Genetic Analysis of *Enhancer of split*, a Locus Involved in Neurogenesis in *Drosophila melanogaster*

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ABSTRACT

Enhancer of split (*E(spl)*), one of the neurogenic loci of Drosophila, is uncovered by the deletion $Df(3R)E(spl)^{R\text{-}B251}$ with breakpoints at 96F8 and 96F13. We describe here the results of a genetic analysis of this chromosomal interval. Thirty-one mutations in genes of this region were recovered during various programs of mutagenesis. In addition, we included the spontaneous mutations $E(spl)^{D}$ and *groucho* (*gro*), which are known to map to this region, in our study. These 33 mutations define four lethal complementation groups, one of which includes $E(spl)^{D}$ and *gro*. Mutations of the *E(spl)* group behave as complementing and noncomplementing pseudoalleles, defining different functions. Alleles are classified according to their complementation behavior in two different ways: with respect to their viability as heterozygotes with other lethal alleles and with respect to *gro* and to $E(spl)^{D}$. The phenotypes of these mutations and the pattern of heteroallelic complementation speak in favor of a considerable genetic complexity of the *E(spl)* locus.

THE neurogenic (NG) genes of *Drosophila melanogaster* are involved in the process of segregation of neural and epidermal lineages (POULSON 1937; LEHMANN *et al.* 1981, 1983; DIETRICH and CAMPOS-ORTEGA 1984; HARTENSTEIN and CAMPOS-ORTEGA 1986). Loss of function of any of the NG genes leads to the commitment of all NG ectodermal cells to the neural fate. Results of different embryological studies indicate that cell-cell interactions mediate the separation of these two cell lineages in grasshoppers (DOE and GOODMAN 1985) and fruit flies (TECHNAU and CAMPOS-ORTEGA 1986, 1987). The available data suggest that some of the products of the NG genes participate in a process of cell communication, by means of providing a regulatory signal necessary for epidermogenic commitment of the NG ectodermal cells. Particularly noteworthy are experiments in which cells from the NG region of NG mutants were isochronically transplanted into the NG region of the wild type; these experiments distinguish the function of *Enhancer of split* [*E(spl)*], as being required in the receiving cell, from those of the remaining NG genes, which appear to be necessary to provide a source for the regulatory signal (TECHNAU and CAMPOS-ORTEGA 1987).

LEHMANN *et al.* (1983) found that *E(spl)* is a NG locus. The locus received its name from the allele $E(spl)^{D}$ (recovered by M. GREEN; see LINDSLEY and GRELL 1968; KNUST *et al.* 1987). This is a dominant allele that interacts with *split* (*spl*), an allelomorph of *Notch* (*N*), which is another NG gene (WELSHONS 1956, 1965). LEHMANN *et al.* (1983) induced revertants of the $E(spl)^{D}$ phenotype and found that such revertants frequently correspond to *E(spl)* loss-of-function mutations. The latter were found to be embryonic lethal and to cause the neuralizing syndrome, *i.e.*, they lead to a neural fate for all the cells of the NG ectoderm. The $E(spl)^{D}$ allele has effects on neurogenesis that are opposite to those of its revertants, and it therefore was assumed to represent a gain-of-function state of a gene that controls the separation of epidermal and neural lineages (KNUST *et al.* 1987).

We present here results suggesting that *E(spl)* is actually a complex of interrelated gene functions, rather than a single gene. We report on the genetic organization of the chromosomal interval 96F8 to 96F13, which is uncovered by $Df(3R)E(spl)^{R\text{-}B251}$ (KNUST *et al.* 1987) and includes the locus of *E(spl)*. The results described below suggest that several genetic functions are related to *E(spl)*, and that the role that this gene plays in neurogenesis requires the participation of more than one of these genetic functions.

MATERIALS AND METHODS

Strains: Strains of *Drosophila melanogaster* were raised on standard medium. *spl* is described in LINDSLEY and GRELL (1968); see RESULTS for a description of *groucho* (*gro*). Balancer chromosomes used were *TM1, Me* and *TM3, Ser Sb* (see LINDSLEY and GRELL 1968). $Df(3R)E(spl)^{8D06}$ was induced in a *st e* chromosome with EMS (JÜRGENS *et al.* 1984). The remaining mutations were recovered either in a *st e* chromosome as lethals over $Df(3R)E(spl)^{R\text{-}B251}$ or in an $E(spl)^{D}$ chromosome as revertants of the enhancing effect upon *spl* (LEHMANN *et al.* 1983; KNUST *et al.* 1987). Oregon R served as wild-type strain.

Isolation of lethals over $Df(3R)E(spl)^{R\text{-}B251}$: Flies of both sexes (*st e*, 0–24 hr after hatching) were collected and

irradiated with 5000 rad (X-rays, 1250 rad/min, 0.3 mm Al filter). Two days after irradiation the females were discarded and the irradiated males were mated for 4 days to virgin females of the genotype *TM1/TM3, Ser*. Single males of the F_1 generation, carrying an irradiated third chromosome over *TM3, Ser Sb*, were mated to 2–4 *e* $Df(3R)E(spl)^{R\text{-}B251}/TM1, Me$ virgins. The progenies of these single pair matings were screened for lethality of the mutagenised *st e* chromosome over *e* $Df(3R)E(spl)^{R\text{-}B251}$. Chromosomes carrying lethals were kept balanced over *TM1, Me*.

Another group of *st e* flies of both sexes (0–24 hr after hatching) were collected, starved for 4 hr in empty vials and transferred to vials containing pieces of filter paper impregnated with a solution of 5% sucrose, 50 mM KPO_4 (pH 6.8) and 30 mM ethyl methane sulfonate (EMS; see LEWIS and BACHER 1968). After 24 hr the flies were transferred to vials with standard food for 2 days. Males were then separated and mated to virgin females homozygous for *th st cp in ri* p^P. As in the previous protocol, the male progeny were then mated singly to 2–4 virgins of the genotype *e* $Df(3R)E(spl)^{R\text{-}B251}/TM1, Me$, and lethals over *e* $Df(3R)E(spl)^{R\text{-}B251}$ were isolated and established as balanced stocks for further study.

All mutations recovered as lethal when heterozygous with $Df(3R)E(spl)^{R\text{-}B251}$ were generically called *l(3)R-B251*, whereas each individual mutation of this group is named by a protocol number, *e.g., l(3)B93, l(3)L11*, etc.

Reversion of $E(spl)^D$: Revertants of $E(spl)^D$ were obtained by irradiating (same conditions as above) homozygous e^s $E(spl)^D$ males and crossing them to homozygous *spl* females, as described previously (LEHMANN *et al.* 1983; KNUST *et al.* 1987). In addition, the same protocol was used to attempt reversion of $E(spl)^D$ by treating e^s $E(spl)^D$ males with 30 mM EMS using the procedure of LEWIS and BACHER (1968). Revertants were detected on the basis of a conspicuous reduction, or even a complete abolition, of the enhancement of the *spl* phenotype that is normally caused by one copy of $E(spl)^D$ in the genome, *i.e.*, in revertants, the eyes were larger than in their non revertant siblings. Putative third chromosomal revertants were used to establish single lines by crossing them to *TM1/TM3, Sb Ser*; they are designated *R* followed by a protocol number.

