A Tripartite Interaction Among Alleles of Notch, Delta, and Enhancer of split During Imaginal Development of Drosophila melanogaster

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ABSTRACT

A dramatic example of a phenotypic interaction that involves neurogenic loci during Drosophila imaginal development is the synergistic impact of split (spl), a recessive allele of the Notch locus, and E(spl), a dominant gain-of-function allele of the Enhancer of split locus, on morphogenesis of the compound eye. Screens for mutations that relieve the enhancing effect of E(spl) on spl have yielded three classes of mutations: intragenic revertants of the E(spl) allele, extragenic suppressors that are allelic to the neurogenic gene Delta (Dl) and unlinked extragenic modifiers. Analysis of the suppression of the spl-E(spl) interaction by various Dl alleles indicates that this modification is sensitive to the dosage of the Dl locus. This tripartite interaction illustrates the combinatorial action of N, Dl and E(spl) during imaginal development.

Phenotypic interactions that involve members of the zygotic neurogenic gene set suggest that products of three genes may interact directly during embryonic and imaginal development of Drosophila melanogaster. These interactions involve alleles of Notch (N, 1-3.0, 3C7), Delta (Dl, 3-66.2, 92A1-2) and Enhancer of split [E(spl), 3-89.1, 96F11-14] (Welschons 1956; Vassin, Vielmetter and Campos-Ortega 1985; De la Concha et al. 1988; Alton et al. 1989).

Comparison of the development of wild-type embryos and neurogenic mutant embryos indicates that neurogenic loci participate in the regulation of the segregation of the ectoderm into neural and epidermal lineages (Poulson 1937, Lehmann et al. 1983). Cell transplantation (Technau and Campos-Ortega 1987) and ablation (Doe and Goodman 1985) experiments imply that at least some neurogenic loci provide functions that mediate cell-cell interactions central to establishment of the epidermal lineage within the ectoderm. Molecular analyses of N (Wharton et al. 1985; Kidd, Kelley and Young 1986) and Dl (Vassin et al. 1987, Koczynski et al. 1988) imply that products encoded by these genes are structurally analogous to proteins known or believed to be involved in cell-cell communication in other organisms. Analysis of the E(spl) region (Hartley, Press and Artavanis-Tsakonas 1988) has led to the identification of another gene product that is essential for embryonic neurogenesis and that could also be involved in mediating cell-cell interactions.

The first imaginal genetic interaction to be described that involves an allele of a zygotic neurogenic gene is between mutations in N and E(spl) (Welschons 1956). As the name implies, E(spl) was originally identified on the basis of a dominant gain-of-function allele, E(spl)Dl, that leads to a severe reduction in the size of the compound eye in males hemizygous for the recessive N allele spl (spl). More recently, genetic analysis has revealed a set of pairwise interactions among alleles of N, E(spl) and Dl that affect development of the wing (Vassin, Vielmetter and Campos-Ortega 1985). The observation that adults heterozygous for loss-of-function mutations that affect any one of these three loci exhibit dominant wing phenotypes reveals the sensitivity of wing development to the dosage of each of these genes. Enhancement or suppression of the haploabnormal phenotype associated with any member of this triumvirate can apparently result from the appropriate alteration of the gene dosage of any other member of this set. The existence of these phenomena suggests that these three loci exert combinatorial effects on imaginal ectodermal development.

Embryonic interactions that involve neurogenic genes are observed in animals heterozygous for loss-of-function mutations that affect E(spl) and either N or Dl. Such animals exhibit embryonic lethality and partially penetrant neural hypertrophy even though they still possess one wild type copy of each mutated locus (Vassin, Vielmetter and Campos-Ortega 1985). This result is unanticipated because adults heterozygous for a loss-of-function mutation in any one of these loci exhibit neither reduced viability nor the embryonic neurogenic phenotype (Poulson 1937; Lehmann et al. 1983). The same study (Vassin, Vielmetter and Campos-Ortega 1985), as well as one by de la Concha et al. (1988), demonstrated that the...
phenotype of embryos hemizygous for loss-of-function mutations in \( N \) can also be modified by increasing either the zygotic dosage of \( Dl \) or the maternal dosage of \( E(spl) \). The interpretation of these studies must be qualified by noting that the duplications and deficiencies employed alter the dosage of a number of loci in addition to the specific genes in question. Evidence that we present suggests a new interpretation for previous data regarding inferred embryonic interactions between either \( N \) or \( Dl \) and rearrangements that affect \( E(spl) \).

