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deltex, a Locus Interacting with the Neurogenic Genes, *Notch*, *Delta* and *mastermind* in *Drosophila melanogaster*

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ABSTRACT

The *Notch* locus of *Drosophila melanogaster*, which codes for a transmembrane protein sharing homology with the mammalian epidermal growth factor, is one of a small number of zygotically acting genes, the so called neurogenic loci, which are necessary for the correct segregation of neural from epidermal lineages during embryogenesis. In an attempt to identify genes whose products may interact with that of *Notch*, we designed a genetic screen aimed at identifying supressors of certain *Notch* mutations which are known to affect the extracellular epidermal growth factor homologous domain of *Notch*. Mutations in two neurogenic loci were identified as suppressors: *Delta*, whose product was recently shown to interact with *Notch* and *mastermind*. In addition, a third, *X*-linked gene was shown capable of acting as a suppressor. We show that this gene is the *deltex* locus, characterize the phenotype of *deltex* mutations, and demonstrate both a maternal and zygotic action of the locus. All *deltex* alleles behave as recessive viables affecting wing, ocellar and eye morphology. There are allele specific interactions between *deltex* and various *Notch* alleles; for example, *deltex* mutants with a reduced dosage of wild-type *Notch* die as pupae. *deltex* also interacts with *Delta* and *mastermind* in a fashion that is formally analogous to its interaction with *Notch*. These results emphasize the special relationship between *Notch*, *Delta* and *mastermind* suggested by previous work and indicate that *deltex* is likely to play an important role in the same genetic circuitry within which these three neurogenic loci operate.

IN our attempts to understand the molecular mechanisms underlying the decision of an embryonic cell to choose between a neural or epidermal developmental pathway, we have been studying a group of Drosophila genes, collectively known as the zygotic neurogenic loci. When mutated to the null state, each of the zygotic neurogenic loci, *Notch* (*N*), *Delta* (*Dl*), *mastermind* (*mam*), *Enhancer of split* (*E*(*spl*)), *neuralized* (*neu*), and *big brain* (*bib*), results in a hypertrophied nervous system at the expense of ventral and lateral epidermal structures. This effect is due to the misrouting of epidermal precursor cells into a neuronal pathway (POULSON 1937; LEHMANN *et al.* 1983; JÜRGENS *et al.* 1984; WIESCHAUS *et al.* 1984; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDIG 1984). Most of the zygotic neurogenic loci have been shown to be pleiotropic affecting both embryonic as well as postembryonic developmental stages (*e.g.*, WELSHONS 1965; SHELLENBARGER and MOHLER 1978; DIETRICH and CAMPOS ORTEGA 1984).

The molecular analysis of *Notch* has demonstrated that it codes for a transmembrane protein sharing homology with the mammalian epidermal growth factor (EGF), suggesting that the *Notch* protein is involved in cell interactions (WHARTON *et al.* 1985; KIDD, KELLY and YOUNG 1986; JOHANSEN, FEHON and ARTAVANIS-TSAKONAS 1989; KIDD *et al.* 1989). This led to the proposition that *Notch* and some or all of the neurogenic loci may code for elements of a cell interaction mechanism essential for the differentiation of various Drosophila tissues (WHARTON *et al.* 1985; reviewed in ARTAVANIS-TSAKONAS 1988). This hypothesis is supported by developmental and genetic studies which suggest that a variety of developmental events such as the differentiation of the embryonic neural ectoderm, ommatidial development or bristle formation, all known to depend on cell interactions, are also affected by *Notch* mutations (WELSHONS 1965, 1971; SHELLENBARGER and MOHLER 1978; HELD and BRYANT 1984; DOE and GOODMAN 1985a,b; TECHNAU and CAMPOS-ORTEGA 1986; TOMLISON and READY 1987; CAGAN and READY 1989).

The concept of an involvement of *Notch* in a cell interaction mechanism implies several interacting components. Indeed, the predicted structures of both *Delta* and (*E*(*spl*)) are also consistent with an involvement in cellular interactions (VÄSSIN *et al.* 1987; HARTLEY, PREISS and ARTAVANIS-TSAKONAS 1988; KOPCZYNSKI *et al.* 1988; PREISS, HARTLEY and ARTAVANIS-TSAKONAS 1988). In an attempt to uncover other elements which are involved in the same cell interaction mechanism in which *Notch* participates, a genetic screen was carried out to identify genes whose products may interact with *Notch* protein (XU *et al.* 1990). Specifically, we have searched for suppressors of the lethality associated with certain heteroallelic combi-

nations of the *Abruptex* (*Ax*) alleles (WELSHONS 1971; FOSTER 1975; PORTIN 1975), a group of dominant *Notch* mutations affecting postembryonic development and associated with point mutations in the extracellular EGF-like domain of the protein (HARTLEY, XU and ARTAVANIS-TSAKONAS 1987; KELLEY *et al.* 1987). This genetic screen led to the identification of independent mutations in two autosomal loci which were demonstrated to be alleles of the two neurogenic loci *Delta* and *mastermind*. The results of the screen, along with the phenotypic interaction analyses of *Notch*, *Delta* and *mastermind*, strongly indicate that *Notch* and these two loci are integrated in the same genetic circuitry, possibly through direct protein-protein interactions (XU *et al.* 1990). Consistent with the genetic data, recent molecular analysis has indicated that the gene products of *Notch* and *Delta* may be directly interacting via their extracellular domains and that the intermolecular association between these two proteins is strong enough to promote cell aggregation (FEHON *et al.* 1990).

Besides the autosomal *Delta* and *mastermind* mutations, the genetic screen also recovered twenty three *X*-linked suppressors. Twenty-one of them are lethal *Notch* alleles, while the other two are viable alleles. In this paper we demonstrate that one of the viable *X*-linked suppressors is allelic to *deltex*, a viable mutation previously identified by MORGAN, STURTEVANT and BRIDGES in 1922. Phenotypic analyses and complementation tests of the existing *deltex* mutations indicate that they define a single complementation group on the *X* chromosome. We have further characterized the adult and the maternal effect embryonic phenotypes associated with these *deltex* alleles. By examining interactions between *deltex* and the neurogenic loci, we show that in addition to *Notch*, *deltex* also interacts with *Delta* and *mastermind*, thus suggesting a close relationship between the products of these four genes.

MATERIALS AND METHODS

Strains and crosses: Stocks were maintained and crosses were performed on a standard cornmeal-molasses-yeast-agar medium containing 0.2% propionic acid or Tegosept as mold inhibitors. All cultures were maintained at 25° unless specific temperatures were mentioned. Genetic markers and strains not specifically mentioned are found in LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987, 1990).

The origin and the meiotic recombination mapping of dx^{ENU}: The mutation (dx^{ENU}) was originally induced in the *y w* Ax^{9B2} chromosome by ENU (XU *et al.* 1990) and then was recombined onto the Ax^{E2} sn^3 chromosome. It was determined to be proximal to Ax^{9B2} mutation. The meiotic recombination position of this mutation was then determined by the following crosses: *y cv v f* virgins were mated to Ax^{E2} dx^{ENU} sn^3/Y males. The F_1 *y cv v f*/Ax^{E2} dx^{ENU} sn^3 heterozygous females were then mated to *y cv v f*/*Y* males. Three hundred F_2 male offspring from the cross were sorted according to their phenotypes. Only one out of 14 of the *y cv* sn^3/Y offspring had the new phenotype, thus the position of the new mutation(dx^{ENU}) was placed between *cv* and sn^3. Eight recombinants between *cv* and dx^{ENU} (1 *y cv* dx^{ENU} sn^3/Y and 7 $Ax^{E2}v$ *f*/*Y*) were recovered among the 184 *y* males and 116 y^+ males. The recombination distance between *cv* and dx^{ENU} was estimated between 2.7 cM (8/300) to 3.8 cM (7/184). Three $Ax^{E2}dx^{ENU}$ *v f*/*Y*, three Ax^{E2} *v f*/*Y* and three Ax^{E2} *cv v f*/*Y* F_2 males were individually mated with *y w* Ax^{9B2}/*FM7C* virgins, and only the crosses involving Ax^{E2} dx^{ENU} *v f*/*Y* males produced viable Ax^{E2}/Ax^{9B2} females. To finally prove that the *deltex* mutation is responsible for rescuing the negative complementation between Ax^{E2}/Ax^{9B2}, an independent *deltex* mutation (*dx*) was recombined onto the Ax^{E2} sn^3 chromosome, and shown to be capable of giving viable Ax^{E2}/Ax^{9B2} females (*y*Ax^{E2}*dx*/*FM7C* virgins *x y w* Ax^{9B2}/Y males).

