Genet. Res., Camb. (1989), **53**, *pp.* 23–26 *With 2 text-figures Printed in Great Britain*

Interaction of Hairless, Delta, Enhancer of split and Notch genes of *Drosophila melanogaster* as expressed in adult morphology

MARIITTA SIRÉN AND PETTER PORTIN*

Laboratory of Genetics, Department of Biology, University of Turku, SF-20500 Turku 50, Finland

(*Received 17 June 1988 and in revised form 30 January 1989*)

Summary

The interaction of three neurogenic loci *viz.* Delta, Enhancer of split and Notch, and a related gene, Hairless, of *Drosophila melanogaster* was investigated at the adult morphology level by measuring the effects of the mutations of the three other genes on the expression of the recessive lethal antimorphic Abruptex mutations of the Notch locus. The Abruptex mutations were also coupled in *cis* or *trans* with facet-glossy or split mutations of the Notch locus. In some of the experiments, the genotype of the fly was homozygous for either facet-glossy or split mutation or their wild type alleles but heterozygous for the Abruptex. Facet-glossy is located in a large intron of the locus, whereas split is located in the same exon as Abruptex. In all compounds studied, Delta suppressed the expression of Abruptex while Hairless and Enhancer of split enhanced it. The interactions of the four genes studied were allele specific, suggesting an interaction at the protein level. The comparison of the results presented in this study on the interaction of the neurogenic genes with other results on the same subject suggests that the interactions are similar in embryonic and imaginal development.

1. Introduction

In *Drosophila melanogaster* a group of so-called neurogenic genes, promote correct regulation of the initial steps in the development of both central and peripheral nervous system. Members of this group of genes included Notch (*N*, 1–3·0), Delta (*Dl*, 3–66·2), and Enhancer of split (*E*(*spl*), 3–89·0). A related gene, Hairless (*H*, 3–69·5), is also involved in the development of the nervous system.

Amorphic mutations of these genes are embryonic lethals, the embryos dying because of hypertrophy of the neural tissue, *i.e.* complete neuralization of the whole neurogenic region of the ectoderm. This was shown by Poulson (1937, 1941) for the Notch gene, by Lehman *et al.* (1981, 1983) for the Delta gene and by Lehman *et al.* (1983), and Knust *et al.* (1987) for the Enhancer of split gene.

The Hairless gene is known to be related to some of the neurogenic loci in that, for example, *H* mutations reduce the phenotype caused by heterozygosity for *N*, *viz.* notching of wings (Lindsley and Grell, 1968). Hairless mutations cause absence of bristles on the head, thorax and abdomen as well as interruption of wing veins in the heterozygous condition. Homozygous *H* mutations are larval lethals (Lindsley and Grell, 1968).

In heterozygous condition *Dl* mutations cause delta-like thickenings of the wing vein ends, and *E*(*spl*) mutations cause enhancement of the expression of the split (*spl*) allele of the Notch locus. The *E*(*spl*) allele used in this study causes this enhancement in both heterozygous and homozygous condition, the effect being stronger in the latter case. Thus, this allele is not homozygous lethal (Lindsley and Grell, 1968; see also Welshons, 1965).

Of the neurogenic genes, Enhancer of split probably has a central position in neurogenesis, because its action is cell-autonomous in the ectoderm of the embryos, as was shown by cell transplantation experiments (Technau and Campos-Ortega, 1987). Other neurogenic genes were nonautonomous. Therefore, it was suggested by these authors that the function of the *E*(*spl*) gene is required at the receptor side of the hypothetical signal chain of the neurogenic genes.

The cloning and sequencing of the Notch locus by Wharton *et al.* (1985) and Kidd *et al.* (1986), and of the Delta gene by Vässin *et al.* (1987) suggest a similar product for both genes. The proposed encoded proteins are probably transmembrane proteins with mammalian Epidermal Growth Factor-like (EGF-like) repeats. It is believed that these transmembrane

* Corresponding author.

proteins mediate cell-cell contacts, thus making possible the developmental decision between the epidermal and neuroblastic pathways for the ectodermal progenitor cells of the neurogenic region of the embryo.

Recessive lethal Abruptex (*Ax*, 1–3·0) mutations at the Notch locus produce in heterozygous condition a phenotype which makes it possible to measure the expression of the Notch gene at the adult morphological level. Abruptex mutations, namely, cause interruption of wing veins and the reduction in number of bristles in the head and thorax.