We have isolated loss-of-function mutations that affect \( E(spl) \) in order to further characterize the transheterozygous embryonic lethal interaction between alleles of \( Dl \) and \( E(spl) \). A common method of obtaining loss-of-function mutations for a locus within which one initially possesses only dominant gain-of-function mutations involves reversion of the dominant phenotype associated with the mutation in question (Anderson, Jurgens and Nusslein-Volhard 1985). Reversion of such mutations most frequently involves inactivation of the aberrant expression of the locus and concomitant generation of a loss-of-function mutation that affects the gene of interest. To our surprise, we found that screens for mutations that lead to reversion of the dominant \( E(spl) \) double mutant phenotype yielded not only intragenic lesions that affect \( E(spl) \), but also a set of linked extragenic suppressors of this interaction. Combined genetic, cytogenetic and physical analyses indicate that these suppressor mutations are \( Dl \) alleles. These results reveal that the morphogenesis of the ectodermally derived adult eye is sensitive to the combined action of the products of \( N, Dl \) and \( E(spl) \). We also deduce that the role of the \( E(spl) \) function, per se, in heterozygous embryonic lethal interactions between mutations that affect \( E(spl) \) and either \( N \) or \( Dl \) is questionable. However, these lethal interactions may involve one or more loci that map in proximity to and are distinct from \( E(spl) \).

**MATERIALS AND METHODS**

**Drosophila stocks:** The markers employed have been described in Lindsay and Grell (1968) unless otherwise noted. A stock isogenic for a single third chromosome marked with \( E(spl)^{pl} \) (map position 89.1, Lehmann et al. 1983) and \( tx \) (map position 91.0, Lindsay and Grell 1968) was generated in our laboratory by meiotic recombination and appropriate crosses, beginning with an isogenic third chromosome carrying the \( E(spl)^{pl} \) allele derived from a stock provided by William Welshons (Iowa State University). A compound first chromosome marked with mutations in yellow, white and forked \((C(1)RM, y w f; abbreviated ywf=\) was employed in some procedures. The \( Dl \) alleles \( J \) and \( Df(3R)Cha^{mp} \) and the isogenic stock \( tx e^{+} \) to have been described in Alton et al. (1988). The balancer chromosomes \( TM6B, Hu e T b c a \) (TM6B) and \( In(3R)C, Sh e l(3)y \) (IS) and other \( Dl \) alleles are described in Alton et al. (1989) unless otherwise noted. The \( N \) allele \( 81k1 \) has been described in Grimwade et al. (1985).

**Mutagenesis:** Males from an isogenic \( E(spl)^{pl} \) \( tx \) stock were collected and mutagenized, then mated (Figure 1) to virgin females from a stock marked with mutations in Notch and ebony \( (spl^{e^{+}}) \) and brooded as described in Alton et al. (1988). The resulting first generation \( (F_1) \) male progeny \((46,100 \text{ third chromosomes from EMS mutageneses and } 24,800 \text{ third chromosomes from X-ray mutageneses}) \) that enclosed before 19 days postoviposition were scored for phenotypic reversion of the reduction in eye size associated with the \( spl/y^{pl} E(spl)^{tx e^{+}} \) genotype (Figure 2). Males partially or fully reverted for the phenotype associated with this genotype were crossed to \( y^{wf=\} \) virgin females to assess transmissibility of the revertant phenotype. Stocks isogenic for single third chromosomes of the genotype \( Dl^{+} E(spl)^{tx} \) or \( E(spl)^{-} \) \( tx \) were constructed with \( TM6B \) or \( IS \) balancers; final stocks generated were free of first chromosomes carrying the \( spl \) mutation. Allele designations (Table 1): \( BE, \) Bloomington EMS-induced; \( BX, \) Bloomington X-ray-induced; \( GE, \) Cambridge EMS-induced; \( CS, \) Cambridge Spontaneous.

**Complementation tests:** Tests for viability of specific allele combinations generally involved the mating of virgin females and males from stocks in which lethal-bearing chromosomes were balanced over \( TM6B \). Parents were mated in

![Diagram](https://via.placeholder.com/150)

**Figure 1.—Mutagenesis scheme used to recover phenotypic revertants of the \( spl/Y;E(spl)^{\circ \circ} \) \( tx/e^{+} \) double mutant phenotype. Details are given in MATERIALS AND METHODS. Asterisk indicates mutagenized chromosome.**

**Terminology:** Normal development of the compound eye leads to the establishment of a geometric array of ommatidia that serve as the primary mediators of photoreception in the adult fly (Ready, Hanson and Benzer 1976). We will use the term "facet" to refer to the external manifestation of partial or complete ommatidial development within the compound eye. We employ this less specific term in our descriptions because ultrastructural studies of the eye in adult males of the genotype \( spl/Y^{e^{+}} e^{+} \) and \( spl/Y;E(spl)^{\circ \circ} \) \( tx/e^{+} \) indicate that ommatidial development is deranged, although the facets observed by scanning electron microscopy are substantially normal in these animals (D. W. Williams, T. R. Tokarski and M. A. T. Muskavitch, unpublished data).

**Scanning electron microscopy:** Adult flies of the appropriate genotypes were collected and dehydrated serially in 80% (v/v, one wash), 95% (v/v, one wash) and 100% (v/v, three washes) ethanol in water. Specimens were then critical point dried in a Pelco model H Unit, then examined and photographed using a Cambridge Stereoscan 250 MK2 scanning electron microscope.