The origins of dx, dx^{SM} *and* dx^P: The original *deltex* allele, *dx*, was isolated by virtue of its recessive wing vein phenotype which resembles the dominant phenotype of *Delta* mutations on the third chromosome and was therefore named *deltex* (delta in the *X* chromosome, MORGAN, STURTEVANT and BRIDGES 1922). dx^{SM} was a spontaneous mutation isolated in a F_2 culture of a cross between a wild-type male and a balancer female carrying a *mei-9*^{L} mutation, and was kindly provided by ABRAHAM SCHALET. A nonjumper mutation (nj^{P76}; S. N. KRISHNAN, personal communication) was also carried on the same chromosome. dx^P was isolated from the dx^{fl} stock by recombining off the Sxl^{fPb} allele from that chromosome in the following crosses (GOLUBOVSKY 1983; MAINE *et al.* 1985): a dx^{fl} *f*/*FM6* virgin was mated to a *y cho* sn^3/Y male. The F_1 dx^{fl} *f*/*y cho* sn^3 heterozygous virgins were mated to *y cho* sn^3/Y males. Individual F_2 males, which had both *deltex* and *singed* phenotypes, were tested for the ability to produce homozygous females by crossing to *FM6*/*FM7C* virgins. Two such recombinant males were recovered and gave homozygous females.

The strains and crosses for complementation tests between deltex alleles: y dx^{ENU} sn^3 virgins *x y* dx^{ENU} sn^3/Y, dx^{SM} nj^{P76} t^2v/Y, *ec dx*/*Y* or dx^P sn^3/Y males.

Ax^{E2} dx^{ENU} sn^3/FM6 virgins × Ax^{E2} dx^{ENU} sn^3/Y, *y* dx^{ENU} sn^3/Y, dx^{SM} nj^{P76} t^2 *v*/*Y* or *ec dx*/*Y* males.

dx^{SM} nj^{P76} t^2 *v*/*FM6* virgins × dx^{SM} *NJP76* t^2 *v*/*Y* or *ec dx*/*Y* males.

dx^P sn^3 virgins × *y* dx^{ENU} sn^3/Y males.

The strains and crosses for the data in Figure 1 were as follows: The crosses involving the fa^{swb} mutation were: w^a fa^{swb} virgins × *y* dx^{ENU} sn^3shY males–F_1: *y* dx^{ENU} sn^3/w^a fa^{swb} females × w^a fa^{swb}/Y males–F_2: w^a fa^{swb} dx^{ENU} sn^3/Y males × w^a fa^{swb} virgins.

The crosses involving the fa^g mutation were: *y* dx^{ENU} sn^3 virgins × fa^g *rb*/*Y* males–F_1: *y* dx^{ENU} sn^3/fa^g *rb* females × *y* dx^{ENU} sn^3/Y males–F_2: fa^g *rb* dx^{ENU} sn^3/Y males × fa^g *rb* virgins.

FM6/*FM7C* virgins × fa^g *rb* dx^{ENU} sn^3/Y males–F_1: *FM7C*/fa^g *rb* dx^{ENU} sn^3 virgins × fa^g *rb* dx^{ENU} sn^3/Y males.

The crosses involving the fa^{no} mutation were: *FM7C*/*w spl* dx^{ENU} sn^3 virgins × fa^{no} *rb*/*Y* males–F_1: *w spl* dx^{ENU} sn^3/fa^{no} *rb* virgins × fa^{no} *rb*/*Y* males–F_2: fa^{no} *rb* dx^{ENU} sn^3/Y.

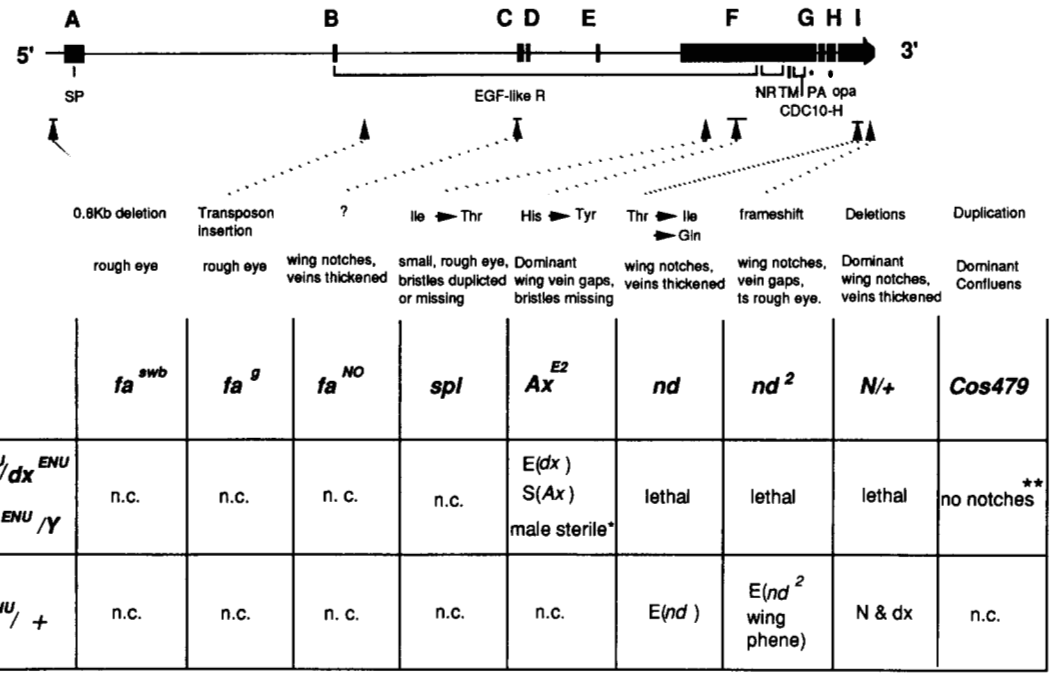
The crosses involving the *spl* mutation were: *y* dx^{ENU} sn^3 virgins × *w spl*/*Y* males–F_1: *y* dx^{ENU} sn^3/w *spl* females × *y* dx^{ENU} sn^3/Y males–F_2: *w spl* dx^{ENU} sn^3/Y males × *w spl* virgins.

FM6/*FM7C* virgins × *w spl* dx^{ENU} sn^3/Y males–F_1: *FM7C*/*w spl* dx^{ENU} sn^3 virgins × *w spl* dx^{ENU} sn^3/Y males.

The crosses involving the *Abruptex* mutations were: *y* dx^{ENU} sn^3 virgins × Ax^{E2} sn^3/Y males–F_1: *y* dx^{ENU} sn^3/Ax^{E2} sn^3 females × *y* dx^{ENU} sn^3/Y males–F_2: Ax^{E2} dx^{ENU} sn^3/Y males × Ax^{E2} sn^3 virgins.

FM6/*FM7C* virgins × $Ax^{E2}dx^{ENU}$ sn^3/Y males–F_1: *FM7C*/Ax^{E2} dx^{ENU} sn^3 virgins × Ax^{E2} dx^{ENU} sn^3 males.

y Ax^{E2} *cv vf* virgins × *ec dx*/*Y* or dx^{SM} nj^P:$t7^6$ t^2 *v*/*Y* males–



	fa^{swb}	*fa*^{g}	*fa*^{NO}	*spl*	*Ax*^{E2}	*nd*	*nd*^{2}	*N/+*	*Cos479*
dx^{ENU}/*dx*^{ENU} or *dx*^{ENU}/*Y*	n.c.	n.c.	n. c.	n.c.	E(*dx*) S(*Ax*) male sterile*	lethal	lethal	lethal	no notches**
dx^{ENU}/ +	n.c.	n.c.	n. c.	n.c.	n.c.	E(*nd*)	E(*nd*^{2} wing phene)	N & dx	n.c.