Complementation analysis: Strains carrying *l(3)R-B251* mutations were tested by crossing to other *l(3)R-B251* mutations, to *E(spl)* alleles previously recovered as $E(spl)^D$ revertants, and to $E(spl)^D$, *gro* and $Df(3R)E(spl)^{8D06}$. To quantitatively assess semi-lethality, which was observed in several cases, 250–400 adult progeny of each cross were classified according to genotype and the difference between the number of expected segregants and those actually observed was determined. To assess the reliability of these determinations, several controls were carried out by crossing females carrying second and third chromosomal mutations, seemingly unrelated to *E(spl)*, with males carrying one of the mutations under study. We used *l(2)ff225a cn bw* (a second chromosomal lethal affecting compound eye development; see CAMPOS-ORTEGA 1980), *l(3)Me109* (FERRUS and GARCIA-BELLIDO 1976), *Df(3R)red, mwh jv trd* and $Df(3)ry^{75}, kar^2, ry^{75}$ (LINDSLEY and GRELL 1968), and crossed them with *l(3)B48, gro* and $E(spl)^D$. Differences between expected and observed segregants in the control crosses were determined as above.

Preparation of embryos: Embryos were staged according to criteria described in CAMPOS-ORTEGA and HARTENSTEIN (1985). Cuticle preparations were made with fully developed embryos, generally older than 48 hr, following the procedure of VAN DER MEER (1977). Stainings were made on whole mounts at stages 14–16, either with an antibody against horseradish-peroxidase (anti-HRP, purchased from Sigma) following the procedure described by JAN and JAN (1982), or with fuchsin according to ZALOKAR and ERK (1977). The genotypes of the mutant embryos were inferred from the fractions of animals that expressed abnormal phenotypes.

RESULTS

Lethals over $Df(3R)E(spl)^{R\text{-}B251}$ and other *E(spl)* mutations

Mutagenesis of *st e* sperm, using either X rays or EMS, led to the recovery of 19 recessive lethals over $Df(3R)E(spl)^{R\text{-}B251}$, called *l(3)R-B251* mutations (Tables 1 and 2). The lethality caused by 15 of them was fully penetrant over any of six available *E(spl)* deletions (alleles *R1, R2, R-B251, R23.1, R-A7.13.2* and *8D06*, listed in Table 2); four of the mutations (*l(3)L5, l(3)B103, l(3)B105* and *l(3)B115*) produced a variable number of escapers when heterozygous with individual deletions (Table 1).

In addition to the *l(3)R-B251* lethals, a number of other mutations were available for study. First of all, we used the dominant mutation $E(spl)^D$. The reader is referred to KNUST *et al.* (1987), for a discussion of the phenotypic traits associated with $E(spl)^D$, its relationships to *spl*, and the effects of increasing the ploidy of $E(spl)^+$.

Second, we studied a total of eleven *E(spl)* loss-of-function mutations, which have been recovered over the years as revertants of the enhancing effect upon *spl* of $E(spl)^D$, that is, by mutating the $E(spl)^D$ allele (using the protocol of LEHMANN *et al.* 1983; see KNUST *et al.* 1987). Eight of these eleven revertants have already been described in KNUST *et al.* (1987); the other three were induced more recently. All extant loss-of-function *E(spl)* alleles that were recovered as revertants of $E(spl)^D$ were induced by X-ray mutagenesis. No *E(spl)* loss-of-function mutations were recovered as revertants of $E(spl)^D$ following mutagenesis with EMS among 5×10^4 mutagenised chromosomes (this work), although several *Dl* and *neu* alleles, among others, were recovered as EMS-induced, second-site $E(spl)^D$ revertants (our unpublished observations; see KNUST *et al.*, 1987 concerning X-ray-induced second-site $E(spl)^D$ revertants). Second-site revertants can be distinguished from *E(spl)* loss-of-function mutations in that they map to other chromosomal positions and in that the former are, at least partially, viable when heterozygous with *E(spl)* deletions, whereas the latter are generally lethal. Among the eleven revertants studied, *R-H2.1* gave rise to a few escapers in heterozygosity with some of the deletions.

Third, we also studied the allele $Df(3R)E(spl)^{8D06}$, recovered by JÜRGENS *et al.* (1984) from EMS-treated

chromosomes as an embryonic lethal affecting epidermal development. This mutation is actually associated with a deletion having breakpoints at 96F7 and 97A6 (this study).

Fourth, we used the mutation *groucho* (*gro*), which behaves genetically as a recessive visible, hypomorphic mutation of *E(spl)* (KNUST *et al.* 1987). $E(spl)^D/gro$ flies are phenotypically wild type. This observation supports the allelic relationship of these two variants, for normally 45–50% of the $E(spl)^D/+$ flies show a mild roughening of the compound eyes. Since $E(spl)^D$ is a gain-of-function mutation (KNUST *et al.* 1987), the complementation of its dominant effects over the wild-type allele by *gro* suggests that *gro* is a weak *E(spl)* loss-of-function mutation (see next section). Homozygosity for *gro* produces defects at the supraorbital border that vary between increased epidermal pigmentation (penetrance 100%) and prominent bushes of supraorbital bristles (penetrance approximately 25%). This latter trait can be increased to 70–80% penetrance by selection. Other phenotypic traits of homozygous *gro* flies are rather erratic in their appearance and largely dependent on particular lines. For example, the number of palpal vibrissae is frequently increased, and there are notches and protrusions at the wing margin and other defects in the wing veins. No increased embryonic lethality was found to be associated with homozygosity for *gro* (Table 2). Although there is only a single *gro* allele available, the described phenotypic traits must be due to *gro*, rather than to background effects not related to this mutation, since individual, or all, of these phenotypic traits have been observed in flies heterozygous for *gro* with any of many different *E(spl)* mutations of various origins (see below).

Complementation analysis of *l(3)R-B251* and *E(spl)* mutations

***Df(3)E(spl)$^{R\text{-}B251}$* uncovers four lethal complementation groups:** Crosses were carried out between flies of the 19 *l(3)R-B251* strains, the 11 revertants of $E(spl)^D$, and the alleles $Df(3R)E(spl)^{8D06}$, *gro* and $E(spl)^D$ (Tables 1 and 2). As a first step, lethality or viability of the corresponding transheterozygous flies was scored (Table 1). Background effects could be readily excluded whenever the studied mutations had been induced in different genetic backgrounds, or following different experimental protocols, *i.e.*, as $E(spl)^D$ revertants in $E(spl)^D$ chromosomes, and as EMS and X-ray-induced lethals in *st e* chromosomes. In other cases, however, *e.g.*, when crossing flies from the same genetic background, these effects could not be excluded. Since *E(spl)* is maternally expressed (VÄSSIN, VIELMETTER and CAMPOS-ORTEGA, 1985; KNUST *et al.* 1987; KNUST, TIETZE and CAMPOS-ORTEGA, 1987), most of the crosses were reciprocal in order to detect possible maternal effects. Slightly different results were known to occur concerning crosses that involve *E(spl)* deletions (KNUST *et al.* 1987). With the exception of crosses between *F4.4* and *gro* (see below), no major difference was found in reciprocal crosses involving the newly induced *l(3)R-B251* mutants and thus, no further distinction was made between the results of such reciprocal crosses.