**Complementation tests:** Tests for viability of specific allele combinations generally involved the mating of virgin females and males from stocks in which lethal-bearing chromosomes were balanced over \( TM6B \). Parents were mated in...
vials and pupal (before day 14 postoviposition) and adult
(before day 19 postoviposition) progeny were scored for
exhibition of the Tubby+ phenotype. The appearance of
such animals indicated that a given allele pair was viable to
the presence of Stubble Tubby+ adult progeny was
taken as an indicator of viability of the allele pair. For
complementation tests involving N, crosses designed to yield
maternal contribution of the N allele were performed with
N^{814}/FM7 virgin females and E(spl)^{y}/TM6B males. Crosses
designed to yield paternal contribution of N involved
E(spl)^{y}/TM6B virgin females and N^{814}/Y;Dp(1;2)w^{+}1985/+ males (GRIMWADE et al. 1985). The presence of Bar+ Tubby+ adult progeny in the former crosses and Bar+ Curly+ Tubby+ adult progeny in the latter crosses was taken as an indication of the viability of the allele pair. A minimum of 100 adult progeny were scored for each cross. Criteria for viability of allele combinations were: lethality, less than 5% of expectation; semilethality, between 5% and 15% of expectation; viability, greater than 15% of expectation.

**Meiotic separability:** Males from stocks heterozygous for reversion-bearing chromosomes and the balancer TM6B (IS for BX23) were mated to virgin females from the yw = stock. Virgin female progeny with the Tubby+ phenotype (Stubble+ for BX23) were mated to males of the genotype spl/Y in bottles. Male progeny of this cross were scored for occurrence of the reduced eye phenotype associated with the spl/Y:E(spl)^{y} genotype (Figure 2B). If the phenotypic reversion were due to a modifying mutation extragenic to the E(spl) locus, then we predicted that meiotic recombination would lead to separation of the modifying mutation from the E(spl)^{y} allele and subsequent expression of the severe double mutant phenotype in the progeny. Since we could reliably detect only one product resulting from such recombination events, we calculated the apparent separability of the modifying mutation from E(spl)^{y} as a fraction equal to 2n^v/n^t in which n^v equals the number of males exhibiting the spl/Y:E(spl)^{y} phenotype and n^t equals the total number of males scored. A minimum of 600 male progeny were scored for each reversion-bearing chromosome. Since the separability value approximates the meiotic map distance between E(spl) (map position 89.1) and the modifying locus, we anticipated a value on the order of 0.23 for modifying mutations within Dl (map position 66.2). Conversely, intragenic reversion of the E(spl)^{y} allele (or a modifying mutation mapping very close to the locus) would yield a separability approximating zero. Those alleles listed in Table 1 for which separability values of <0.01 are given yielded no apparent recombinants among over 600 male progeny scored in each case.

**Phenotypic criteria:** Phenotypic reversion of the spl/Y; E(spl)^{y} tx eye phenotype was classified as: weak, 50–150 facets/eye; moderate, 150–250 facets/eye; strong, 250–350 facets/eye or full, 350–450 facets/eye. Terminal cuticular phenotypes were assessed in mixed populations of embryos resulting from brooding of balanced stocks of the genotype Dl- E(spl)^{y} tx/TM6B, for extragenic suppressors, or E(spl)^{y} tx/TM6B, for apparent intragenic revertants. Phenotypes for embryos homozygous for mutated Dl alleles were classified as weak hypomorph, intermediate hypomorph or amorph as described in ALTON et al. (1988). Phenotypes of embryos homozygous for mutated E(spl) alleles were classified as: mild, equivalent to the weak hypomorph class of Delta+ phenotypes; moderate, equivalent to the intermediate hypomorph class of Delta+ phenotypes; and severe, corresponding to loss of ventral, lateral and the majority of dorsal cuticle (only two small patches of unfused postero-dorsal cuticle are usually observed).

**Other procedures:** Drosophila culture conditions, methods of cytological analysis, meiotic mapping of Delta+ wing phenotypes, cuticular preparations and direct immunofluorescence analysis of embryo whole mounts have been described in ALTON et al. (1988).

**RESULTS AND DISCUSSION**

**Identification of two classes of phenotypic revertants:** Hemiogyosity for the spl mutation (spl/Y, Figure
The alleles listed were isolated in our laboratory with the exception of R1 and R2 (Lehnmann et al. 1983) and 8D06 (Jurgens et al. 1984). Cytological analysis of the E(spl) alleles listed above yielded the following cytogenetic definitions: BE22, BE25, BX22 and BX37 [normal]; BX21 [T(2;3)4;96F10-12 (Preiss, Hartley and Artavanis-Tsakonas 1988)]; BX23 [not determined]; BX36 [Df(3R)86F2;97B1]; R1 [Df(3R)86F2;97A associated with Inv(3R)97A;99C (ibid.)]; R2 [Df(3R)96F5;97A9 (ibid.)]. 8D06 [Df(3R)96F;97A3-4 (ibid.)].