FIGURE 1.—Allelic specific interactions between *deltex* and *Notch* loci. The genomic organization of the 40-kb-long *Notch* locus is schematically shown on the top of the figure: the nine exons, labeled from A to I, give rise to a 10.2-kb mRNA, and are indicated by solid bars connected by lines representing the intronic regions. The various key domains of the corresponding *Notch* protein are denoted underneath by lines and brackets: SP, signal peptide; EGF-like R, the 36 EGF-like repeats; NR, cysteine-rich repeats present in both *Notch* and *lin-12*; TM, transmembrane domain; CDC10-H, five repeats of yeast CDC10 homologous sequence (BREEDEN and NASMYTH 1987); PA, nucleotide phosphate-binding sequence homology; opa, opa repeat (for more details see WHARTON *et al.* 1985). The approximate positions (arrows) of the molecular lesions and the phenotypes of different *Notch* alleles are also depicted. The specific *Notch* allele of the flies (either homozygous or hemizygous) is given in the top row of the figure, whereas the relevant constitution of the *deltex* locus is presented in the first column. The observed phenotypes of *Notch* allele and *deltex* mutation in the double mutant combinations are presented as: n.c., both *Notch* allele and *deltex* phenotypes are present and not changed; S, suppressed; E, enhanced. The particular phenotypes which are enhanced or suppressed are specially indicated after E or S. For example, E(nd^2 wing phene) means that only the wing phenotype of nd^2 is enhanced. Lethal = such double mutant flies are not viable; * = in addition to dx^{ENU}, dx^{SM} and *dx* were also tested; ** = the wings of these flies are never nicked, but the extra vein materials phenotype is stronger than either dx^{ENU} or *Notch* duplication (Cos479) alone. For details of strains and crosses, see MATERIALS AND METHODS.

F_1: *y* Ax^{E2} *cv v f/ec dx* or *y* Ax^{E2} *cv v f/*dx^{SM} nj^{P76} t^2 *v* females × *y* Ax^{E2} *cv v f/Y* males–F_2: *y* Ax^{E2} *dx/Y* or *y* Ax^{E2} dx^{SM} nj^{P76} t^2 *v/Y* sterile males.

F_2: *y* Ax^{E2} *cv v f/y* Ax^{E2}*dx* virgins × *FM7C/Y* males–F_3: *y* Ax^{E2} *dx/FM7C* virgins × *ec dx/Y* or *y* Ax^{9B2} sn^3*/Y* males.

The crosses involving the *nd* and nd^2 mutations were: w^a *nd* or nd^2 virgins × *y* dx^{E2} sn^3*/Y* males–F_1: w^a *nd/y* dx^{ENU} sn^3 or nd^2*/y* dx^{ENU} sn^3 virgins × *FM7C/Y* males–F_2: w^a *nd* dx^{E2} sn^3*/FM7C* or nd^2 dx^{E2} sn^3*/FM7C* virgins × w^a *nd/Y*, nd^2*/Y*, *y* dx^{ENU} sn^3*/Y*, *y* $w^a N^{5419}$*/Y*; *Cos479/+* or *FM7C/Y* males

The crosses involving the *Notch* deficiency N^{5419} were: *y* w^a N^{5419}*/FM6* virgins × *y* dx^{ENU} sn^3*/Y* males–F_1: *y* w^a N^{5419}*/y* dx^{ENU} sn^3 virgins × *FM7C/Y* males–F_2: *y* w^a N^{5419} dx^{ENU} sn^3*/FM7C* virgins × *y* dx^{ENU} sn^3*/Y*, *y* w^a N^{5419}*/Y;Cos479/+* or *FM7C/Y* males–F_3: *y* w^a N^{5419} dx^{ENU} sn^3*/Y;Cos479/+* males × *C(1)RM,y w f* virgins.

y dx^{ENU} sn^3 virgins × *y* w^a N^{5419} dx^{ENU} sn^3*/Y;Cos479/+* males.

The crosses involving the *Notch* transformant *Cos479* were: *y* dx^{ENU} sn^3 or *ec dx* virgins × *y* w^a N^{5419}*/Y;Cos479/+* males.

C(1)A, y;Cos479/+ virgins × *y* dx^{ENU} sn^3*/Y* or *ec dx/Y* males.

The strains and crosses for the data in Figure 2 were as follows: The crosses involving the *Notch* deficiency N^{5419} were: (see strains and crosses for Figure 1).

The crosses involving the Dl^{9P39} mutation were: *y* dx^{ENU} sn^3 or *ec dx* virgins × Dl^{9P39}*/TM1* males.

y dx^{ENU} sn^3sh*FM7C* virgins × Dl^{9P39}*/TM1* males.

The crosses involving the mam^{IL115} mutation were: *y* dx^{ENU} sn^3 or *ec dx* virgins × *cn bw sp* mam^{IL115}*/CyO* males.

y dx^{ENU} sn^3*/FM7C* virgins × *cn bw sp* mam^{IL115}*/CyO* males.

The crosses involving the neu^{IF65} mutation were: *y* dx^{ENU} sn^3 or *ec dx* virgins × neu^{IF65}*/TM3Ser* or Ax^{E2} sn^3*/Y;*neu^{IF65}*/TM1* males.

The crosses involving the bib^{0D05} mutation were: *y* dx^{ENU} sn^3 or *ec dx* virgins × *cn bw sp* bib^{0D05}*/CyO* males.

The crosses involving mutations of the *E(spl)* locus were: *y* dx^{ENU} sn^3 or *ec dx* virgins × $l(gro)^{X1}$*/TM6B*, $E(spl)^{BX22}$*/TM6B* and $e^4E(spl)^{E73}$ *tx/TM6B* males.

Immunocytochemistry: Embryos were dechorionated in 50% Clorox solution and fixed in a mixture of heptane and 4% paraformaldehyde for 30 min at room temperature. Vitelline membranes were removed in mass by the heptane-methanol method of MITCHISON and SEDAT (1983). Embryos were washed for 1 hr in BSN (balanced salt solution pH 6.95; 0.04 M NaCl, 0.05 M KCl, 0.01 M $MgSO_4$, 6 mM $CaCl_2$, 10 mM Tricine, 20 mM glucose, 50 mM sucrose, 0.2% bovine serum albumin with 3% normal goat serum, and 0.1% saponin) followed by overnight incubations with 1:2 dilutions of 22C10 and INV4D9 monoclonal antibodies (Mab) at 4°. Washing was performed with phosphate-buffered saline (PBS) three times followed by an additional preincubation for 1 hr in BSN. Horseradish peroxidase (HRP)-conjugated goat anti-mouse immunoglobulin G (Jackson Laboratories) was used at a 1:500 dilution in BSN

	$\frac{N^{54l9}}{+}$	$\frac{Dl^{9P39}}{+}$	$\frac{mam^{IL115}}{+}$	$\frac{neu^{IF65}}{+}$	$\frac{bib^{OD05}}{+}$	$\frac{E(spl)^{*}}{+}$
$\frac{dx}{dx}$ or $\frac{dx}{Y}$	pupal lethal	pupal lethal**	pupal lethal**	Viable, dx	Viable, dx	Viable, dx
$\frac{dx}{+}$	N & dx	Dl & dx	weak dx	wt	wt	wt

FIGURE 2.—The genetic interactions between *deltex* and zygotic neurogenic loci. The effects of mutations at different zygotic neurogenic loci on the *deltex* flies are summarized. The top row indicates the genetic constitution of the neurogenic locus and the left-most vertical column indicates the genetic constitution of the *deltex* locus. In the case of the *Notch* deficiency, only dx^{ENU} was tested. Both dx^{ENU} and *dx* were tested with the mutations from the rest of zygotic neurogenic loci. The observed phenotypes are presented as: pupal lethal, such flies die as pupae; Viable, dx, such flies are viable and exhibit *deltex* phenotypes; N & dx, such flies exhibit both *Notch* and *deltex* phenotypes; wt, no notable adult mutant phenotype is associated with these flies; * = given the complexity of the E(*spl*) region, three different mutations were used in the tests. They were a large deletion $l(gro)^{X1}$, a small deletion $E(spl)^{BX22}$ and a point mutation $E(spl)^{E73}$ (PREISS, HARTLEY and ARTAVANIS-TSAKONAS 1988). ** = about 10–15% of *y* dx^{ENU} $sn^3/Y;Dl^{9P39}/+$ flies and *y* dx^{ENU} sn^3/Y; *cn bw sp* $mam^{IL115}/+$ flies develop into adults. For details of strains and crosses, see MATERIALS AND METHODS.

for 2 hr at room temperature followed by washing in PBS. HRP reactions were performed in 0.5 mg/ml DAB and 0.015% hydrogen peroxide for 3–5 min. Anti-HRP staining was performed utilizing fluorescein conjugated antiperoxidase antibodies (Cappel). Embryos were treated as above. Incubations were performed overnight using a 1:500 dilution, followed by rinsing with PBS. Embryos staining with 4,6-diamidino-2-phenylindole (DAPI) were treated as above and incubated with 100 ng/ml DAPI in BSN for 10 min, followed by rinsing with PBS. All embryos were mounted in 2% *n*-propyl gallate in 70% glycerol.

