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

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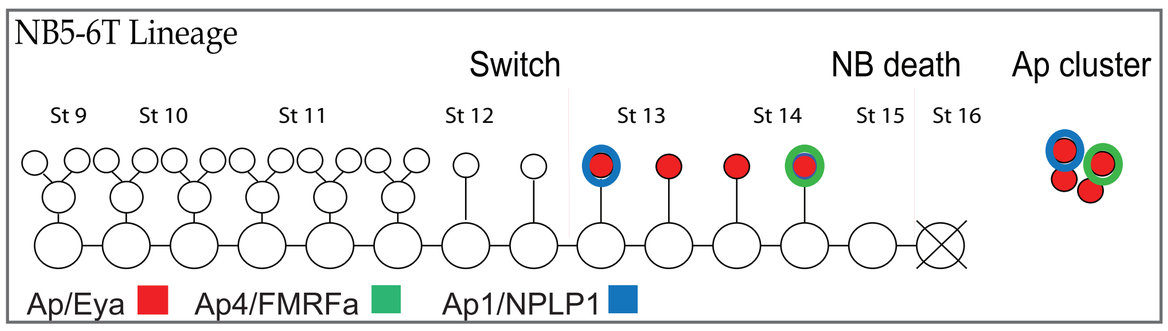
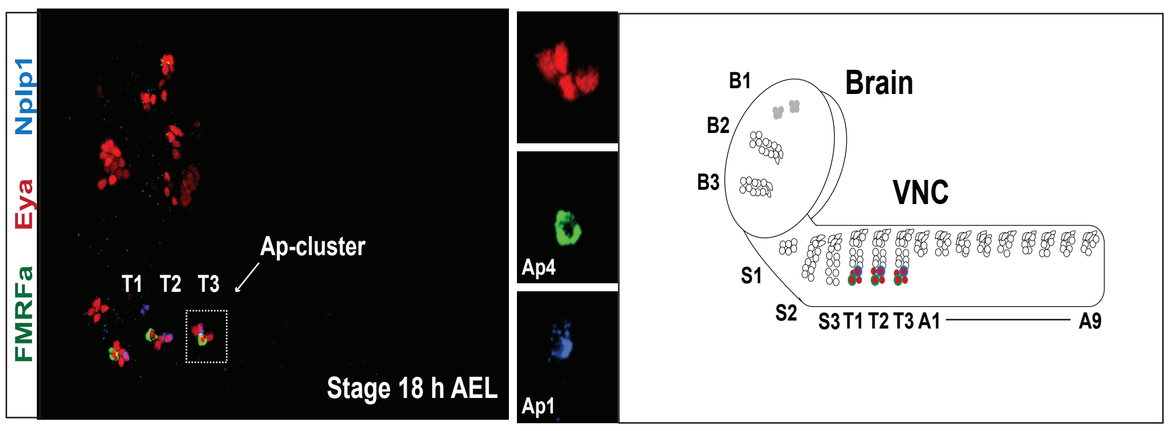
Abstract

The *Drosophila* embryonic neuroblast NB5-6 generates a set of four neurons expressing the Apterous 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.

The neuroblast 5-6T lineage

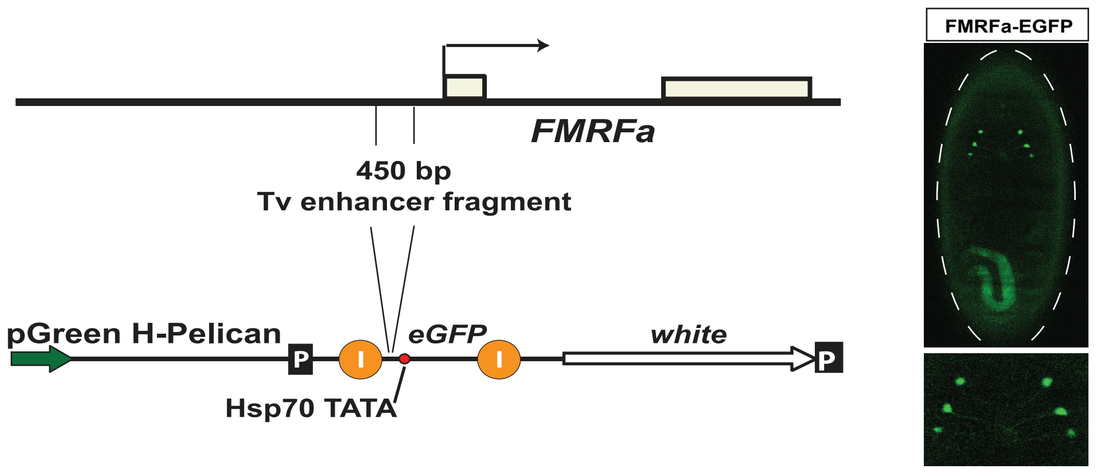


In *Drosophila*, embryonic NBs lineages have been found to be stereotypical with respect to: the Cell fate, lineage size and lineage topology.