* W = weak, M = moderate, S = strong, F = full; see MATERIALS AND METHODS for criteria for each class.

† Neurogenic phenotype of embryos homozygous for the allele listed [each Dl allele was linked to E(spl) and Dl] in a cross in which the Dl allele is contributed maternally; L = lethal viability (≤50% of expectation), V = viable (viability >65% of expectation); see MATERIALS AND METHODS for a description of the crosses performed.

∞ Approximate frequency of separation of the E(spl) allele from any modifying mutation on the third chromosome; see MATERIALS AND METHODS for procedures and interpretation.

† Correlated molecular lesion defined within the Dl locus (Kopczynski et al. 1988).

‡ Associated with a chromosomal translocation.

2A) leads to a reduction in the size of the eye to approximately 370 facets (Table 2) and to distinct disorganization of the facet array and duplication of some interommatidial bristles. In comparison, the eye of a wild-type adult male (Figure 2D) contains approximately 600 facets (Table 2) in a regular geometric array. The dominant gain-of-function allele E(spl) fails to affect the size of the eye (Table 2) or the regularity of the ommatidial array in males (Figure 2C) or females carrying only wild-type N alleles. However, males of the genotype spl/Y;E(spl) exhibit eyes (Figure 2B) that consist of only 45 facets (Table 2). This interaction is sufficiently strong that the split− phenotype is expressed in a pseudodominant fashion in females of the genotype spl+/E(spl) (Figure 2H). The eye in such females is reduced in size to that of a spl/Y male (Table 2) and is similarly disorganized. These quantitative and qualitative data illustrate the synergistic effect of the spl and E(spl) mutations on the development of the compound eye.

We utilized this synergistic phenotype as the basis for a screen in which we isolated a set of 34 revertants of the spl/Y;E(spl) phenotype (Figure 1, Table 1). The extent of phenotypic reversion varies widely between marginal improvements in the phenotype and complete reversions of the spl/Y;E(spl) phenotype to one approximating the spl/Y phenotype. Parallel effects are exerted on the pseudodominant
expression of the split− phenotype observed in females heterozygous for spl and E(spl)Δ (data not shown). Analyses described below indicate that 28 of these apparent reversions were correlated with third chromosomeal mutations. We also obtained a set of six dominant modifiers of the spl-E(spl)Δ interaction that map to the second chromosome; these will not be considered below.

Genetic lesions correlated with reversion of the spl/Y;E(spl)Δ tx/eΔ* phenotype fall into two groups based on phenotypes observed in animals heterozygous for the mutated chromosome and a wild-type third chromosome. Class I mutations (21 alleles) yield wing venation phenotypes similar to those associated with heterozygosity for Dl mutations, and class II mutations (seven alleles) yield wild type wing venation. Complementation tests reveal that chromosomes that carry class I mutations are invariably lethal when heterozygous with Dl3 (Table 1) and, with one exception (BX43), lethal or semi-lethal when heterozygous with Df(3R)ChaM9 (data not shown). When contributed paternally, chromosomes that carry class II mutations are each viable over both of these Dl alleles (Table 1). These phenotypic properties and complementation patterns raise the possibility that a significant fraction of the phenotypic reversions are the result of mutations that affect Dl, not E(spl).

**Dl is a modifier of the spl-E(spl)Δ interaction:** The first apparent reversion of the E(spl)Δ mutation we identified resulted from the class I mutation CS20 (Figure 2F), which arose spontaneously during the construction of the isogenic E(spl)Δ tx stock. Meiotic mapping of the mutation responsible for the wing phenotype associated with the CS20-bearing chromosome in relation to the third chromosome markers spineless (ss, map position 58.5), ebony (e, map position 70.7) and rough (ro, map position 91.1) revealed that the dominant wing venation phenotype associated with CS20 maps to a position 62% distal from ss within the ss-e interval, corresponding to approximate map position 66 (58 recombinants within the ss-e interval were assessed). Parallel mapping of Dl3 revealed that the associated wing venation defect maps at a position 64% distal from ss within the ss-e interval, corresponding to approximate map position 66 (50 recombinants within the ss-e interval were assessed). Males carrying recombinant chromosomes obtained during the CS20 meiotic mapping experiment were mated to spl; E(spl)Δ virgins to determine the cosegregation frequency of the dominant wing phenotype and the apparent reversion of the spl/Y;E(spl)Δ tx/eΔ* phenotype. Within a set of 11 recombinant chromosomes tested (four yielding DeltaΔ− phenotypes and seven yielding DeltaΔ+ phenotypes), phenotypic reversion of the spl/Y;E(spl)Δ tx/eΔ* phenotype correlated exactly with the dominant wing phenotype that maps on the CS20 chromosome. These data strongly suggest that the phenotypic reversion observed for the CS20 chromosome reflects an extragenic suppression of the spl-E(spl)Δ interaction by a mutation within Dl.