Scanning electron microscopy: Whole flies which either crawled out of puparia by themselves or were helped out of puparia by dissection were desiccated, coated with gold-palladium, and viewed with ISISS-40 scanning electron microscope.

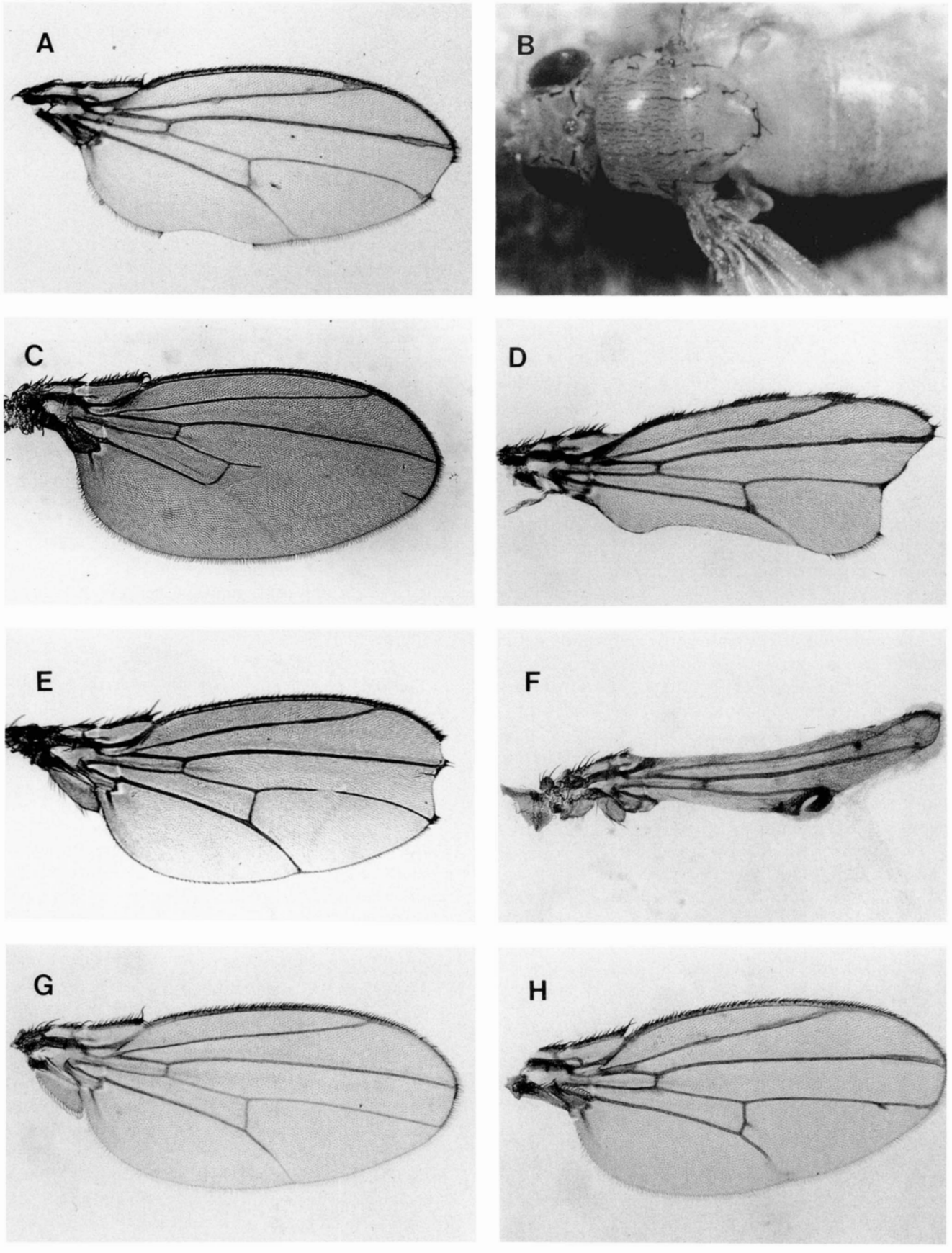
RESULTS

Mutations in *deltex* can suppress the negative complementation between *Abruptex* alleles: Recombination and complementation analysis of the mutant chromosome carrying one of the viable, *X*-linked suppressor of the Ax^{E2}/Ax^{9B2} negative complementation, revealed that this mutation affects a gene other than *Notch*. This suppressor has a recessive wing as well as an ocellar phenotype (Figure 3, A and B). Meiotic mapping placed this mutation about 3 cM proximal to *crossveinless* (*cv*) on the *X* chromosome (see MATERIALS AND METHODS). The phenotype and the map position of this mutant were consistent with it being an allele of the previously described locus *deltex* (*dx*) (MORGAN, STURTEVANT and BRIDGES 1922). Indeed, genetic complementation tests showed that the suppressor is allelic to the *deltex* (*dx*) mutation and was called *deltex-ENU* (dx^{ENU}), reflecting the fact that it was induced by nitrosoethylurea (ENU) (XU *et al.* 1990).

To test if preexisting *deltex* alleles were also capable of rescuing Ax^{E2}/Ax^{9B2} flies, the original *deltex* allele, *dx*, was recombined onto the Ax^{E2} chromosome. Since Ax^{E2} $dx/Ax^{9B2}/+$ flies were found to be viable (see MATERIALS AND METHODS), the dominant suppression of the *Ax* negative complementation is not peculiar to the dx^{ENU} allele. These observations were extended by showing that a different lethal combination of two *Ax* alleles, namely Ax^{E2} and Ax^{75C24}, could also be rescued by dx^{ENU} ($Ax^{75C24}+/Ax^{E2}$ dx^{ENU}).

Adult phenotypes of *deltex* mutations: Besides the newly induced mutation dx^{ENU}, three additional *deltex* alleles are available: *dx*, dx^{SM} and dx^{P}. All four mutations behave genetically as recessive viables and define a single complementation group on the *X* chromosome (see MATERIALS AND METHODS for complementation crosses). dx^{SM} and dx^{ENU} are both fully penetrant strong alleles. The adult phenotype associated with these *deltex* mutations consists of extra wing vein material especially at the distal ends of the wing veins (deltas), and frequently nicked wing margins and tips (Figure 3A). In addition to the wing phenotype, their

FIGURE 3.—Phenotypes and phenotypic modifications of *deltex* mutations. A, The wing of a *y* dx^{ENU} sn^3/Y male: note the notches and extra vein material along the veins. B, The ocellar region of a *y* dx^{ENU} sn^3/Y male: note the fusion of ocelli with the lack of ocellar bristles. C, The wing of an Ax^{E2} sn^3/Y male: note the wing vein gaps at the posterior ends of the fourth and fifth longitudinal veins. D, The wing of an Ax^{E2} dx^{ENU} sn^3/Y male: the phenotype (wing vein gaps) of the Ax^{E2} mutant is completely suppressed, whereas the *deltex* phenotypes are strongly enhanced. E, The wing of a nd^2 female: shows distal wing notches. F, The wing of a nd^2 dx^{ENU} sn^3/nd^2 + + female: a severe wing material loss phenotype is observed in this genetic combination. The w^a *nd* dx^{ENU} $sn^3/+$ *nd* + + flies exhibit a similar phenotype. G, The wing of a *y* dx^{ENU} $sn^3/Y;H/+$ male: note that the wing phenotype of *deltex* is completely suppressed by the *Hairless* mutation. H, The wing of a *y* dx^{ENU} $sn^3/Y;Cos479/+$ male: note that the wing nicking phenotype of *deltex* is completely suppressed by a *Notch* duplication.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ocelli are closer or are fused and the hairs and bristles in the region appear abnormal or are missing (Figure 3B). The phenotypes of *dx* are weaker than the previous two, primarily showing only extra vein material at the wing tips. Wing notchings and ocelli abnormalities occur infrequently. dx^P is the weakest allele and adults display only a weak vein phenotype at the wing tips.