The present study examines the interactions between Abruptex mutations and Delta, Enhancer of split, and Hairless. Measurements of the interactions were made on adult morphology so that we could compare the interactions of the neurogenic genes on imaginal development with their interactions in embryogenesis which have been studied by Vässin *et al.* (1985), and de la Concha *et al.* (1988).

Our results suggest that the effects of *H*, *Dl*, and *E(spl)* on *N* are allele specific thus occurring most likely at the protein level. A model for the interaction of these genes will be presented, and the results are compared with other results on the interaction of neurogenic loci. The comparison suggests that the interactions are similar in embryonic and imaginal development.

2. Materials and Methods

The expression of the Abruptex mutation of the Notch gene was investigated in genotypes in which the Ax^{59d5} mutation was coupled in *cis* or *trans* with fa^{g} or *spl*. In some of the experiments, the X chromosome was homozygous for fa^{g} or *spl* or their wild type alleles but heterozygous for Ax^{59d5}. The third chromosome was either wild, heterozygous for *H*, Dl^{l} or $E(spl)^{D}$ or homozygous for $E(spl)^{D}$. Likewise, the expression of Abruptex was measured in genotypes in which $Ax^{59b8\cdot1}$ was coupled in *cis* or *trans* with *spl*, or the fly was homozygous for *spl* or its wild type allele while being heterozygous for $Ax^{59b8\cdot1}$. Again, the third chromosome was either homozygous wild, heterozygous for *H*, Dl^{l} or $E(spl)^{D}$ or homozygous for $E(spl)^{D}$.

The Abruptex mutations used in the study cause absence of orbital, dorsocentral and scutellar bristles as well as interruption of wing veins III, IV and V.

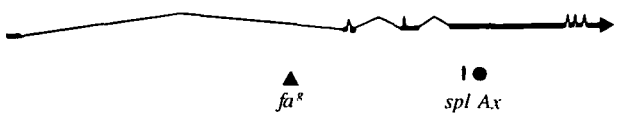

Fig. 1. The molecular fine structure map of the Notch locus indicating the positions of fa^{g}, *spl*, and $Ax^{59b8\cdot1}$ and Ax^{59d5} (designated *Ax*). Thin lines are introns, and heavy lines are exons. fa^{g} is an insertion, while *spl* as well as *Ax* mutations map as points. Data from Artavanis-Tsakonas *et al.*, 1984; Hartley *et al.*, 1987; Kelley *et al.*, 1987; Kidd *et al.*, 1983; Kidd and Young, 1986.

The molecular fine structure map of the Notch locus is presented in Figure 1. Abruptex mutations map as points in the EGF-like domain repeat in the large exon of the locus. Split (*spl*) also maps as a point in the same exon as *Ax*, while facet-glossy (fa^{g}) is an insertion mutation in the large intron of the locus (Artavanis-Tsakonas *et al.*, 1984; Kidd and Young, 1986; Hartley *et al.*, 1987; Kelley *et al.*, 1987).

The expression of Abruptex was measured on approximately 50 females in each case as follows. The numbers of orbital, dorsocentral, and scutellar bristles were counted. The number of wing vein breaks was counted, and their total length measured microscopically from wings mounted in a drop of water under a cover slip. The factor by which the Abruptex phenotype was either enhanced or suppressed was calculated from the mean of the factors of enhancement or suppression of the three polyphenes studied, *viz.* absence of bristles, number and length of wing vein breaks.

The flies were raised on a standard *Drosophila* medium containing semolina, agar-agar, syrup and both dried and fresh yeast at 25 °C. The statistical significance of the results was tested by analysis of variance of the primary measurements.

3. Results

The Delta mutation strongly suppressed the expression of Abrubtex in all the gene compounds tested (Table 1). The Hairless mutation strongly enhanced the expression of Abruptex in all the compounds which we were able to construct (Table 1). The Enhancer of split mutation also enhanced the expression of Abruptex in all compounds tested, although not as strongly as Hairless. The enhancement increased with increasing dose of the $E(spl)^{D}$ mutation (Table 1).

Particularly in the cases of Hairless and Delta, and to a lesser extent in the case of $E(spl)^{D}$, the effects of these mutations on Notch were quantitatively dependent on its allele constitution. The results were statistically significant in all instances.