Two different mutations that were lethal with complete penetrance when heterozygous with each other were taken as members of the same complementation group; a third mutation was considered to be a member of the same group when it was completely lethal when heterozygous with at least one of the other two mutations. Semi-lethality was observed in some cases. As described in the MATERIALS AND METHODS section, the percentage of dead progeny from each cross was calculated indirectly, by means of determining the difference between the numbers of expected and actually observed adult segregants. To assess semilethal effects, control crosses were carried out in which second and third chromosomal lethals, apparently unrelated to *E(spl)*, were crossed with some of the mutations under study. The numbers of expected and observed segregants were found to differ in less than 4% in the progeny of 13 different control crosses; only in one case (progeny from the cross *mwh jv trd/TM1* × $E(Spl)^D/E(spl)^D$) was this difference greater (about 20%). Thus, in order to account for possible background effects we allowed in our experiments a larger safety margin and considered differences between segregants larger than 40% as indicative of partial lethality.

Using these criteria, four different lethal complementation groups, C1–C4, were defined. Two of these groups (C1 and C3) are represented by single alleles (*l(3)B103* and *l(3)B105*, respectively), C2 comprises two alleles (*l(3)A3* and *l(3)B17*), whereas C4 comprises 22 alleles (including *gro* and $E(spl)^D$). The allele *l(3)L5* was found to be fully lethal only when heterozygous with *R2* (a cytologically visible deletion; KNUST *et al.* 1987); escapers developed as heterozygotes with any of several of the other mutations (Table 1). Therefore, *l(3)L5* could not be unambiguously classified as lethal allele of any of the four complementation groups. Although none of the alleles of the C1–C3 groups was completely lethal when heterozygous with alleles of the C4 group, a rate of semilethality ranging between 40% and 60% of the progeny was found in some of the crosses between C1–C3 and C4 alleles. In a few cases, *e.g.*, crosses of the allelomorphs *l(3)A3* or *l(3)B17* with the translocation *R14.8*, or of *l(3)B105* with *l(3)B48*, semilethality was higher, between 70% and 80% (Table 1).

Heteroallelic complementation of lethality permits to distinguish different types of C4 alleles: Mutations of the C4 group exhibited a complex pattern

TABLE 1

Complementation matrix of *E(spl)* and *l(3)R.B251* mutations[a]

	8D06	*R1*	*R2*	*R23.1*	*R-B251*	*R-A7.13.2*	*R-A7.1*	*R14.8*	*R-C1.4c*	*R-F4.4*	*L11*	*L9*	*B37*	*B48*	*B93*	*B88*	*B7*	*R-F6.2*	*A4*	*B12*	*B94*	*B95*	*B102*	*B128*	*B115*	*R-H2.1*	*L5*	$E(spl)^D$	*gro*	*A3*	*B17*	*B103*	*B105*
8D06	+																															
R1	+	+																														
R2	+	+	+																													
R23.1	+	+	+	+																												
R-B251	+	+	+	+	+																											
R-A7.13.2	+	+	+	+	+	+																										
R-A7.1	+	+	+	+	+	+	+																									
R14.8	+	+	+	+	+	+	+	+																								
R-C1.4c	+	+	+	+	+	+	+	+	+																							
R-F4.4	+	+	+	+	+	+	+	+	+	+																						
L11	+	+	+	+	+	+	+	+	+	+	+																					
L9	+	+	+	+	+	+	+	+	+	+	+	+																				
B37	+	+	+	+	+	+	+	+	+	+	+	+	+																			
B48	+	+	+	+	+	+	+	+	+	+	+	+	+	+																		
B93	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+																	
B88	+	+	+	+	+	+	+	+	+	+	+	+	66	+	+	+																
B7	+	+	+	+	+	+	+	+	+	92	+	+	50	+	+	60	+															
R-F6.2	+	+	+	+	+	+	76	82	73	65	−	−	−	−	−	−	53	+														
A4	+	+	+	+	+	+	−	−	−	nd	−	nd	50	52	91	44	57	nd	+													
B12	+	+	+	+	+	+	−	48	−	nd	−	nd	−	67	47	77	63	nd	69	+												
B94	+	+	+	+	+	+	−	71	−	nd	47	nd	42	−	+	+	40	nd	54	69	+											
B95	+	+	+	+	+	+	−	44	−	nd	46	nd	−	75	−	−	52	nd	+	+	49	+										
B102	+	+	+	+	+	+	−	58	−	nd	−	nd	42	nd	44	+	71	nd	+	+	54	+	+									
B128	+	+	+	+	+	+	−	60	60	nd	63	nd	+	−	nd	58	+	nd	nd	−	nd	nd	nd	+								
B115	+	+	96	+	+	+	50	75	59	nd	52	nd	+	53	94	nd	+	nd	−	−	83	42	73	nd	+							
R-H2.1	+	98	+	+	77	+	57	50	−	97	56	−	−	−	50	60	84	89	nd	nd	nd	nd	nd	nd	nd	+						
L5	nd	97	+	−	96	nd	40	−	82	−	nd	nd	83	85	nd	nd	85	57	nd	nd	−	nd	67	70	66	−	+					
$E(spl)^D$	+	+	+	+	+	+	92	70	60	91	−	74	73	60	54	88	+	70	−	−	−	−	−	−	nd	72	−	8[b]				
gro	90	94	86	76	94	93	−	82	87	93[c]	69	75	−	−	67	69	−	−	−	−	−	−	−	−	nd	−	−	−	−			
A3	+	+	+	+	+	+	−	78	−	nd	−	nd	52	66	58	50	56	nd	54	43	56	53	−	nd	−	nd	−	nd	nd	+		
B17	+	+	+	+	+	+	−	79	−	nd	nd	nd	43	43	43	44	71	nd	−	40	55	51	68	−	50	nd	nd	−	−	+	+		
B103	+	+	83	+	+	+	−	44	−	nd	−	nd	56	−	43	52	50	nd	40	59	48	48	−	nd	65	nd	−	nd	nd	55	50	+	
B105	+	+	+	+	+	60	40	−	−	nd	−	nd	−	82	52	44	67	nd	48	42	40	−	45	nd	nd	nd	42	nd	nd	42	40	−	+

[a] The table indicates lethality or viability of the progeny of crosses between the mutants under discussion. Females are indicated in the ordinate. +, 100% lethality; −, completely viable; nd, not determined; numbers indicate percentage of lethality.
[b] Lethals from homozygous $E(spl)^D$ parents (KNUST *et al.* 1987).
[c] Crosses made at 18°.

of heteroallelic complementation with respect to their viability in crosses between different alleles (Table 1). Two main types of alleles could be distinguished. The first type comprises seven alleles: *R1, R2, 8D06, R23.1, R-B251, R-A7.13.2* (carrying cytologically visible deletions; refer to Table 2), and *l(3)B7* (EMS induced). All of them are either partially or totally lethal when heterozygous with any of the remaining lethal mutations of the C4 group. The second type comprises alleles that are fully viable when heterozygous with at least one of the other lethal C4 alleles. With the exception of the seven alleles of the previous type, all other mutations of the C4 complementation group belong to this category. The alleles of the second type can be further subdivided in two classes. One comprises variants that are totally or partially lethal when heterozygous with *R-A7.1*, and the other class comprises the remaining six variants (*l(3)A4, l(3)B12, l(3)B94, l(3)B95, l(3)102, l(3)128*), which are fully viable as heterozygotes with *R-A7.1* (Table 1).

Allele *R-F4.4*, a cytologically normal $E(spl)^D$ revertant, represents a somewhat exceptional case, for it was found to be (totally or partially) lethal in all crosses in which it was involved, with the exception of crosses with *l(3)L5*; in addition, as already mentioned, allele *l(3)L5* could not be unambiguously classified, since it was either fully viable or produced escapers in heterozygosity with all other mutations, with the exception of the deletion *R2*.