All four *deltex* mutants have a weak rough eye phenotype which is not obvious under the dissecting microscope. Scanning electron micrographs revealed that eye morphology is characterized by infrequently missing or duplicated bristles as well as an irregular array of ommatidia (Figure 4, A and B). The visual ability of the *deltex* mutations was tested by subjecting dx^{ENU} flies to an edge test (LIPSHITZ and KANKEL 1985). According to this test the dx^{ENU} flies do not show any visual abnormality (data not shown).

Since some of the *deltex* flies have spread wings, these flies were subjected to a flight testing assay (CHASE 1986). The original *deltex* allele, *dx*, displays a temperature sensitive flightless phenotype. Mutant flies grown at 25° cannot fly while those grown at 18° behave normally. In contrast, dx^{ENU} and dx^{SM} flies are incapable of flying at both temperatures. Finally, the weakest *deltex* allele, dx^P, does not show any flight defect in this test.

The maternal effect embryonic defects of *deltex* mutations: Although *deltex* mutations are viable, we have found that about 40% of the eggs laid by homozygous dx^{ENU} females fail to hatch ($y\ dx^{ENU}\ sn^3/y\ dx^{ENU}\ sn^3$ virgins × $y\ dx^{ENU}\ sn^3$ males). In contrast, heterozygous dx^{ENU} females ($y\ dx^{ENU}\ sn^3/y\ cho\ sn^3$ virgins $x\ y\ dx^{ENU}\ sn^3$ males) laid normal looking eggs, and more than 99% of the eggs from such a cross hatched, indicating full viability of the homo- or hemizygous *deltex* embryos. Respectively, about 45%, 40% and 16% of the eggs laid by homozygous dx^{SM}, *dx* and dx^P females also failed to hatch ($dx^{SM}\ nj^{P76}\ t^2\ v/dx^{SM}\ nj^{P76}\ t^2\ v$, *ec dx/ec dx* or $dx^P\ sn^3/dx^P\ sn^3$). Even though the exact percentages of unhatched eggs between these *deltex* mutations are not directly comparable since each *deltex* allele is associated with different genetic background, these data indicate that *deltex* is associated with a maternal embryonic effect.

To further characterize the *deltex* maternal effect, eggs laid by homozygous dx^{ENU} females ($y\ dx^{ENU}\ sn^3/y\ dx^{ENU}\ sn^3$) were collected and examined. About 6% of the eggs were either obviously shorter than normal eggs or had defective dorsal appendages (data not shown). As revealed by cuticular preparations, the majority of the unhatched eggs (eggs that were collected and aged for more than 24 hr at 25°) did not develop any cuticular structures, whereas a small fraction of them had defective or wild-type looking cuticles (data not shown). Abnormally developed embryos could also be recognized by staining with anti-HRP antibody which is known to label neurons in Drosophila embryos (JAN and JAN 1982). Although the number of the anti-HRP-positive cells varies in such embryos, all of them had fewer stained cells than wild-type and, in addition, the anti-HRP-positive cells appeared disorganized (Figure 5, A and B). This phenotype is clearly different from the neurogenic phenotype of null mutations of any zygotic neurogenic locus, in which hypertrophy of the nervous system is reflected by a massive anti-HRP staining pattern.

Embryos from the same cross were examined with two additional markers. First, they were labeled with monoclonal antibody (Mab) 22C10, an antibody shown to recognize primarily peripheral nerves (FUJITA *et al.* 1982). As shown in Figure 5, C and D, it seems that the structures recognized by MAb 22C10 were also affected in embryos with mutant phenotype. We consistently saw that MAb 22C10 recognized fewer and apparently disorganized cells in these embryos. In order to visualize non-neuronal structures, we examined the embryos with a Mab (INV4D9) which recognizes the product of the segmentation gene engrailed (PATEL *et al.* 1989). In this case abnormally developed embryos at germband extension stage, as well as at older stages, were easily recognized by their staining pattern (Figure 5, E and F). As seen in Figure 5F, it is clear that these embryos had fewer cells expressing engrailed.

The data obtained with the three markers suggest that tissues in the affected embryos may be degenerating. Staining with DAPI, a dye which stains all nuclei, revealed that 4–5-hr embryos from homozygous dx^{ENU} females have fewer, irregularly arranged nuclei (Figure 5, G and H). As can be seen in Figure 5H, many cells in the embryo have large, abnormally shaped nuclei, suggesting they were undergoing nuclear degeneration. It was also noticed that some of the old embryos (25–30 hr at 25°) had very few DAPI-positive cells (data not shown). The DAPI staining pattern therefore is also consistent with the notion of degenerative events.

Allele-specific interactions between *deltex* and *Notch*: The dominant suppression of the negative complementation between *Abruptex* alleles by *deltex* mutations has already been described. A single copy of a *deltex* mutation has no apparent effect on a homozygous Ax^{E2} fly. For example, the genotype $Ax^{E2}\ dx^{ENU}/Ax^{E2}$ + is phenotypically indistinguishable from Ax^{E2}/Ax^{E2}. In contrast, homozygous or hemizygous *deltex* mutations completely suppress the Ax^{E2} phenotype and at the same time the *deltex* phenotype appears to be enhanced by the Ax^{E2} mutation (Figure 3, C and D). In addition, we observed that *deltex, Abruptex* double mutations have a pronounced effect on male fertility: hemizygous $Ax^{E2}\ dx^{ENU}$ males have low fertil-