We then determined the frequency with which each of the other putative suppressing class I mutations could be separated from the E(spl)Δ mutation by meiotic recombination (Table 1). We found that 18 of the 21 chromosomes carry suppressing mutations that separate from the E(spl)Δ mutation with an average frequency of 0.24 ± 0.02 (±SE, n = 18). Cytogenetic analysis of the three chromosomes that exhibit very low frequencies of separation (BX38, BX41 and BX43) reveals that each has suffered a translocation involving one breakpoint within the 92A interval. Since the meiotic map distance between Dl and E(spl)Δ is approximately 23 map units and the cytological location of Dl is 92A1-2, these data are consistent with the hypothesis that class I mutations are Dl alleles.

Molecular analysis of class I mutations reveals that at least nine suppressing mutations are correlated with physical alterations within Dl (Table 1; KOPCZYNSKI et al. 1988). Each of these 21 chromosomes yields a terminal embryonic phenotype in homozygotes that involves the neural hypertrophy and reduction of the epidermis characteristic of loss-of-function mutations in zygotic neurogenic loci (LEHMANN et al. 1983). Dl is the only known neurogenic locus that is located at a distance from E(spl)Δ that would be consistent with the meiotic separability exhibited by the majority of suppressing class I mutations.

Finally, we find that the suppression of the spl-E(spl)Δ interaction by class I mutations can be mimicked by the deficiency DlM2 (Table 2) and other Dl alleles tested (data not shown). While a number of independent amorphic Dl alleles (3, BE32, Df(3R)bxΔ110 and Df(3R)ChaM9; ALTON et al. 1988) tested act as suppressors of this interaction, a set of independent hypomorphic Dl alleles assayed (BE34, ibid.; BX8 and HD82, ALTON et al. 1989) do not. These results imply that suppression of the spl-E(spl)Δ interaction is dependent on the degree of reduction in the function of the Dl locus and is not necessarily mediated by a specific class of Dl alleles.

In contrast to a previous report (KNUST et al. 1987), we find no alleles of the neurogenic locus **neutralized** (neu, 3-50.5, 86CD) among 21 independent class I mutations we have isolated. Each of the class I mutations we have characterized is viable in heterozygous combination with the amorphic allele neuΔ655 (LEHMANN et al. 1983) (data not shown).

Genetic analyses in *Drosophila* and other organisms have demonstrated that phenotypic interactions between mutations in independent loci often identify genes that encode functionally related products. Some mutations that fail to complement β-tubulin mutations
map to α-tubulin genes (Stearns and Botstein 1988) or loci that appear to encode products involved in microtubule function (Regan and Fuller 1988), and numerous interactions can be defined among loci known or believed to encode products involved in the structure and function of muscle (Park and Horvitz 1986, Homyr and Emerson 1988). Such intergenic interactions may be allele-specific or dosage-dependent for different pairs of loci, respectively. The interactions we observe among N, Dl and E(spl) mutations may therefore reflect the functional relatedness of products derived from these genes during imaginal development.

Intragenic reversion of E(spl) 

All class II mutations are viable in transheterozygous combinations with Dl2 or Df(3R)Cha AG when either of these Dl alleles is contributed maternally (Table 1). This suggests that class II mutations are genetically distinct from the class I mutations. All attempts to recover the E(spl) allele from each of the class II revertant chromosomes by meiotic recombination failed (Table 2), which implies that these mutations are intragenic revertants of E(spl).

The BX21 mutation is associated with a translocation that affects the chromosomal interval 96F10-12, while the BX36 mutation is correlated with a large deficiency (96F1;97B1) that removes this same interval (Table 1). The cytogenetic analysis of the previously isolated E(spl) mutations R1, R2 (Lehmann et al. 1983) and 8D06 (Jurgens et al. 1984) indicates that each of these alleles is correlated with an extensive deficiency that affects the same region of the chromosome (Table 1). Therefore, two of the class II mutations, as well as these three previously identified alleles, are correlated with rearrangements that affect the chromosomal interval within which E(spl) maps (Ziemer et al. 1988; Preiss, Hartley and Artavanis-Tsakonas 1988). Complementation tests indicate that BX21, BX22 and BX36 each fail to complement groucho (gro, map position 90) a recessive mutation that appears to be an allele of E(spl) (ibid., Knust et al. 1987). These combined data indicate that these three class II mutations are alleles of E(spl).