The Ap neurons are visualized with the cell-specific markers *Eya* and *FMRFa-GFP*. In the thoracic segments the last four neurons generated by NB5-6T is the Ap cluster neurons, which are defined by their expression of the LIM-homeodomain TF Apterous (Ap), and the co-factor *Eyes absent (Eya)*. Two of the Ap cluster neurons, Ap1 and Ap4, are peptidergic and express the neuropeptides *Nplp1* and *FMRFa*, respectively. Model summarizing data from several previous studies, which have identified a number of regulatory genes acting to specify the Ap cluster neurons [1].

Genetic screen



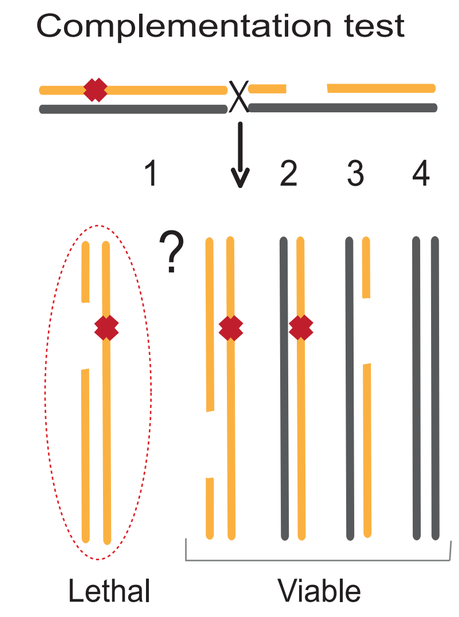
EMS mutagenized and homozygozied for the mutant chromosome had got TV-GFP insertion.
Further, they were screened for alternation on *FMRFa-GFP* expression at the late embryonic stage [2].

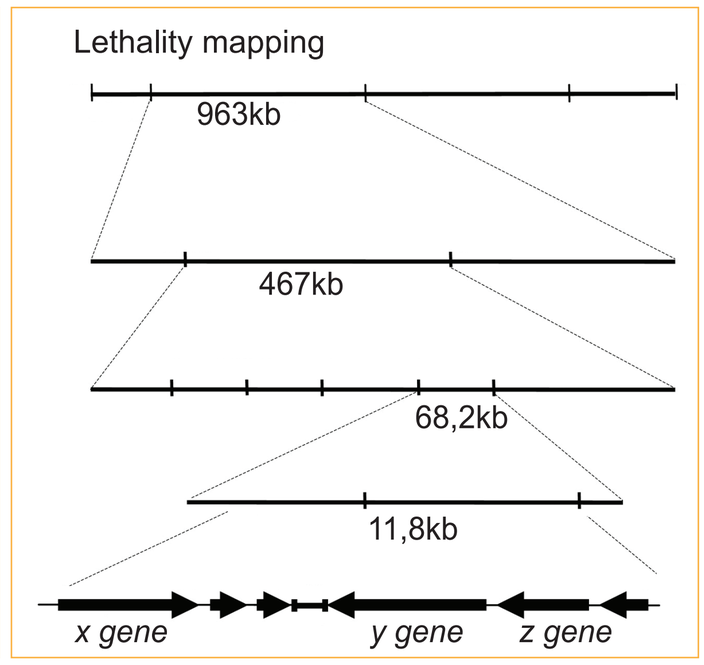
7 mutant lines had shown weak or loss of *FMRMa* expression

- 1 mutant line has fewer neurons express *Eya*
- 6 mutant strains has extra neurons with *Eya* Expression