C4 alleles can be classified in four subgroups with respect to *gro* and $E(spl)^D$: Still another classification of C4 alleles was possible after studying progenies of the crosses with *gro* and $E(spl)^D$ (Table 2). In these cases we did not consider lethality or viability only, but also other phenotypic traits associated with these latter two mutations, *e.g.*, the various defects described above for *gro* homozygotes, as well as roughening of the compound eye, dominant embryonic lethality and maternal effects, which are associated with $E(spl)^D$ (KNUST *et al.* 1987). Allelism of *gro* and *E(spl)* mutations (mentioned in the previous section) was further supported by the crosses with the other alleles of the C4 group (Table 2). All *E(spl)* mutations associated with chromosomal aberrations were found to cause poor viability when heterozygous with *gro*; estimated lethality ranged from 75 to 94% of the transheterozygotes. Approximately 90% of the transheterozygous progeny of *R-F4.4* mothers and *gro* fathers died when the animals developed at 18°–20°; many escapers developed at higher temperatures (25°–29°) (Tables 1 and 2). In the reciprocal cross (*gro* mothers and *R-F4.4* fathers), the viability of

TABLE 2

Pattern of complementation of *E(spl)* alleles

Allele	Lethality with $E(spl)^D$ (%)	Lethality with *gro* (%)	Penetrance with *gro*[a] (%)	Cytology	Origin	Isolated as
R1	100	94	100	inv+del	X rays	rev
R-B251	100	94	95	del	X rays	rev
R-A7.13.2	100	93	100	inv+del	X rays	rev
8D06	100	90	95	del	EMS	Lethal
R2	100	86	100	del	X rays	rev
R23.1	100	76	95	del	X rays	rev
R-F4.4	91	40–93[b]	100	Normal	X rays	rev
B88	88	69	70	Normal	EMS	Lethal
L9	74	75	71	Normal	X rays	Lethal
R14.8	70	82	100	trans	X rays	rev
R-C1.4c	60	87	90	trans	X rays	rev
B93	54	67	47	Normal	EMS	Lethal
L11	–	69	80	trans	X rays	Lethal
B7	100	–	0	Normal	EMS	Lethal
R-A7.1	92	–	11	Normal[c]	X rays	rev
B37	73	–	52	Normal	EMS	Lethal
R-H2.1	72	–	1	Normal	X rays	rev
R-F6.2	70	–	0	Normal	X rays	rev
B48	60	–	10	Normal	EMS	Lethal
L5	–	–	13	inv+del	X rays	Lethal
A4	–	–	0	nd	EMS	Lethal
B12	–	–	0	nd	EMS	Lethal
B94	–	–	0	nd	EMS	Lethal
B95	–	–	0	nd	EMS	Lethal
B102	–	–	0	nd	EMS	Lethal
B128	–	–	0	nd	EMS	Lethal
B115	nd	nd	nd	nd	EMS	Lethal
$E(spl)^D$	8[d]	–	0[e]	Normal	spont	
gro	–	–	100	Normal	spont	

[a] Refers to the penetrance of the visible *gro* phenotype in heterozygosity with the indicated alleles.
[b] Lethality of 45% at 25° and 93% lethality at 18°; *F4.4* provided by mothers in both cases.
[c] Carries a 34-kb DNA deletion, cytologically not visible.
[d] Number of dead embryos directly determined from egg collections of homozygous $E(spl)^D$ parents (KNUST *et al.*, 1987).
[e] $E(spl)^D$*gro* is phenotypically wild-type (see text).
–, No significant lethality detectable; del, deletion; inv, inversion; trans, translocation; rev, isolated as revertant of $E(spl)^D$; lethal, isolated as lethal over *R-B251* [except *8D06* (JÜRGENS *et al.*, 1984)]; spont, spontaneous; nd, not determined.

transheterozygous animals was apparently not affected. The viability of the mutations *l(3)L9* and *l(3)L11* was also strongly impaired when heterozygous with *gro*. Escapers from these crosses exhibited the *gro* phenotype at a very high level of penetrance and expressivity. *R-A7.1/gro* animals were fully viable and showed the *gro* phenotype in only 11% of the transheterozygotes. Other alleles uncovered some of the phenotypic traits associated with *gro* in a variable number of cases (Table 2). However, several other mutations (*e.g., R-F6.2, R-H2.1, l(3)B7, l(3)A4, l(3)B12, l(3)B94, l(3)B95, l(3)B102* and *l(3)B128*) complemented the visible phenotype of *gro*. Some of the latter alleles were also fully viable when heterozygous with $E(spl)^D$ (Table 2). We should point out that these latter mutations were actually found to behave over $E(spl)^D$ like the wild-type allele: as mentioned above, the dominant expression of $E(spl)^D$ when heterozygous with the wild-type causes 45–50% of the heterozygous progeny to exhibit mild roughening of the compound eyes.

On the basis of these results, four subgroups of alleles can be distinguished among the members of the C4 group. The first subgroup consists of alleles that complement the visible phenotype of *gro* and are poorly viable when heterozygous with $E(spl)^D$, *e.g., R-F6.2, R-H2.1, l(3)B7*; alleles *R-A7.1* and *l(3)B48* represent intermediate cases, in that they complement *gro* almost completely and are poorly viable over $E(spl)^D$. The second subgroup comprises alleles *l(3)L11* and *l(3)L5*, which behave like the wild-type when heterozygous with $E(spl)^D$ and uncover with incomplete penetrance some of the phenotypic traits of *gro*. The third subgroup comprises alleles that complement *gro* and behave like the wild-type when heterozygous with $E(spl)^D$ (*e.g., l(3)A4, l(3)B12, l(3)B94, l(3)B95, l(3)B128*). Finally, the fourth subgroup comprises all remaining alleles, *i.e.*, those

which complement neither *gro* nor $E(spl)^D$ (see Table 2).

Phenotypes of *l(3)R-B251* and other *E(spl)* mutations

To study the embryonic phenotypes of the mutants, cuticle preparations of putative homozygotes for each of the *l(3)R-B251* mutations were made. In addition, stainings of mutant embryos were carried out with an anti-HRP antibody that in insects recognizes a neural antigen (JAN and JAN 1982).

Only deletions lead to severe, fully penetrant neural hyperplasia: The most conspicuous phenotypic abnormality exhibited by *E(spl)* loss-of-function mutants is the neural hyperplasia that results from the neuralization of the NG ectoderm (LEHMANN *et al.* 1983). Homozygotes for any of the revertants, with the exception of *R-H2.1*, as well as for the alleles *8D06*, *l(3)L9* and *l(3)L11*, develop neural hyperplasia (Table 3; Figure 1). However, expressivity and penetrance are variable for the different mutants. Neuralization is severe and fully penetrant in homozygotes for six of these eleven revertant chromosomes, that is, *R1*, *R2*, *R-B251*, *R-A7.13.2*, *R23.1* and *R-A7.1*, as well as in homozygotes for *8D06*; all of these chromosomes carry deletions of the region under study. We cannot distinguish chromosomal aberrations in the polytene chromosomes of *R-A7.1*; however, this variant has been characterized at the molecular level and found to carry a deletion of about 34 kb of DNA in the *E(spl)* region (KNUST, TIETZE and CAMPOS-ORTEGA 1987). It is noteworthy that, although following the terminology of LEHMANN *et al.* (1983) the phenotype of most *R-A7.1* homozygous embryos is extreme, this phenotype is not as severe as that of embryos homozygous for any of the larger, cytologically visible chromosomal deletions; on the other hand, *R-A7.1* embryos with weaker phenotypes occur frequently (see examples of severe and weak neural hyperplasia of *R-A7.1* embryos in Figure 1, B and C). Embryos homozygous for either of the two translocations *R-C1.4c* and *R14.8* exhibit a neuralized phenotype with incomplete penetrance (approximately 70% and 40%, respectively, of the putative homozygotes) and rather erratic expressivity, between weak and intermediary [Figure 1E; see LEHMANN *et al.* (1983) for criteria to classify the neural hyperplasia]. Only a few (5–10%) of the putative homozygotes for the revertants *R-F4.4* and *R-F6.2* and for the X-ray-induced variants *l(3)L9* and *l(3)L11* exhibit weak neuralization, as shown by anti-HRP stainings (Figure 1, D and F).