Inter se complementation analysis among the seven newly isolated class II mutations and three preexisting E(spl) alleles reveals the existence of a number of qualitatively distinct alleles that affect E(spl) (Figure 3). The chromosome bearing BX37 is homozygous viable and viable over all other mutations tested. BE22 and BE25 are similarly viable over all other mutations tested, but the chromosomes bearing each of these alleles are lethal in homozygotes. These homozygotes exhibit a mild neurogenic phenotype (Table 1). We have not yet determined whether this phenotype results from some partial impairment of the wild-type E(spl) function or from accessory lethal mutations on each chromosome. Certain pairwise combinations of alleles BX21, BX22 and BX23, which yield neurogenic phenotypes in homozygotes ranging from mild to moderate (Table 1), exhibit inter se complementation behavior that varies depending on which allele is contributed maternally. The BX23 BX21 allele pair exhibits impaired viability only when BX23 is contributed maternally, and the BX22 BX23 allele pair exhibits lethality only when BX22 is contributed maternally. The latter case can be explained by invoking the maternal effect that has been reported to be associated with E(spl) (Knust et al. 1987) and noting that BX22 is a more severely affected allele than BX23 (Table 1). The explanation for the former case may be analogous if the variegation associated with the translocation BX21 is such that BX21 can be less severely affected than BX23 in a significant fraction of females in the crosses performed. Allele BX36 behaves exactly as the preexisting deficiencies R1, R2 and 8D06. It is viable in combination with BX37, BE22 and BE25 and lethal when heterozygous with all other alleles tested. The alleles we have isolated therefore range from a putative exact revertant (BX37) to complete loss-of-function mutations.

We note that the severity of the neurogenic phenotype differs for independent mutations each of which appears to affect the E(spl) locus (Table 1). BX22 has been correlated with a 14-kilobase (kb) deletion and an immediately adjacent 14-kb inversion in distal 96F (Preiss, Hartley and Artavanis-Tsakonas 1988). Embryos homozygous for this mutation exhibit a moderate phenotype. BX36, and the other large, multiband deficiencies that remove the locus, yield a severe phenotype in homozygotes involving an
even greater reduction in the epidermis than that associated with amorphic \textit{Dl} mutations.

Other authors have argued that the \textit{E(spl)} locus is genetically complex and is comprised of multiple functions that map within the distal portion of cytological interval 96F (Ziemer et al. 1988) based, in part, on the observation that large deficiencies that affect 96F yield more severe neurogenic phenotypes than smaller deficiencies that affect 96F. We observe the same trend. However, \textit{BX22} eliminates or severely truncates the DNA that encodes each of six transcripts; among these six, only two, which appear to have the same coding capacity and arise from a single transcription unit (Hartley, Preiss and Artavanis-Tsakonas 1988), have been directly demonstrated to play a role in embryonic neurogenesis to date (Preiss, Hartley and Artavanis-Tsakonas 1988). The more severe neurogenic phenotype associated with larger deficiencies must therefore reflect the elimination of an independent locus or loci, in addition to those affected by \textit{BX22}, that also affect ectodermal differentiation and map(s) in close proximity to the \textit{E(spl)} locus.

Vital interactions among \textit{N}, \textit{Dl} and mutations that affect \textit{E(spl)} may not involve \textit{E(spl)}, \textit{per se}: Given the reported maternal-dependence of the heterozygous lethal interactions that occur among \textit{N}, \textit{Dl} and \textit{E(spl)} (Vasslin, Vielemetter and Campos-Ortega 1985), we conducted complementation tests among alleles of these loci by performing each cross in two directions: paternal or maternal contribution of each \textit{E(spl)} allele (Table 3). We found that \textit{BX36} and other large deficiency alleles of \textit{E(spl)} are semilethal or lethal in heterozygous combination with either \textit{Dl}\textsuperscript{'} or \textit{Df(3R)ChaM9} when the \textit{E(spl)} allele is maternally contributed to zygotes. The extent of the reduction in viability is correlated with the extent of the reduction in \textit{Dl} function in these crosses (Table 4). Fully penetrant lethality is observed only when the \textit{Dl} allele present is one of a number of amorphic alleles tested. This result implies that this lethal interaction is dependent on relative gene dosage and is not mediated by a specific class of \textit{Dl} alleles. We do not, however, observe a significant reduction in the viability of heterozygotes that carry any of the other \textit{E(spl)} mutations tested, independent of the direction of the cross (Table 3).

We have also analyzed the allele- and maternal-dependence of the lethal phenotypic interaction observed in zygotes heterozygous for mutations that affect \textit{N} or \textit{E(spl)}. We find that heterozygotes that carry the amorphic mutation \textit{N}\textsuperscript{81kl} exhibit reduced viability only when they also carry a third chromosomal mutation that involves extensive deletion of material encompassing \textit{E(spl)} (e.g., \textit{BX36}, \textit{R1}, \textit{R2} or \textit{8D06}), just as we observe for the \textit{Dl-E(spl)} interaction.

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<th>\textit{Dl}\textsuperscript{'} male</th>
<th>\textit{Dl}\textsuperscript{'} female</th>
<th>\textit{Df(3R)ChaM9}\textsuperscript{+} male</th>
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\textbf{TABLE 3}

Viability* of animals heterozygous for \textit{E(spl)} and \textit{Dl} or \textit{N} mutations

Heterozygotes were generated by crossing sets of flies, from balanced stocks, heterozygous for different single neurogenic gene mutations; see MATERIALS AND METHODS for a description of the crosses performed. Crosses involving the alleles \textit{Dl}\textsuperscript{'} and \textit{Df(3R)ChaM9} were performed in both polarities, i.e., the \textit{Dl} mutation was contributed either paternally ("Dl\textsuperscript{'} male") or maternally ("Dl\textsuperscript{'} female").