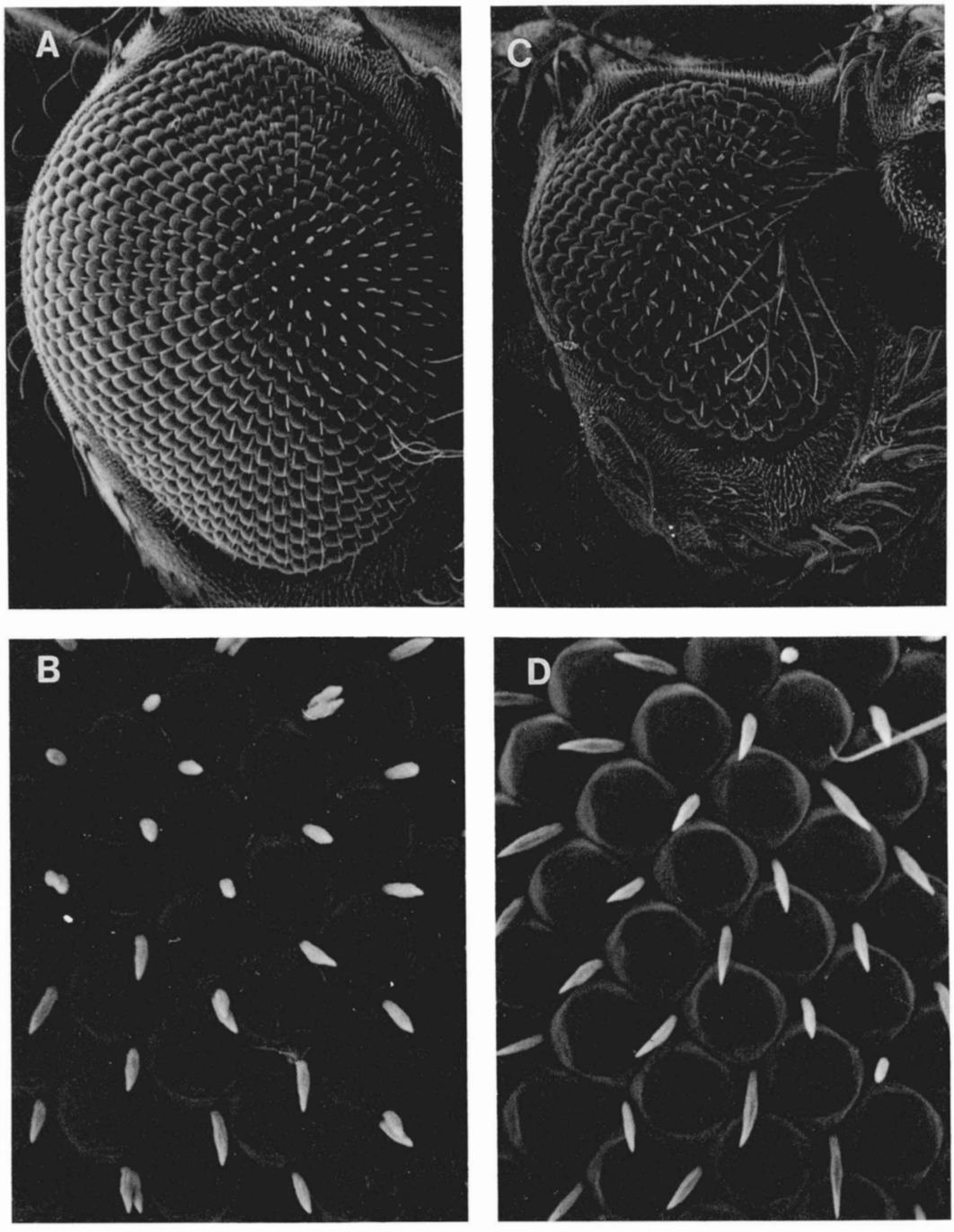
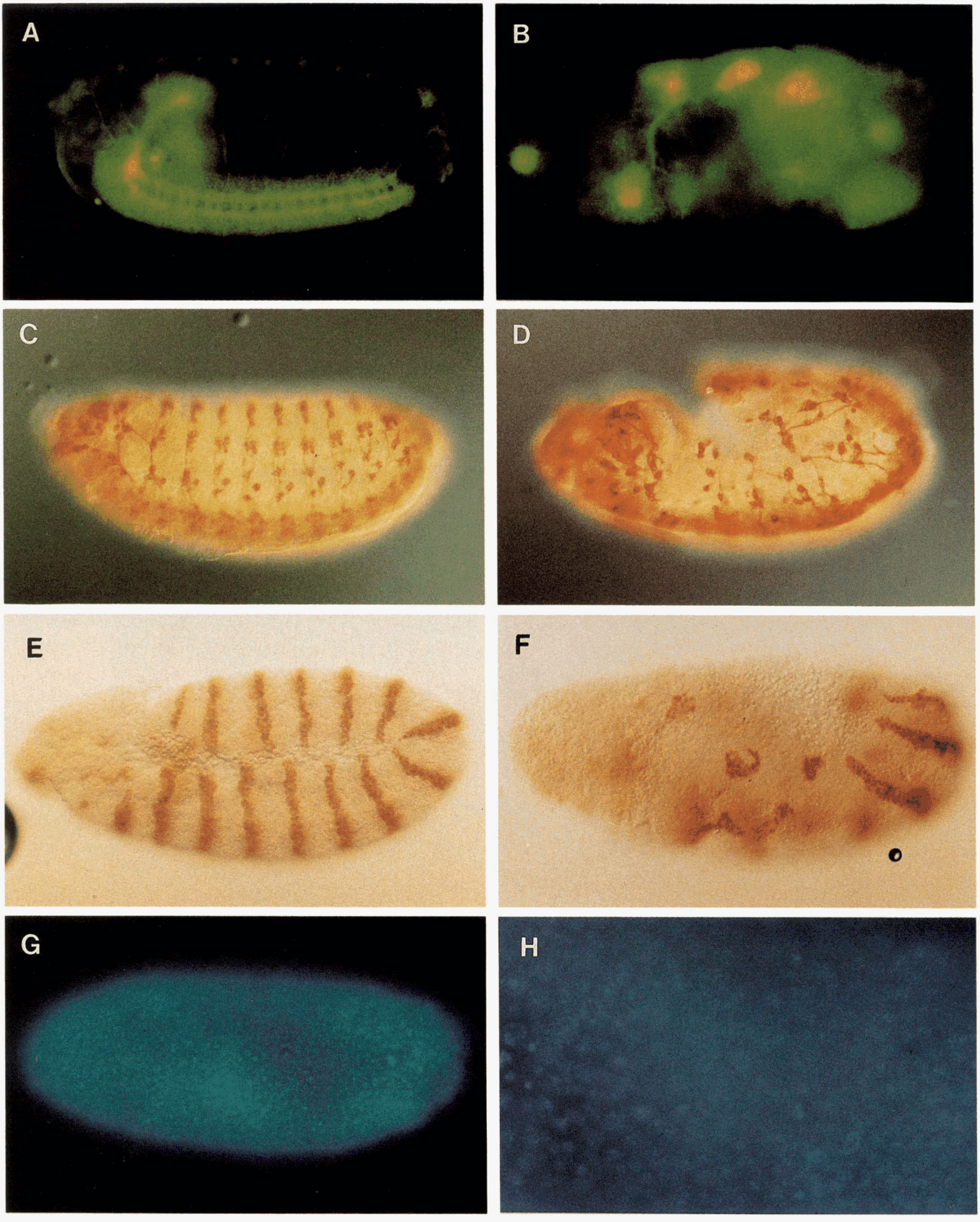


FIGURE 4.—The eye defects associated with dx^{ENU} mutant adults and with dead pupae caused by reducing *Notch* dosage in *deltex* mutants. A, An eye of $y\ dx^{ENU}\ sn^3$ female: note the relatively normal size of the eye. B, An area of the same eye in A: note the duplicated and missing brisltes between facets. C, An eye of $y\ w^a N^{54l9}\ dx^{ENU}\ sn^3/y\ +\ +\ dx^{ENU}\ sn^3$ dead female: note the size of the eye is smaller than wild type. D, An area of the same eye as in C, showing square facet array which is remincent of an ey^R mutant eye (HARTMAN and HAYES 1971).

ity while the hemizygous $Ax^{E2}\ dx$ (or dx^{SM}) males are completely sterile.

The relationship between *deltex* and *Notch* was examined further by undertaking a systematic phenotypic analysis of double mutant combinations between dx^{ENU} and various *Notch* alleles. To achieve this, *Notch* mutations affecting different domains of the gene, were recombined onto the chromosome carrying dx^{ENU} (see MATERIALS AND METHODS). Possible synergistic effects between *Notch* and *deltex* were monitored in homozygous, hemizygous, as well as heterozygous, combinations and the results are summarized in Figure 1. The top part of the figure is a schematic representation of the *Notch* gene. The approximate position, in relation to coding regions (dark bars), of the lesions associated with each of the *Notch* mutations examined, is indicated and a brief phenotypic description of each mutant is also given.

dx^{ENU} does not show any noticeable interaction with fa^{swb}, fa^g or *split*. These three mutants affect, respec-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tively, the 5′ untranscribed region, the second intron and the 14th EGF-like repeat of the *Notch* locus (KIDD and YOUNG 1986; HARTLEY, XU and ARTAVANIS-TSAKONAS 1987; KELLEY *et al.* 1987; MARKOPOULOU, WELSHONS and Artavanis-Tsakonas 1989; RAMOS *et al.* 1989), and all three have pronounced effects in the eye (WELSHONS 1965; KEPPY and WELSHONS 1975). Hemizygotes of double mutants of dx^{ENU} with fa^{swb}, fa^{g} or *split*, display both the *deltex* and *Notch* phenotypes with no significant modification (fa^{swb} dx^{ENU}/Y, fa^{g} dx^{ENU}/Y, or *spl* dx^{ENU}/Y). In contrast, a striking interaction is seen between dx^{ENU} and the two notchoid mutations. Both *nd* and nd^2 have been previously shown to be caused by mutations affecting the intracellular part of the *Notch* protein (XU *et al.* 1990). The hemizygous double mutants *nd* dx^{ENU} or nd^2 dx^{ENU} never reach adulthood despite the fact that each of the single mutants is viable. The lethal period of such double mutants has not yet been determined. Furthermore, one copy of the dx^{ENU} mutation strongly enhances the notchoid wing phenotype and visa versa (*nd* dx^{ENU}/*nd* +, nd^2 dx^{ENU}/nd^2 +, *nd* dx^{ENU}/+ dx^{ENU}, nd^2 dx^{ENU}/+ dx^{ENU}; see Figures 1 and 3, E and F). Interestingly, a *Notch* mutation, fa^{no}, which has a similar phenotype as *notchoid* alleles (WELSHONS 1965), does not show any notable phenotypic interaction with dx^{ENU}. Hemizygotes of double mutations of fa^{no} and dx^{ENU} (fa^{no} dx^{ENU}/Y) are viable and display both fa^{no} and *deltex* phenotypes.