Most of the embryonic progeny of *l(3)B7*/+ crossed to itself are phenotypically wild type. However, 5–8% of these embryos exhibit hypoplasic defects in the ventral cord, particularly a lack of some of the connectives and fusion of commissures (Figure 2). We do not know whether the defective embryos are homozygous for *l(3)B7*. However, we predict that these defects are the same as those found among the progeny of $E(spl)^D$ (KNUST *et al.* 1987) due to the similarities between both alleles (see below).

TABLE 3

Phenotypes of *E(spl)* alleles

Allele	Neural hyperplasia	Effect on *spl*
R1	Extreme	Suppression
R-B251	Extreme	Suppression
R-A7.13.2	Extreme	Suppression
8D06	Extreme	Suppression
R2	Extreme	Suppression
R23.1	Extreme	Suppression
R-A7.1	Extreme	Suppression
R14.8	Weak[a,b]	Suppression
R-C1.4c	Weak[a,b]	Suppression
L9	Variable[a,b]	No effect
L11	Variable[a,b]	No effect
R-F6.2	Variable[a,b]	Enhancement
R-F4.4	Variable[a,b]	Weak enhancement
B7	Weak hypoplasia[b]	Weak enhancement
R-H2.1	No effect[b]	Enhancement
B88	?[b]	No effect
B37	No effect	No effect
B115	No effect	No effect
L5	No effect	No effect
B48	?	No effect
B93	?	No effect
A4	No effect	No effect
B12	No effect	No effect
B94	No effect	No effect
B95	No effect	No effect
B102	No effect	No effect
B128	No effect	No effect
$E(spl)^D$	Hypoplasia	Enhancement
gro	No effect	No effect

[a] Only a few embryos are neuralized.
[b] Fully penetrant neural hyperplasia when heterozygous with any *E(spl)* deletion.

None of the remaining *E(spl)* alleles causes, when homozygous, neural hyperplasia (Table 3). Homozygotes for either of the alleles *R-H2.1*, *l(3)A4*, *l(3)B95*, *l(3)B102*, *l(3)B115* or *l(3)B128* appear to be morphologically normal. However, homozygosity for *l(3)L5*, *l(3)L9*, *l(3)L11*, *l(3)B12*, *l(3)B37*, *l(3)B48*, *l(3)B88*, *l(3)B93* or *l(3)94* leads to other morphogenetic abnormalities; for example, dorsal epidermal defects, incomplete head involution, or incomplete germ band shortening are observed (Figure 2). These defects will not be described here in detail, since they are apparently unrelated to the neural defects of the other mutants.

The incomplete penetrance of the neural hyperplasia caused by several *E(spl)* mutations, *i.e.*, *l(3)L9*, *l(3)L11*, *l(3)B7*, *l(3)B88*, *R-F6.2* and *R-F4.4*, can be increased to 100% when these mutations are heter-