4. Discussion

In the present study it was observed that the effects H^{-}, Dl^{-}, and $E(spl)^{D}$ on *N* were quantitatively dependent on the allelic status of the Notch gene. This suggests that the interaction between *N* and the three other genes occurs at the level of protein products encoded by the respective genes, and not at the level of transcription, for example. This notion is in particular supported by the observation that fa^{g} or *spl* in *cis*- or *trans*- position with *Ax* alters the interaction of *N* with *H* and *Dl*.

A model for the interaction of the four genes studied, based on the results of the present study, is presented in Figure 2. When considering the model, it should be remembered that the recessive lethal

https://doi.org/10.1017/S0016672300028330

Table 1. *Effect of Hairless, Delta and Enhancer of split mutations on the expression of Abruptex in different facet-glossy – Abruptex and split – Abruptex combinations of the Notch locus*

Genotype of the Notch locus	Effect of Hairless	Effect of Delta	Effect of $E(spl)^D/+$	Effect of $E(spl)^D/E(spl)^D$
$Ax^{59a5}/+$	enh. 2·9 ×	suppr. 3·3 ×	enh. 1·4 ×	enh. 2·2 ×
$Ax^{59b8\cdot1}/+$	enh. 2·9 ×	suppr. 5·7 ×	enh. 1·1 ×	enh. 2·2 ×
$fa^g\ Ax^{59a5}/++$	enh. 7·4 ×	suppr. 161 ×	enh. 2·1 ×	enh. 2·4 ×
$fa^g+/+Ax^{59a5}$	enh. 3·4 ×	suppr. 4·0 ×	enh. 1·4 ×	enh. 1·6 ×
$fa^g\ Ax^{59a5}/fa^g+$	enh. 6·1 ×	suppr. 70 ×	enh. 1·1 ×	enh. 1·4 ×
$spl\ Ax^{59b8\cdot1}/++$	enh. 2·6 ×	suppr. 4·5 ×	enh. 1·4 ×	enh. 1·6 ×
$spl+/+Ax^{59b8\cdot1}$	—	suppr. 10·5 ×	enh. 1·5 ×	enh. 2·1 ×
$spl\ Ax^{59b8\cdot1}/spl+$	—	suppr. 4·3 ×	enh. 1·5 ×	enh. 2·2 ×
$spl\ Ax^{59a5}/++$	enh. 3·6 ×	suppr. 3·2 ×	enh. 1·4 ×	enh. 1·7 ×
$spl\ +/+\ Ax^{59a5}$	—	suppr. 21 ×	enh. 1·5 ×	enh. 1·9 ×
$spl\ Ax^{59a5}/spl+$	—	suppr. 2·7 ×	enh. 1·7 ×	enh. 2·1 ×

enh. = enhancement, suppr. = suppression, — = impossible to construct. The figures represent the factor by which the phenotype was either enhanced os suppressed. The unexpected large factors in the case of *Dl* (160, 70, and 21) are explained by the fact that control genotypes gave a very little expression of the wing venation phenotype of *Ax*.

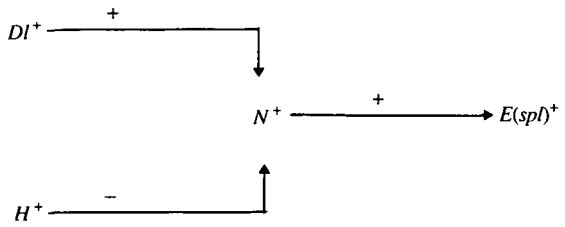

Fig. 2. A model for the functional interactions between the wild type alleles of Hairless (H^+), Enhancer of split ($E(spl)^+$), Delta (Dl^+), and Notch (N^+). The symbols + and − at the arrows indicate positive or negative influences on the activity of the corresponding genes, and the size of the symbol indicates the relative intensity of each effect.

Abruptex mutations of the Notch locus are antimorphic gain of function mutations, as was shown by Portin (1981) from gene dosage studies. In the model the *E(spl)* gene has been presented as located on the receptor side of the hypothetical signal chain of the genes studied. This is based on the observation of Technau and Campos-Ortega (1987) that the *E(spl)* gene was cell autonomous in the ectoderm of the embryos, while the other neurogenic genes were nonautonomous (see Introduction).