The *deltex* phenotypes were also found to be partially suppressed by an extra copy of *Notch*. The wings of dx^{ENU} flies which also carry a duplication for the *Notch* locus (Cos479; RAMOS *et al.* 1989) are never nicked (Figure 3H), and have thickened veins. Since both *deltex* as well as supernumery copies of *Notch* confer a similar vein phenotype, it is unclear whether the phenotype is contributed by the *deltex* mutation, the extra copy of the *Notch* gene or both. The ocellar phenotype of these flies is either completely or partially suppressed. In addition we also noticed that the *Hairless* (*H*) mutation, which has been shown to interact with some of the neurogenic loci (VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985), does suppress the *deltex* adult phenotypes (wings and ocelli) in a dominant fashion while its own phenotype remains unchanged (*y* dx^{ENU} sn^3/Y; *H*/+ Figure 3G).

***deltex* interacts with neurogenic mutations *Notch*, *Delta* and *mastermind*:** Genetic analysis has shown that wild-type development is very sensitive to the dosage of the *Notch* locus: a female with three, rather than two, copies of *Notch* has a *Confluens* phenotype, consisting of irregularly thickened wing veins (WELSHONS 1965); a female with only one copy of *Notch* displays wing notching, and flies completely lacking *Notch* activity die as embryos displaying the neurogenic phenotype. We have shown that an extra copy of the *Notch* gene suppresses the *deltex* phenotype. We have further found that the haplo-insufficient behaviour of *Notch*, underlying the dominant adult wing notching phenotype, also manifests itself in the interaction between *Notch* and *deltex*.

From the crosses of double heterozygous *N* deficiency and dx^{ENU} virgins to hemizygous dx^{ENU} males (*y* w^a N^5;54^{19} dx^{ENU} $sn^3/FM7C$ × *y* dx^{ENU} sn^3/Y) or homozygous dx^{ENU} virgins to hemizygous *N* and dx^{ENU} males carrying a *Notch* duplication on their third chromosome (*y* dx^{ENU} sn^3 × *y* w^a N^{5419} dx^{ENU} sn^3/Y; *Cos479*/+), we noticed that flies homozygous for dx^{ENU} and heterozygous for the *Notch* deficiency (N^{5419} dx^{ENU}/+ dx^{ENU}) were missing from the progeny. We observed that one-third of the pupae from the first cross failed to eclose. By dissecting the pupal cases, we could not detect any obvious morphological abnormalities. The *yellow*, *singed* bristle phenotypes, the *deltex* ocellar phenotype and the dominant thoracic bristle phenotype associated with *Notch* were clearly visible in these dead pupae, indicating that they represented the missing class of the cross, *i.e.* the offspring which were heterozygous for *N* and homozygous for dx^{ENU}. A closer examination of the dead offspring heterozygous for *N* and homozygous for dx^{ENU} revealed that their eyes are significantly smaller than the wild type (Figure 4C). Scanning electron micrographs show that the small eyes bear the infrequent missing or duplication bristle phenotype of *dx* mutants. In addition, the ommitidia have a square shape instead of the hexagonal shape of wild-type eyes. Bristles are seen at each corner of a square-shaped ommitidium (Figure 4D).

The genetic screen for supressors of the *Abruptex* negative complementation has shown that in addition

FIGURE 5.—The embryoic phenotypes of eggs produced by homozygous dx^{ENU} females. A, A *y* dx^{ENU} sn^3 embryo which was produced by dx^{ENU} parents shows an anti-HRP-staining pattern that is similar to wild type. B, A *y* dx^{ENU} sn^3 embryo from the same cross which was at a similar age as the one in A, and shows abnormal anti-HRP-staining pattern: note there are fewer anti-HRP-stained cells and the stained cells are disorganized. C, A *y* dx^{ENU} sn^3 embryo which was produced by dx^{ENU} parents shows a staining pattern of Mab 22C10 that is similar to wild type. D, A *y* dx^{ENU} sn^3 embryo from the same cross which was at a similar age as the one in C, and shows an abnormal MAb 22C10 staining pattern: note the embryo has less MAb 22C10-positive cells than the one in C and, in addition, they are disorganized. E, A *y* dx^{ENU} sn^3 embryo which was produced by dx^{ENU} parents shows a staining pattern of MAb INV4D9 that is similar to wild type. F, A *y* dx^{ENU} sn^3 embryo from the same cross which was at a similar age as the one in E, and shows abnormal MAb INV4D9 staining pattern: note there are no MAb INV4D9-positive staining cells in most of the embryo. G, A 4–5-hr-old *y* dx^{ENU} sn^3 embryo which was produced by dx^{ENU} parents shows an abnormal DAPI-staining pattern: note the nuclei are disorganized. H, An area of the embryo in G showing many abnormal nuclei which may undergo degeneration.

to *deltex*, *Delta* and *mastermind* are the other two loci capable of suppressing the negatively complementing *Abruptex* alleles. We have asked whether *deltex* can interact with *Delta* and *mastermind* as well as with the remaining zygotic neurogenic loci by examining the phenotypes of double mutants. The results of this analysis are summarized in Figure 2.

No interaction was observed when *deltex* mutations were combined with mutations from the *E(spl)*, *bib* or *neu* loci (see MATERIALS AND METHODS). Interestingly however, we found that both *mam* and *Dl* behave similarly to *Notch*: namely, *deltex* mutants with only one wild type copy of *Dl* or *mam* can also lead to pupal lethality ($dx^{ENU}/Y;mam^{IL115}/+$ and $dx^{ENU}/Y;Dl^{9P39}/+$). However, unlike *deltex* females carrying one copy of a *Notch* deficiency, the lethal phenotype of *deltex* mutants carrying one copy of *Delta* or *mastermind* mutations is not completely penetrant. We found that about 10–15% of the $dx^{ENU}/Y;mam^{IL115}/+$ flies and the $dx^{ENU}/Y;Dl^{9P39}/+$ flies eclosed. In both cases, a small eye phenotype, which is weaker than the one seen in the analogous *Notch* doubly mutant combinations, was detected in the dead pupae as well as in the escaped flies. In addition, flies which are double heterozygous for *dx* and *N,Dl* or *mam* show a weak dominant *dx* phenotype. We have not yet determined whether the penetrance of the pupal lethality is dependent on the specific *Dl* or *mam* alleles. We also tested the original *dx* mutation which, as previously mentioned, is a weak *deltex* allele, with the zygotic neurogenic mutations except *Notch*. We found this allele to behave similarly to dx^{ENU} in terms of interacting with *Delta* and *mastermind*. However the number of escapers in the lethal combinations between *dx* and mam^{IL115} or Dl^{9P39} is significantly higher than the corresponding combinations with dx^{ENU}. These observations suggest, again, functional links among *Notch*, *Delta* and *mastermind*, and emphasize the sensitive dosage relationship between these loci.