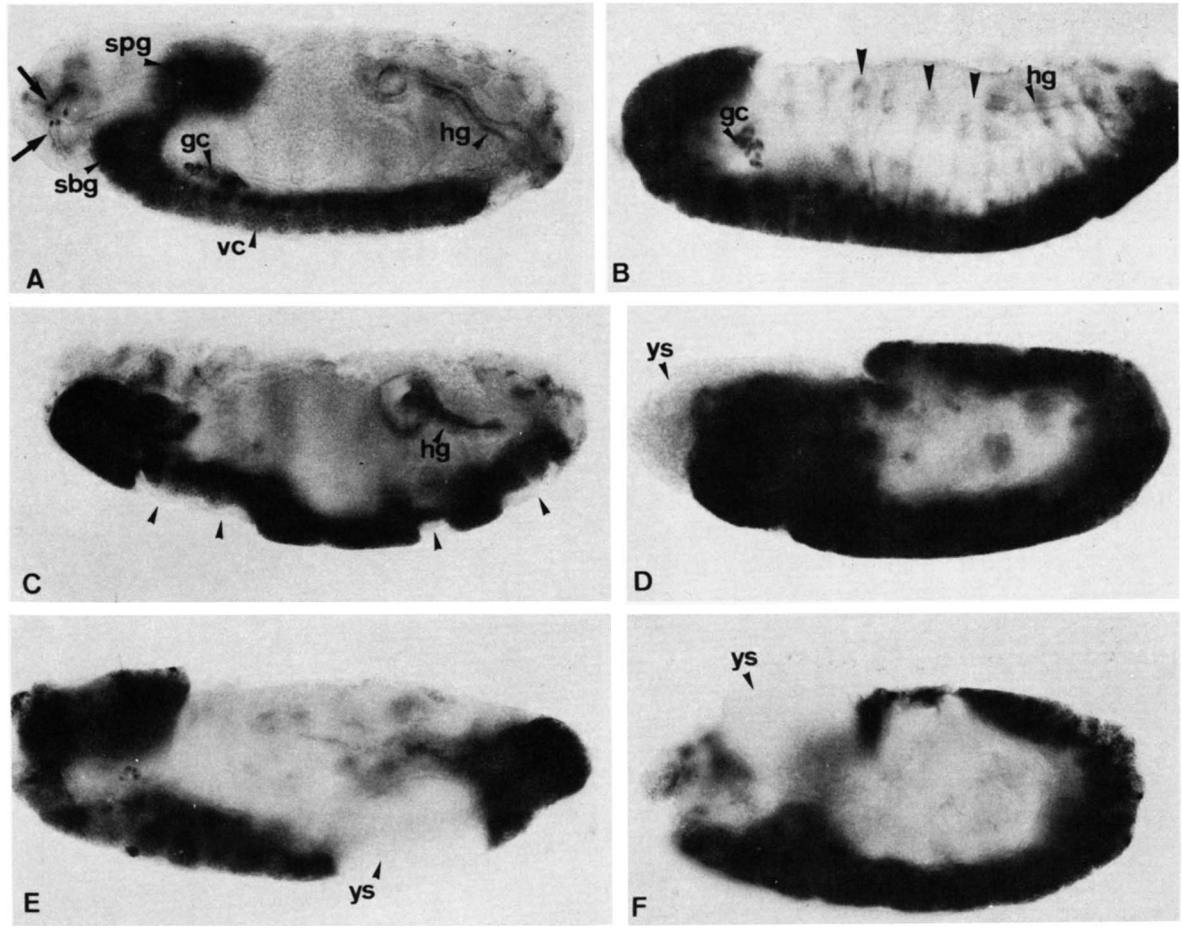

FIGURE 1.—Shows examples of neuralized embryos, stained with an anti-HRP antibody. *A* is a wild-type control. *gc:* garland cells; *hg:* hindgut; *spg:* supraoesophageal ganglion; *sbg:* suboesophageal ganglion; *vc:* ventral cord. The arrows point to the epiphysis (top) and hypophysis (bottom), respectively, two prominent sensory organs within the foregut. *B* shows a homozygous $E(spl)^{R\text{-}A7.1}$ embryo, exhibiting an extreme degree of neuralization, with highly hyperplasic CNS. The three arrowheads point to some of the segmental sensory organs and nerves (out of focus). *gc:* garland cells; *hg:* hindgut. *C* is homozygous $E(spl)^{R\text{-}A7.1}$, with a weak neural hyperplasia. Arrowheads point to ventral remnants of the epidermis that have escaped neuralization. *D* is one of the few *l(3)L11* embryos with neural hyperplasia. Notice that, although the CNS is clearly hyperplasic, the phenotype is rather aberrant, as compared to that of the embryo in *B*. In this embryo, germ band shortening has not taken place and the yolk sack (*ys*) has not been included in the midgut but it protrudes anteriorly through the hyperplasic neural tissue. The embryos shown in *E* (putative homozygous for $T(3;4)E(spl)^{R\text{-}C1.4c}$), and *F* (putative homozygous for $E(spl)^{R\text{-}F6.2}$) exhibit similar aberrant phenotypes. In the embryo shown in *E* the yolk sack (*ys*) protrudes ventrally, in the embryo in *F* it protrudes dorsally and anteriorly. This latter embryo exhibits additionally a defective germ band shortening.

ozygous with any of the *E(spl)* deletions. *R-H2.1*, which in homozygotes does not produce any obvious morphological defects, also develops weak to intermediate neuralization with complete penetrance under these circumstances (Figure 3). It is remarkable that all these latter alleles are lethal when heterozygous with *R-A7.1*, and that none of the alleles that are viable over *R-A7.1* do affect neurogenesis in any noticeable way (Tables 1 and 3). This indicates that *E(spl)* functions related to neurogenesis are comprised within the DNA deleted in *R-A7.1*.

Mutations of the complementation groups C1–C3 are also embryonic lethals. We have studied the phenotypes associated with homozygosity for C1–C3 mutations and found that *l(3)A3* and *l(3)B17* (C2 alleles) lead to rather unspecific head defects; homozygous *l(3)B103* (C1) embryos are U-shaped, probably because of defective germ band shortening; and *l(3)B105* (C3) does not cause any apparent morphogenetic defect of the homozygous embryos (data not shown).

Effects of $E(spl)^{-}$ mutations on the *spl* phenotype: Most of the revertants and *8D06*, when heterozygous with $E(spl)^{+}$, completely suppress the phenotype of *spl* males (as first described by KNUST *et al.* 1987; refer to Tables 3 and 4). Translocations *R-C1.4c* and *R14.8* do not completely suppress the *spl* phenotype (KNUST *et al.* 1987). Although isolated as $E(spl)^{D}$ revertants, alleles *R-F6.2*, *R-H2.1* and *R-F4.4* enhance the *spl* phenotype. The *spl* enhancement is almost

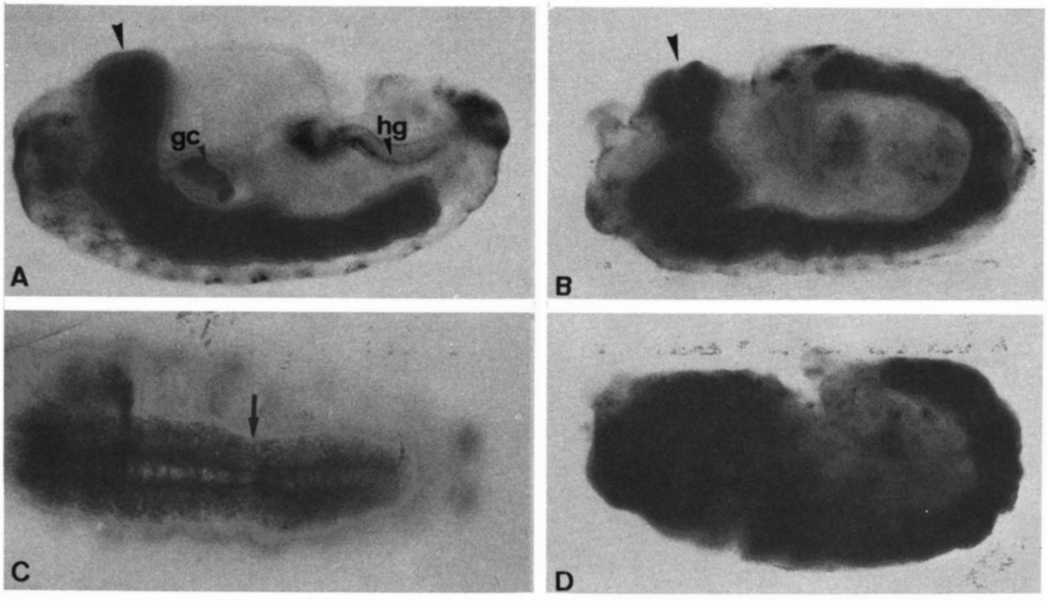

FIGURE 2.—Examples of neural abnormalities associated with alleles of complementation group C4. *A* is *l(3)B37; B* is *l(3)L5*. The phenotype of both embryos is similar. No neural hyperplasia is detectable in these embryos; *arrowheads* point to supraoesophageal ganglia protruding through an epidermal defect. *C* is *l(3)B7*. The arrow points to a defective, hypoplasic region in the ventral cord. *D* is *l(3)B94*. Notice that germ band shortening has been abnormal in the embryos at *B* and *D*, giving them a U-shaped appearance.