In previous studies on the interaction of neurogenic loci in embryos, Vässin *et al.* (1985) and de la Concha *et al.* (1988) found that duplication of the Dl^+ gene enhanced the expression of N^-. Duplication of $E(spl)^+$ also suppressed the expression of N^- in both studies. In the study of de la Concha *et al.* (1988), it was observed that H^- suppressed the expression of N^-. These results are in agreement with those of the present study taking into account the fact that $E(spl)^D$ and lethal *Ax* mutations are gain of function mutations (Portin, 1981, Knust *et al.*, 1987). Therefore, not unexpectedly, it is concluded that the interactions of the neurogenic genes are similar in embryonic and imaginal development. Further, our results indicate that gain of function mutations (*Ax*) of the *N* locus have similar effects on the interaction of neurogenic genes as loss of function (N^-) mutations.

This work was supported by The Academy of Finland.

References

Artavanis-Tsakonas, S., Grimwade, B. G., Harrison, R. G., Markopoulou, K., Muskavitch, M. A. T. Schlesinger-Bryant, R., Wharton, K. & Yedvobnick, B. (1984). The *Notch* locus of *Drosophila melanogaster*: A molecular analysis. *Developmental Genetics 4*, 233–254.

de la Concha, A., Dietrich, U., Weigel, D. & Campos-Ortega, J. A. (1988). Functional interactions of neurogenic genes of *Drosophila melanogaster*. *Genetics 118*, 499–508.

Hartley, D. A., Xu, T. & Artavanis-Tsakonas, S. (1987). The embryonic expression of the Notch locus of *Drosophila melanogaster* and the implications of point mutations in the extracellular EGF-like domain of the putative protein. *The EMBO Journal 6*, 3407–3418.

Kelley, M. R., Kidd, S., Deutsch, W. A. & Young, M. W. (1987). Mutations altering the structure of Epidermal Growth Factor-like coding sequences at the *Drosophila Notch* locus. *Cell 51*, 539–548.

Kidd, S., Lockett, T. & Young, M. W. (1983). The Notch locus of *Drosophila melanogaster*. *Cell 34*, 421–433.

Kidd, S., Kelley, M. R. & Young, M. W. (1986). Sequence of the Notch locus of *Drosophila melanogaster*: Relationship of the encoded protein to mammalian clotting and growth factors. *Molecular and Cellular Biology 6*, 3094–3108.

Kidd, S. & Young, M. W. (1986). Transposon-dependent mutant phenotypes at the Notch locus of *Drosophila*. *Nature 232*, 89–91.

Knust, E., Bremer, K. A., Vässin, H., Ziemer, A., Tepan, U. & Campos-Ortega, J. A. (1987). The Enhancer of split locus and neurogenesis in *Drosophila melanogaster*. *Developmental Biology 122*, 262–273.

Lehmann, R., Dietrich, U., Jimenez, F. & Campos-Ortega, J. A. (1981). Mutations of early neurogenesis in *Drosophila*. *Wilhelm Roux's Archives of Developmental Biology 190*, 226–229.

Lehmann, R., Jimenez, F., Dietrich, U. & Campos-Ortega, J. A. (1983). On the phenotype of development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Archives of Developmental Biology 192*, 62–74.

Lindsley, D. L. & Grell, E. H. (1986). *Genetic Variations of Drosophila melanogaster*. Washington: Carnegie Institute Publications.

Portin, P. (1981). The antimorphic mode of action of lethal Abruptex alleles of the Notch locus in *Drosophila melanogaster*. *Hereditas 95*, 247–251.

Poulson, D. F. (1937). Chromosomal deficiencies and embryonic development of *Drosophila melanogaster*. *Proceedings of The National Academy of Sciences of U.S.A. 23*, 133–137.

Poulson, D. F. (1941). The developmental effects of a series of Notch deficiencies in the X-chromosome of *Drosophila melanogaster*. *Proceedings of the 7th International Congress of Genetics*, 240–241.

Technau, G. M. & Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*. *Proceedings of The National Academy of Sciences of U.S.A. 84*, 4500–4504.

Vässin, H., Bremer, K. A., Knust, E. & Campos-Ortega, J. A. (1987). The neurogenic gene Delta of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO Journal 6*, 3431–3440.

Vässin, H., Vielmetter, J. & Campos-Ortega, J. A. (1985). Genetic interactions in early neurogenesis of *Drosophila melanogaster*. *Journal of Neurogenetics 2*, 291–308.

Welshons, W. J. (1965). Analysis of a gene in *Drosophila*. *Science 150*, 1122–1129.

Wharton, K. A., Johansen, K. M., Xu, T. & Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell 43*, 567–581.