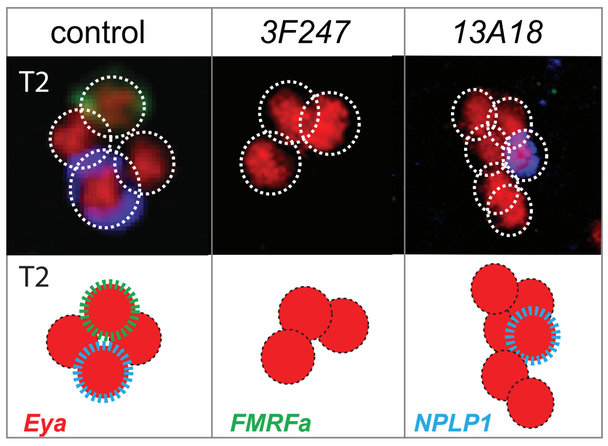
	Mutant	Quantification of AP/Eya expression	Gene
	3F247	loss of Ap/eya Expression	*18wheeler*
Allelic mutants	*4P24*	Extra Ap/eya expression	*Prp8*
Allelic mutants	*7P17*	Extra Ap/eya expressi, Up to 7 neurons	*Prp8*
Allelic mutants	*9P4*	Extra Ap/eya expression (4,27 Ap/eya)	*CG2469*
Allelic mutants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

Genetic mapping





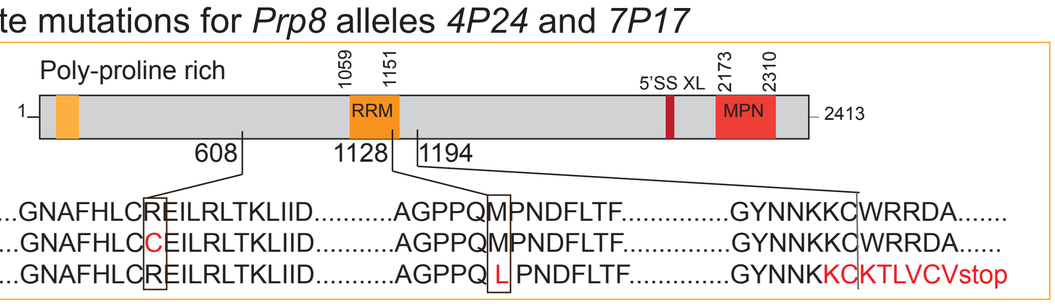
The observed phynotype for second toracic Ap-cluster of *3F247* and *18A13* mutants



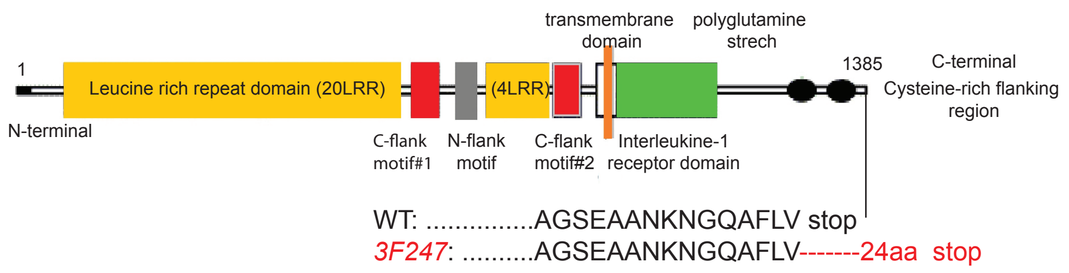
The Ap cluster neurons are showen with the cell-specific markers: *Eya, FMRFa* and *NPLP1* in the 3 thoracic hemisegments of NB5-6 lineage. The phynotype for the second thoracic Ap-cluster of *3F247* and*18A13* mutants are compared to the control.

Molecular mapping

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



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



Summary

1- *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.

2- *Pre-mRNA splicing factor (Prp8)* gene is the most conserved eukaryotic spliceosome factor across evolution. It might be involved in neurodevelopment by controlling alternative mRNA splicing of regulatory molecules playing roles in NBs proliferation. Prp8 might be involved in apoptosis due to its similarity to the Apoptosis Regulated Protein 2.

3- *GATAe* has homology with vertebrates transcription factor *GATA1*, a zinc finger protein involved in DNA binding with a helices domain.

4- *CG2469* is homolog of a component of polymerase-associated factor 1.

5- *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.

Acknowledgements

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References

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2. Ulvklo C, MacDonald R, Bivik C, Baumgardt M, Karlsson D, Thor S. (2012) Control of neuronal cell fate and number by integration of distinct daughter cell proliferation modes with temporal progression. Development.;139(4):678-89.