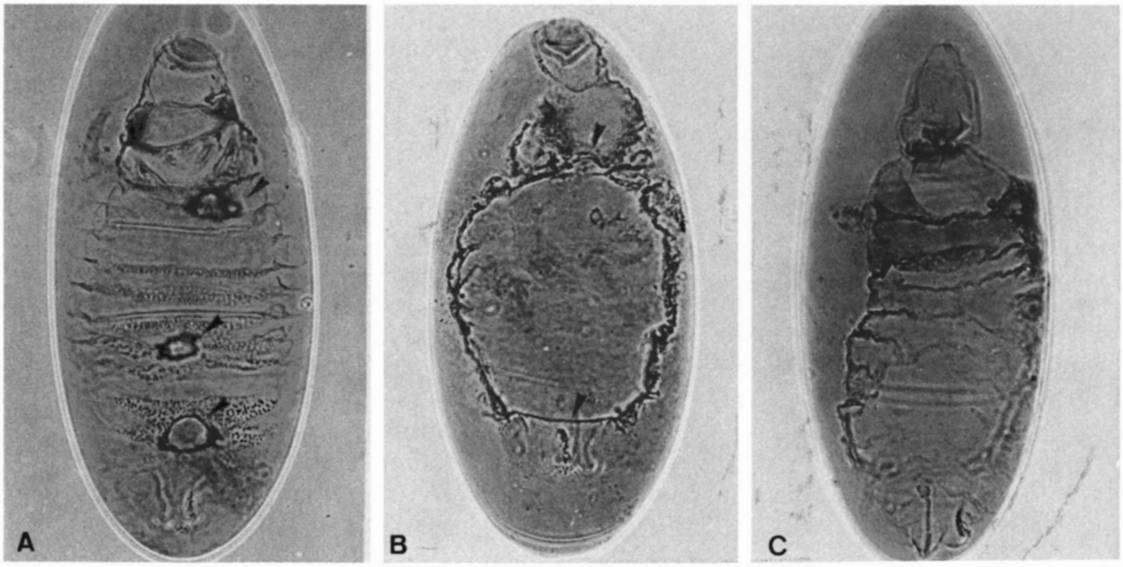

FIGURE 3.—Some of the *E(spl)* alleles cause neuralization with full penetrance while in heterozygosity over $Df(3R)E(spl)^{R\text{-}B251}$. The pictures show cuticle preparations of fully developed embryos exhibiting increasing severity of the phenotype. *A* is $l(3)L11/Df(3R)E(spl)^{R\text{-}B251}$ and shows weak neuralization. The head is defective; *arrowheads* point to holes in the ventral cuticle that are due to neural commitment of the corresponding cells. *B* is $E(spl)^{R\text{-}F6.2}/Df(3R)E(spl)^{R\text{-}B251}$, showing a weak to intermediate phenotype. Most of the cephalic and ventral epidermis is missing (*arrowheads* point to some remnants of ventral cuticle) because of neural commitment of the corresponding cells. *C* is $E(spl)^{R\text{-}F4.4}/Df(3R)E(spl)^{R\text{-}B251}$ with an intermediate phenotype.

fully penetrant (approximately 95%) and fairly pronounced in the former two alleles, comparable to that of $E(spl)^{D}$ (refer to Figure 1 in KNUST *et al.* 1987), but not so strong in the latter allele. With the exception of *l(3)B7*, none of the *l(3)R-B251* mutations was found to modify the phenotype of *spl*. The *spl* phenotype is weakly enhanced (Table 4) in females that are heterozygous for *spl* and *l(3)B7* (Table 4). In order to test whether a normal dosage of $E(spl)^{+}$ compensates the enhancement of the *spl* phenotype caused by these four alleles, we crossed them with flies carrying a duplication of $E(spl)^{+}$ ($Dp(3;3)E(spl)^{+}$ Su^{8}; P. RIPOLL, personal communication). No major modification of the phenotype can be stated in any of the genotypes carrying two $E(spl)^{+}$ copies in combination with any of the four alleles under discussion; the *spl* enhancement persists or is even more pronounced than with only one $E(spl)^{+}$ copy (Table

TABLE 4

Effect on the *spl* phenotype of *E(spl)* alleles in different genetic combinations

Genotype	Compound eyes		No. of bristles[a]
	Size	Roughness	
spl/Y;E(spl)^{D}/+	+ + + +	+ + + +	18 ± 3
spl/Y;R-F6.2/+	+ + + +	+ + + +	20 ± 2
spl/Y;R-F6.2/DpE(spl)^{su8}	+ + + +	+ + + +	18 ± 3
spl/Y;R-H2.1/+	+ + + +	+ + + +	21 ± 2
spl/Y;R-H2.1/DpE(spl)^{su8}	+ + + +	+ + + +	16 ± 3
spl/Y;R-F4.4/+[b]	+ +	+ +	22 ± 2
spl/Y;R-F4.4/DpE(spl)^{su8}	+ + +	+ + +	22 ± 2
spl/Y;B7/+[b]	+ +	+ +	22 ± 2
spl/Y;B7/DpE(spl)^{su8}	+ + +	+ + +	22 ± 3
spl/Y;R14.8/+	wt	+	22 ± 2
spl/Y;R14.8/DpE(spl)^{su8}	+ +	+ +	22 ± 2
spl/Y;+/+	+ +	+ +	25 ± 2
spl/Y;+/DpE(spl)^{su8}	+ + +	+ + +	24 ± 2
+/Y;+/+	wt	wt	32 ± 0

[a] Selected pro- and mesothoracic macrochaetae (humerals, presuturals, notopleurals, supraalars, postalars, scutellars and sternopleurals) were counted in flies (n = 32–40) of indicated genotypes.

[b] Not distinguishable from *spl/Y;+/+*; however, increased roughness of compound eyes clearly visible in females (*spl/+;R-F4.4/+* flies.

wt, wild-type; + to + + + +: increasing roughness and decreasing size of compound eyes, respectively.

4). Therefore, with respect to their interactions with *spl*, all four alleles can be considered as gain-of-function mutations.

DISCUSSION

Lethal complementation groups uncovered by *Df(3R)E(spl)$^{R\text{-}B251}$*: A first, yet tentative conclusion of our study is that the deletion *Df(3R)E(spl)$^{R\text{-}B251}$* uncovers a minimum of four different lethal complementation groups, one of them being the NG locus *E(spl)* itself. The *E(spl)* (C4) group is particularly well represented, whereas the other three complementation groups are represented by only 1–2 alleles. Therefore, it is rather improbable that saturation with lethals of this region has actually been attained. Nevertheless, the number of lethal complementation groups found in our study agrees fairly well with the number of polytene chromosome bands deleted in *Df(3R)E(spl)$^{R\text{-}B251}$*, with breakpoints at 96F8;96F13, suggesting that this deletion cannot uncover many more lethal genes than these four. The discrepancy in the number of alleles in each group may be due to several reasons, such as target size, or easy search for revertants, etc. We should like to point out that partial complementation was found between alleles of the C4 group and mutants of the C1–C3 groups, suggesting that all studied mutants may be members of the same complementation group. However, since with a few exceptions, the observed semilethality was rather low, and since none of the C1–C3 mutants was completely lethal when heterozygous with any of the C4 mutants, it is difficult to decide whether or not the observed interactions are due to background effects and whether the C1–C3 groups, despite being represented by individual alleles, are indeed genes independent from those of the C4 group.

The mating protocol used to recover lethals over *Df(3)E(spl)$^{R\text{-}B251}$* risks the isolation of clusters of premeiotic lesions. Although no attempt was actually made to control in particular the occurrence of clusters, we can exclude them for most of the lethals under discussion. We are certain that all our X-ray-induced lethals arose from independent events, as they were recovered from different experimental series and exhibit different cytological defects. With respect to the 16 EMS-induced variants, independence is obvious between alleles of the series *A* and of the series *B*, recovered at different mutagenesis experiments. Two main arguments to support allelic independence among the mutants of the series *B* are the patterns of complementation and the phenotype of the mutants (see next section) which are rather different in most of the cases. In a few cases, however, *e.g.*, *l(3)B12*, *l(3)B95* and *l(3)B102*, the patterns of complementation were similar and no reasonable evidence could be adduced to support their independent origin.