* Viability: = lethal, -s = semilethal, + = viable; see MATERIALS AND METHODS for numerical criteria for different complementation behaviors.

Neurogenic phenotype of animals homozygous for the \textit{E(spl)} mutation listed: \textit{wt} = wild type; \textit{M1} = mild; \textit{MOD} = moderate; \textit{SEV} = severe. Criteria for the various phenotypic classes listed are described in MATERIALS AND METHODS.

Identical results were obtained in crosses in which the \textit{N}\textsuperscript{81kl} allele was contributed paternally, as described in MATERIALS AND METHODS.

The \textit{BX22} mutation is not lethal in heterozygous combination with \textit{N}\textsuperscript{81kl}, nor are any of the remaining \textit{E(spl)} alleles that have been tested. Crosses that involve \textit{N} mutations and mutations that affect \textit{E(spl)} also reveal that the reduction in viability observed is the same for a given allele pair independent of the direction of the cross in which zygotes are generated. We do observe a marginal number of heterozygous escapers in some crosses involving maternal contribution of large deficiency alleles of \textit{E(spl)} and no escapers in crosses involving maternal contribution (data not shown). However, crosses in both directions yield levels of survival below our lethal criterion (5% of expectation). It appears that the combinatorial zygotic requirement for \textit{N} and functions that map in proximity to \textit{E(spl)} is such that neither the maternal contributions from \textit{N} nor those from loci in the vicinity of \textit{E(spl)} can compensate for the deficit in zygotic expression of these loci.

Neither the \textit{N} nor the \textit{Dl} mutations we have tested are lethal in heterozygous combination with \textit{BX22}, yet some of these same mutations are lethal when heterozygous with large deficiencies that affect \textit{E(spl)} (Table 3). Lethality is therefore observed only when functions in addition to those affected by \textit{BX22} are eliminated from the chromosome. This genetic behavior may reflect the synergistic impact of reduction in the level of \textit{N} or \textit{Dl} function and the function of \textit{E(spl)} and flanking loci, since the \textit{BX22} lesion presum-
ably affects a function or functions essential for E(spl) activity (PREISS, HARTLEY and ARTAVANIS-TSAKONAS 1988). However, we currently favor the more conservative interpretation that the observed heterozygous lethality reflects the combinatorial requirement for products of E(sp1) region (KNUST, TIETZE and CAMPOS-ORTEGA 1987; PREISS, HARTLEY and ARTAVANIS-TSAKONAS 1988). Data from these analyses that are relevant to the interpretation of some of our results are summarized in Figure 4. It is clear that the mutation E(spl)P deletes portions or the entirety of genomic sequences that contribute to at least six distinct transcripts. Within the interval defined by the BX22-associated deletion, only the product that is encoded by the m9/m10 transcription unit and is homologous to β-transducin (HARTLEY, PREISS and ARTAVANIS-TSAKONAS 1988) has been directly shown to be essential for embryonic neurogenesis to date (PREISS, HARTLEY and ARTAVANIS-TSAKONAS 1988). PREISS, HARTLEY and ARTAVANIS-TSAKONAS (1988) have shown that a transposon that contains the intact m9/m10 transcription unit, but no other intact transcription units, can rescue the lethality associated with heterozygosity for the E(spl)P allele and deficiencies that affect the E(spl) region. However, it is not yet known whether the product of this transcription unit, as affected within the context of the E(spl)P chromosome, is sufficient to mediate enhancement of the imaginal splitphenotype.

