampour
Supervisor: Stefan Thor

International Masters Program
Molecular Genetics and Physiology
Department of Physics, Chemistry and Biology
Linköpings universitet
2012-05-25

The neuroblast 5-6 model lineage





In Drosophila, embryonic NBs lineages have been found to be stereotypical with respect to:
the cell fate, lineage size and lineage topology.

Mutant lines analyzed

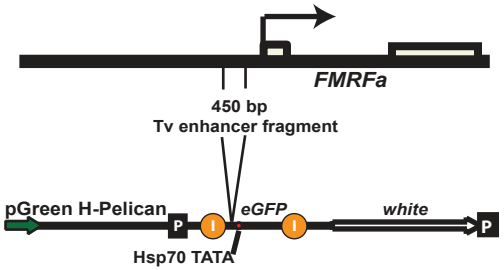
7 mutant lines had shown weak or loss of FMRFa expression

1 mutant line has fewer neurons expressing Eya

6 mutant lines have extra neurons with Eya expression

	Mutant line	Quantification of AP/Eya expression	Gene
Allilic Mutants	*3F247*	Loss of Ap/eya Expression	*18wheeler*
	4P24	Extra Ap/eya expression	*Prp8*
	7P17	Extra Ap/eya expressi, Up to 7 neurons	
	9P4	Extra Ap/eya expression (4,27 Ap/eya)	*CG2469*
	12P23	Extra Ap/eya expression (5,16 Ap/eya)	
	13A18	Extra Ap/eya expression (6,06 Ap/eya)	*CG2034*
	8P13	Extra Ap/eya expression (4,93)	*GATAe*

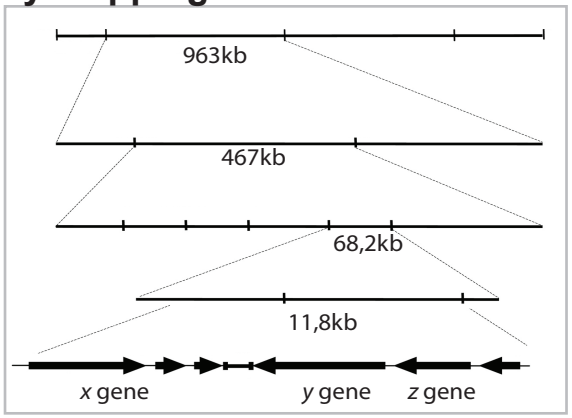
Genetic Screen



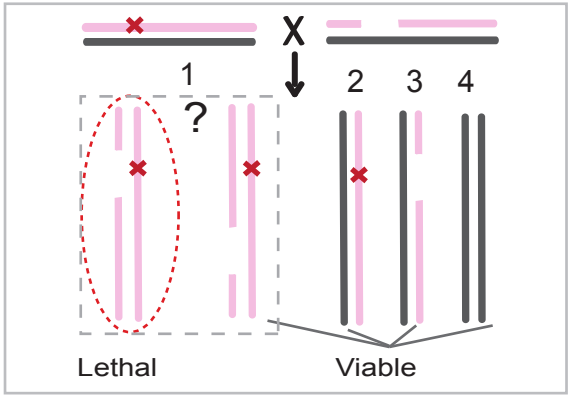
FMRFa-GFP flies were EMS mutagenized and homozygozed for the mutant chromosome. Next, they were screened for alternation of *FMRFa-GFP* expression at the late embryonic stage.

Genetic mapping

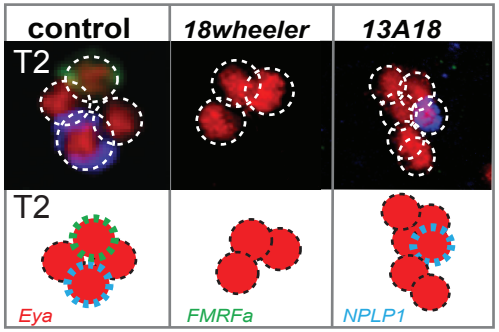
Lethality mapping



Complementation test



Cellular and molecular mapping



The Ap cluster neurons are showen with the cell-specific markers: Eya, FMRFa and Nplp1 in the T2 thoracic hemisegment.
The phynotype for the 2nd thoracic Ap-cluster of *3F247* and *18A13* mutants are compared to the control.

Candidate mutation for *18wheeler* allele *3F247*

wt: .....AGSEAANKNGQAFLV stop
3F247: .....AGSEAANKNGQAFLV-------24aa stop

Candidate mutations for *Prp8* alleles *4P24* and *7P17*

WT: .....FHLCREILRL.....PPQMPND.....KCWRRDA.....
4P24: .....FHLCCEILRL.....PPQMPND.....KCWRRDA.....
7P17: .....FHLCREILRL.....PPQLPND.....CKTLVCVstop
608 1128 1194

Summary

-*18wheeler* is a member of Toll receptor family, with transmembrane activity and cell adhesion traits, regulated by segmentation and homeotic genes. It is predicted to be involved in Slit/Robo signaling pathway.
-*Pre-mRNA splicing factor (Prp8)* gene is the most conserved eukaryotic spliceosome factor across evolution. It might be involved in neuro-development by controlling alternative mRNA splicing of regulatory molecules playing roles in NBs proliferation. Also, Prp8 might be involved in apoptosis due to its similarity to the Apoptosis Regulated Protein 2.
-*GATAe* has homology with vertebrates transcription factor *GATA1*, a zinc finger protein involved in DNA binding with a helices domain.
-*CG2469* is homolog of a component of polymerase-associated factor 1.
-*CG2034* encodes a protein with an unknown biological role
Despite the fact that most of the genes involved in neuro-development are fairly conserved between the fly and vertebrates, the function and molecular pathways in which these genes are involved are currently unknown.