Genetic complexity of *E(spl)*: The main conclusion of our work is that *E(spl)* displays a considerable genetic complexity in that the locus appears to consist of several related genetic functions. We have studied the behaviour of 29 different *E(spl)* alleles and the results clearly point to complexity of this locus [refer to JUDD (1976) for a review on complex loci in Drosophila]. The following arguments indicate genetic complexity of *E(spl)*. First, the *pleiotropic expression of the studied mutants*. Besides encoding essential functions, defects in early neurogenesis, and in the development of compound eyes, wings and bristles, have been found to be associated with *E(spl)* mutations. As judging from the phenotype of the corresponding lethals, some of the essential functions encoded in the *E(spl)* locus are related to neurogenesis, whereas other functions are seemingly unrelated to this process (see below). Second, the *pattern of heteroallelic complementation* allows one to define several distinct functions within the *E(spl)* locus. With respect to heteroallelic complementation, *E(spl)* alleles can be classified in at least two different ways. The first one uses the viability of heterozygotes for two different alleles as classifying criterium. With the exception of *E(spl)^{D}* and *gro*, the remaining 27 *E(spl)* mutations studied are lethal with complete penetrance when homozygous. However, only seven of them are (partially or completely) lethal when heterozygous with any of the *E(spl)* mutations, whereas

the remaining 22 are fully viable when heterozygous with at least one of the other mutations. Six of the members of the first group carry large deletions; hence, it is expected that they uncover all other mutations. The seventh, however, *l(3)B7*, is EMS induced, without any detectable chromosomal lesion. Its lack of complementation with all the other lethals suggests that this variant affects one (or more) function(s) that is (are) shared by all lethals (see below). Still a further subdivision of the 22 alleles of the second group is possible, when considering their viability in heterozygosity with *R-A7.1*, which carries a deletion of approximately 34 kb of DNA within the *E(spl)* region (KNUST, TIETZE and CAMPOS-ORTEGA 1987). Six of the C4 mutants are fully viable with *R-A7.1*, indicating that their genetic lesions are located outside the region deleted in *R-A7.1*, albeit the corresponding lesions do indeed affect *E(spl)* dependent functions, for all of the variants are members of the C4 group.

The second classification of C4 mutants is in four subgroups and takes into account their complementation patterns in heterozygotes with $E(spl)^D$ and *gro*. One of the subgroups is characterized by total or partial lethality with $E(spl)^D$ and complementation of the visible *gro* phenotype; the second is characterized by noncomplementation of the visible *gro* phenotype, or even by lethality when heterozygous with *gro*, and complete viability with $E(spl)^D$; the third subgroup is characterized by complementation of *gro* and wild-type behavior with $E(spl)^D$; and the fourth subgroup includes all remaining alleles, which neither complement *gro* nor $E(spl)^D$. These data, and the behavior of the lethals with *R-A7.1*, indicate the existence of independent functions within the C4 group.

A third, rather strong indication of the genetic complexity of *E(spl)* is provided by the *embryonic phenotype* of the C4 group of mutants, in particular with respect to the neural hyperplasia and epidermal hypoplasia that are characteristic of NG mutations (CAMPOS-ORTEGA 1985), and that also develop in embryos homozygous for any of various *E(spl)* alleles. In the light of our present results, *E(spl)* appears to be actually different from the other NG genes of Drosophila. Whereas mutations that cause severe neural hyperplasia with complete penetrance have been recognized at the molecular level in the genes *N* (ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983), *Dl* (VÄSSIN *et al.* 1987) or *mam* (WEIGEL, KNUST and CAMPOS-ORTEGA 1987), to correspond to DNA lesions within fragments of 1–2 kb, or even smaller, only deletions of the entire *E(spl)* region have been found associated with severe neuralization of all homozygous embryos. It was already mentioned that the allele *R-A7.1* carries a 34-kb deletion (KNUST, TIETZE and CAMPOS-ORTEGA 1987), although we are unable to distinguish cytological abnormalities in the polytene chromosomes of this allele; homozygosity for this allele causes severe and fully penetrant neural hyperplasia, although not as extreme as the cytologically visible deletions, suggesting that these 34 kb of genomic DNA do not contain all *E(spl)* gene functions related to neurogenesis. In contrast with the severe phenotype caused by *E(spl)* deletions, homozygosity for any of six further *E(spl)* loss-of-function alleles, three of them associated with translocations (*l(3)L11*, *R14.8* and *R-C1.4c*) and the other three being X-ray-induced variants not associated with chromosomal breaks (*l(3)L9*, *R-F4.4* and *R-F6.2*) causes only partial, highly variable neural hyperplasia. None of the EMS-induced lethals leads in homozygosity to neural hyperplasia, whereas the X-ray-induced embryonic lethal revertant *R-H2.1*, as well as several other EMS induced C4 lethals (Table 3), do even not cause any morphogenetic defects of the homozygous embryos. These observations strongly suggest that simple lesions in the locus are not sufficient to affect neurogenesis in any severe way and that more than one of the genetic functions of the *E(spl)* region must simultaneously be affected in order to produce a complete neuralization of the NG ectoderm with full penetrance.

We would like to emphasize nine embryonic lethal variants and their relationships to neurogenesis. These are *R-C1.4c*, *R14.8*, *R-F4.4*, *R-F6.2*, *l(3)L9* and *l(3)L11*, which in homozygotes lead to neural hyperplasia with incomplete penetrance, and *R-H2.1*, *l(3)B88* and *l(3)B7*, which do not cause any apparent neural hyperplasia of the corresponding homozygotes. These nine variants are remarkable because all of them develop fully penetrant neural hyperplasia in heterozygosity with any of the *E(spl)* deletions. Hence, with respect to neurogenesis, all but *l(3)B7*, which is a gain-of-function mutation (see below), behave formally as hypomorphs (MULLER 1932). However, since the lethality caused by some of the mutations of this group is fully complemented by others (*e.g.*, *R-C1.4c* is fully viable over *R-H2.1*; *R-F6.2* is fully viable over *l(3)L9*, *l(3)L11* or *l(3)B88*; and *R-H2.1* is fully viable over *l(3)L9*), the mutations must really affect different essential functions. Three of the mutations (*R-C1.4c*, *R14.8* and *l(3)L11*) are associated with translocations; thus, variegation with respect to neurogenesis may be adduced to explain their variable phenotypes. But the mutational lesions of the remaining alleles are not associated with visible chromosomal defects and they do affect neurogenesis in a differential way. This means, the studied region encodes distinct essential functions which exhibit different relationships to neurogenesis. We believe that this behavior supports the functional and genetic complexity of *E(spl)*.

A further point should be stressed, which is also consistent with the complexity hypothesis, in this case

concerning the *relationships between E(spl) and spl.* No EMS induced revertant of $E(spl)^D$ was found after screening a fairly large number of chromosomes (5×10^4); would *E(spl)* be a single gene, we should have recovered several revertants among those chromosomes. This observation is comparable to those discussed above with respect to neurogenesis and suggests that more than one gene function has to be mutated in order to revert $E(spl)^D$.

R-F6.2, R-F4.4 and *R-H2.1*, which were recovered as revertants and are not associated with chromosomal defects, enhance in most cases the phenotype of *spl* flies. This is in contrast to all *E(spl)* loss-of-function mutations associated with deletions, which almost completely suppress the *spl* phenotype. We assume that the enhancement of the *spl* phenotype caused by *R-F6.2, R-F4.4* and *R-H2.1* is due to incomplete reversion of $E(spl)^D$, and that these three alleles still have remnants of the $E(spl)^D$ function, for in the presence of a normal dosage of $E(spl)^+$ all three behave like $E(spl)^D$ with respect to their relation to *spl*. The allele *l(3)B7* exhibits a similar behavior with a duplication of $E(spl)^+$, and it is also similar to $E(spl)^D$ in other respects, indicating that *l(3)B7* is indeed a gain-of-function mutation [refer to KNUST *et al.* (1987) for a discussion on $E(spl)^D$].

Several gene complexes are known to exist in the Drosophila genome exhibiting formal similarity to *E(spl)*. For example, the gene complexes *Bithorax* (LEWIS 1978), *Antennapedia* (WAKIMOTO and KAUFMAN 1981), and the complex genes *cut* (JOHNSON and JUDD 1979) and *Delta* (VÄSSIN and CAMPOS-ORTEGA 1987), to quote only a few, encode several related functions, and their mutants show complex patterns of heteroallelic complementation. It should be of considerable interest to elucidate how the different functions of each of these complexes are regulated.

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