DISCUSSION

A fundamental issue in the analysis of any developmental event deals with the complexity of the genetic circuitry involved in controlling the fate of cells during that event. We are concerned with the question of how many genes are part of the logic that governs neuroblast differentiation in the neurogenic region. The genetic screens of NÜSSLEIN-VOLHARD and WIESCHAUS (1980) for embryonic lethals revealed the existence of six zygotically acting genes which, in a mutant state, can confer neurogenic phenotypes (JÜRGENS *et al.* 1984; WIESCHAUS, NÜSSLEIN-VOLLARD and JÜRGENS 1984; NÜSSLEIN-VOLHARD, WEISCHAUS and KLUDIG 1984). By analogy to other groups of genes which are shown to control specific developmental events, such as the acquisition of anterior-posterior polarity in the embryo, one can argue that the neurogenic genes define elements of a developmental pathway, since they display identical mutant phenotypes. In addition to the zygotic neurogenic loci, there is a considerable number of maternally acting genes which have been shown, by virtue of specific mutant conditions, to confer neurogenic phenotypes (PERRIMON, ENGSTROM and MAHOWALD 1989; SCHÜPBACH and WIESCHAUS 1989). Thus, it is clear that the misrouting of an ectodermal cell into a neural developmental pathway can be influenced by a rather large number of genes. Conversely, a gene involved in the cell interaction mechanism in which *Notch* participates, does not necessarily have to exhibit a neurogenic phenotype. *deltex* was identified by virtue of its interaction with a neurogenic locus rather by its neurogenic phenotype, but the evidence we have gathered in the present work suggests that this gene is intimately related to neurogenic gene function.

deltex was identified during our attempts to dissect the genetic circuitry in which the neurogenic locus *Notch* is integrated because it is capable of suppressing the phenotype of the negatively complementing *Abruptex* alleles (XU *et al.* 1990). The phenotypic analysis we have carried out has shown that *deltex* is important for both embryonic and postembryonic development. The four *deltex* alleles examined here display similar phenotypes which clearly differ in severity, suggesting that at least some of them are hypomorphic alleles. None of these mutations displays a neurogenic phenotype. In this regard, it is worth emphasizing that we do not know what the amorphic phenotype of *deltex* is, since deletions uncovering the cytogenetic location of *deltex* do not exist despite the many mutagenesis screens involving the *X* chromosome. The cytological position of the *deltex* locus was previously assigned between the 6A3·4 to 6F10·11 polytene chromosome region (DEMEREC *et al.* 1942). Using duplications of the region 6C and duplications covering regions proximal to 6C we have recently shown that they do not complement any of the *deltex* alleles (unpublished results), placing the *deltex* locus in the 6A.B region. The finding that the dx^{P} mutation has a *P* element inserted in 6A is consistent with this localization (unpublished results). If we can show that this *P* element disrupts the *deltex* gene, then imprecise excisions of the element will provide us with small deletions in the region which, presumably, will be null alleles of the locus.

Even though all four *deltex* alleles are viable we have seen that the viability can be dramatically reduced by a maternal effect: a certain percentage of the eggs laid by homozygous *deltex* mothers died before hatching. Examination of the unhatched embryos from *deltex* mothers using several antibodies as specific cellular markers as well as a general nuclear marker, indicated

considerable variability of the mutant phenotype. The development of these embryos appeared to arrest at various embryonic stages, and the staining patterns obtained with all the markers revealed considerable cell death and tissue degeneration.

The genetic screen and subsequent analysis of suppressors of the pupal lethality caused by negative complementation between *Abruptex* alleles indicated a sensitive dosage relationship between *Notch* and the three suppressors *Delta*, *mastermind* and *deltex*. It was found that a negatively complementing mutant combination is rescued by simply lowering the gene dosage of *Delta* or *mastermind*. The same appears to be true for *deltex* assuming that the *deltex* alleles at hand are hypomorphic. For instance having one copy, rather than two, of *Delta* rescues the lethal *Abruptex* combination. The study reported here, involving the interaction of *deltex* mutants and mutations of the neurogenic loci revealed sensitive dosage interactions between *deltex* and *Notch*, *Delta* or *mastermind*. We found that the reduction of one dose of *Notch*, *Delta* or *mastermind* genes in *deltex* mutants led to a similar phenotype observed between negative complementating *Ax* alleles, namely pupal lethality at similar developmental stages.

Recent biochemical work has suggested a direct association between the gene products of *Notch* and *Delta*, which was one of the three suppressor genes recovered by the genetic screen. These experiments showed that the association between *Notch* and *Delta* proteins on cell surfaces is strong enough to promote the aggregation of these cells and revealed in addition that the *Delta* expressing cells are able to form aggregates between themselves or with the *Notch* expressing cells whereas the *Notch* expressing cells are only capable of aggregating with the *Delta* expressing cells (FEHON *et al.* 1990). This result suggests that *Notch* molecules may compete with *Delta* molecules, a notion consistent with the dosage sensitive relationship between the two genes revealed by genetic studies. Although there is no reason to believe that *deltex* or *mastermind* encode proteins similar to *Notch* and *Delta* proteins at this point, given the genetic data we observe, it would not be surprising if the molecular analysis of *deltex* reveals that its gene product interacts directly with all or some of these three neurogenic loci.

Besides interacting with the *Abruptex* alleles, *deltex* also interacts with other *Notch* mutations apparently in an allele specific manner. Flies homozygous for dx^{ENU} or any of the two notchoid alleles (*nd* and nd^2) alone are viable. In contrast, double mutant combinations of dx^{ENU} with any one of the notchoid alleles are lethal. However, the double mutations between dx^{ENU} and fa^{no}, which has a phenotype very similar to the *notchoid* alleles is viable. Thus, as far as one could judge from such observation, the lethality of the *deltex notchoid* double mutants is not a simple additive effect between two mutations affecting the same tissues. Both *notchoid* alleles were shown to be point mutations affecting the intracellular, carboxy-terminal part of the *Notch* protein (XU *et al.* 1990). The molecular lesion associated with fa^{no} is not known, but extrapolating from the intragenic meiotic recombination analysis, it is likely that fa^{no} affects the extracellular portion of *Notch* (YEDVOBNICK *et al.* 1985). The question of whether this lethal interaction between *deltex* and the *notchoid* mutations reflects direct molecular interactions with the intracellular part of *Notch* must await the molecular characterization of *deltex*. When considering the specificity of the *deltex*, *notchoid* interactions, it is worth noting that genetic analyses have revealed that mutations from two neurogenic loci *E(spl)* and *mam* as well as from *scabrous* and *vestigial* also enhance the notchoid phenotype (RABINOW and BIRCHLER 1990; XU *et al.* 1990).

An additional feature of the *deltex* phenotype warrants comment: we have shown that the reduction of one dose of *Notch*, *Delta* or *mastermind* genes in a homozygous *deltex* background leads to a late pupal lethality. Scanning electron microscopy of the dissected pupae has shown that the eyes of *deltex* mutants with *N* deficiency are smaller and have a square facet array which, we note, is reminiscent of the phenotype of the eyeless mutation, ey^R (HARTMAN and HAYES 1971). In ey^R, the mutant phenotype seems to be caused by the failure of proper elongation of the secondary pigment cells along the horizontal axis (READY, HANSON and BENZER 1976). The underlying cause of the phenotype is not known.

Since *deltex* mutations affect postembryonic stages and the embryonic phenotype associated with them is not neurogenic, the relationship of *deltex* to the development of the neurogenic region remains unclear. Indeed, we do not have any evidence that *deltex* plays a role in neurogenesis and one could argue that the documented relationship between *deltex* and the neurogenic genes is relevant only postembryonically. Nevertheless, our work has shown that *deltex* displays dramatic interactions with the neurogenic loci. Not only was it found to interact with *Notch* but it was also shown to display strikingly analogous interactions with the two neurogenic loci *Delta* and *mastermind*. In addition, *deltex* mutations behave, *vis à vis* to *Notch*, similarly to *Dl* and *mam* mutations. Like *Dl* or *mam* mutants, *deltex* alleles were shown to be dominant supressors of the lethality caused by negative complementation between the *Abruptex* alleles.

Interpreting the interactions of *deltex* with any one of the neurogenic loci or gaining insight into the biochemical nature of *deltex* and its role in development must await the molecular analysis of this locus.

The work reported here emphasizes the special relationship between *Notch*, *Delta* and *mastermind* suggested by previous work (XU *et al.* 1990) and indicates that *deltex* is likely to play an important role in the same genetic circuitry within which these three neurogenic loci operate.

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