We observe that heterozygotes that carry certain

### TABLE 4

<table>
<thead>
<tr>
<th>Allele</th>
<th>Embryonic phenotype</th>
<th>Trans-heterozygotes as fraction of total progeny (n)</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD9δ</td>
<td>W</td>
<td>0.37 (242)</td>
<td>+</td>
</tr>
<tr>
<td>BE33δ</td>
<td>W</td>
<td>0.34 (326)</td>
<td>+</td>
</tr>
<tr>
<td>BE31δ</td>
<td>W</td>
<td>0.30 (307)</td>
<td>+</td>
</tr>
<tr>
<td>9K23δ</td>
<td>W</td>
<td>0.21 (136)</td>
<td>+</td>
</tr>
<tr>
<td>6B37δ</td>
<td>I</td>
<td>0.22 (140)</td>
<td>+</td>
</tr>
<tr>
<td>HD40δ</td>
<td>I</td>
<td>0.19 (167)</td>
<td>+</td>
</tr>
<tr>
<td>BE38δ</td>
<td>I</td>
<td>0.10 (370)</td>
<td>+</td>
</tr>
<tr>
<td>HD82δ</td>
<td>I</td>
<td>0.06 (217)</td>
<td>+</td>
</tr>
<tr>
<td>HD62δ</td>
<td>I</td>
<td>0.04 (189)</td>
<td>+</td>
</tr>
<tr>
<td>BE32δ</td>
<td>A</td>
<td>0.04 (317)</td>
<td>+</td>
</tr>
<tr>
<td>Df(3R)Dl27δ</td>
<td>A</td>
<td>0.04 (179)</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>0.04 (145)</td>
<td>+</td>
</tr>
<tr>
<td>Df(3R)Ckα49R</td>
<td>A</td>
<td>0.02 (194)</td>
<td>+</td>
</tr>
<tr>
<td>Df(3R)Dl26R</td>
<td>A</td>
<td>0.02 (106)</td>
<td>+</td>
</tr>
<tr>
<td>Df(3R)DlHD26R</td>
<td>A</td>
<td>0.01 (227)</td>
<td>+</td>
</tr>
<tr>
<td>9D27f</td>
<td>A</td>
<td>0.01 (202)</td>
<td>+</td>
</tr>
<tr>
<td>9M46f</td>
<td>A</td>
<td>0.01 (179)</td>
<td>+</td>
</tr>
<tr>
<td>Df(3R)bxδ1179f</td>
<td>A</td>
<td>&lt;0.01 (184)</td>
<td>+</td>
</tr>
<tr>
<td>9P34f</td>
<td>A</td>
<td>&lt;0.01 (155)</td>
<td>+</td>
</tr>
</tbody>
</table>

*Trans-heterozygotes were generated by crossing virgins of the genotype Df(3R)E(sp1)P/kD to males of the genotype Df(3R)E(sp1)P/kTM6B as described in MATERIALS AND METHODS.*

1. Neurogenic phenotype of embryos homozygous or hemizygous for the Dl allele listed: W = weak hypomorph, I = intermediate hypomorph, A = amorph. See MATERIALS AND METHODS for criteria for different complementation behaviors.

2. The maximum fraction of the first generation progeny expected to be trans-heterozygotes was 0.33 for each cross as performed for this analysis. n = total number of progeny scored from a given cross.

3. Viability: + = lethal, = = semilethal, = = viable; see MATERIALS AND METHODS for numerical criteria for different complementation behaviors.

4. YEDVOBNICK et al. (1985).

5. ALTON et al. (1988).

6. LEHMANN et al. (1983).
mutations in N or Dl and mutations that affect E(spl) are inviable when the mutation that affects the E(spl) region deletes a large chromosomal segment, but are viable if the E(spl) mutation present is BX22 (Table 3). This result implies that the lethal interactions observed between N or Dl mutations and large E(spl) deficiencies cannot strictly reflect the combinatorial requirement for N or Dl and any of the functions eliminated by BX22. Therefore, if future analyses indicate that one or more of the functions affected by BX22 are sufficient to mediate enhancement of the split phenotype, as we might expect based on the extent of reversion associated with the BX22 mutation (Table 1), then the lethal interactions we and others observe will not reflect the specific combinatorial requirement for N or Dl and the E(spl) gene. Based on this reasoning, we currently favor the hypothesis that these lethal interactions reflect the combinatorial requirements for N or Dl and functions other than E(spl) that map in proximity to the E(spl) locus.

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**FIGURE 4.**—The molecular map of a chromosomal interval within the E(spl) region. This figure summarizes portions of the molecular data presented by Knust et al. (1987) (reference A) and Preiss, Hartley and Artavanis-Tsakonas (1988) (reference B). The coordinate system is that utilized in reference B, kb, kilobases. The chromosome that carries the E(spl) mutations possesses a deletion (del) of approximately 0.4 kb of chromosomal DNA and an insertion (ins) of approximately 5 kb of DNA in comparison to wild type chromosomes within the intervals indicated by the rightward cross-hatched boxes. The E(spl)BX22 allele is associated with a deletion of approximately 14 kb of chromosomal DNA indicated by the leftward cross-hatched box. The chromosomal segment indicated by the solid bar labelled “EB transposon” has been employed to fully or partially rescue certain mutant phenotypes associated with the E(spl) mutation (reference B). The maximum extents of chromosomal intervals that have been shown to cross-hybridize with particular embryonic transcripts are indicated by open boxes below the coordinate scale. The correspondences between transcript data presented in the two source references (A and B) are: m5, 1.0 kb (A), 1.4 kb (B); m6, 1.4 kb (A), 1.5 kb (B); m7, 1.5 kb (A), 1.5 kb (B); m7 (not reported in A), 1.2 kb (B); m8, 1.0 kb (A), 1.2 kb (B); m9, 3.5 kb (A), 3.6 kb (B); m10, 4.4 kb (A), 4.0 kb (B); m11, 2.1 kb (A), 2.2 kb (B).

**LITERATURE CITED**


Kidd, S., M. W. Kelley and M. W. Young, 1986 Sequence of the Notch locus of Drosophila: relationship of the encoded


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