Functional Interactions of Neurogenic Genes of Drosophila melanogaster

Anador de la Concha, Ursula Dietrich, Detlef Weigel and Jose A. Campos-Ortega

Institut für Entwicklungsphysiologie der Universität zu Köln, Gyrhofstrasse 17, 5000 Köln 41, Federal Republic of Germany

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ABSTRACT

The neurogenic genes of Drosophila melanogaster are involved in the decision of ectodermal cells to take on a neural or an epidermal fate. We present evidence in support of the notion that six of the neurogenic genes are functionally related. We studied the phenotype of embryos lacking one of the neurogenic genes in the presence of an increased dosage of the wild-type allele of another neurogenic gene. Our analysis also included the Hairless locus, whose function is related to that of the neurogenic genes, as well as to many other genes. The effects observed were asymmetric in that triploidy for a given gene modified the phenotype of loss of the function of another gene, but triploidy of the latter gene did not modify the phenotype of loss of the function of the former gene. These asymmetries allowed us to establish a polarity of gene interactions, as well as to order the genes according to the assumed ability of some of them to modify the activity of others. In this sequence, almondx is the first link and Enhancer of split the last one. Our evidence suggests that the function of big brain is independent of the function of the other six. The consequences of this arrangement for the commitment of ectodermal cells are discussed.

In insects, central nervous system development is initiated by the separation of two different cell types, the neuroblasts and the epidermoblasts, from a common pool of cells located in a special neurogenic (NG) region of the ectoderm (POULSON 1950; SANDER 1955; BATE 1976, 1982; HARTENSTEIN and CAMPOS-ORTEGA 1984; TECHNAU and CAMPOS-ORTEGA 1985, 1986; DOE and GOODMAN 1985a). In Drosophila melanogaster, presumptive neuroblasts and epidermoblasts are contiguous while in the ectoderm, and yet, about 25% of the NG ectodermal cells leave the outer germ layer to become committed as neuroblasts, whereas 75% of these cells remain to become committed as epidermoblasts. Experimental evidence from grasshoppers (DOE and GOODMAN 1985b) and fruitflies (TECHNAU and CAMPOS-ORTEGA 1986) indicates that interactions between neighboring cells are involved in this process.

Several genes are presently known, the activity of which is necessary for a correct segregation of neuroblasts and epidermoblasts in Drosophila (POULSON 1937; LEHMANN et al. 1981, 1983; LABONNE and MAHOWALD 1985; PERRIMON, ENGSTROM and MAHOWALD 1984). These genes are called NG genes because the lack of function of any one of them leads to lethality of the homozygous embryos, which exhibit a characteristic neural hyperplasia and epidermal hypoplasia (LEHMANN et al. 1981, 1983). Since the lack of any one of the NG genes leads to the same phenotype, we consider it likely that these genes have the same function during development. In this paper we report on experiments directed toward answering this question.

The approach used to work out functional relationships between the NG genes relies on the following assumption. Increasing of the copy number, and assumedly of the gene product, of one of the NG genes may lead to a modification of the phenotype of lack of another one when these two genes are functionally linked. In a typical experiment, three copies of the wild-type allele of one NG gene are combined, using duplications, with a loss-of-function mutation of another NG gene. This approach was previously used to analyze the interactions of some of the NG genes, chiefly at the imaginal level (CAMPOS-ORTEGA et al. 1984; DIETRICH and CAMPOS-ORTEGA 1984; VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985). We have now extended the research to the remaining genes, concentrating exclusively on the embryonic defects of the homozygous animals, without considering the phenotypes of heterozygous adult animals.

MATERIALS AND METHODS

Strains: All strains used were grown at 21° on standard food. Table 1 shows the genetic variants used in this work. Most of them had been previously described by LINDSLEY and GRELL (1968), LINDSLEY et al. (1972), LEHMANN et al. (1983), and VÄSSIN, VIELMETTER and CAMPOS-ORTEGA (1985). Additional chromosomal aberrations used in this
The chromosomes $\text{neu}^{+} H^{2}, Dp^{I} H^{2} , H^{2}E(spl)^{R1}, Dp \text{ neu}^{+} Dp^{I} H^{2}, Dp^{I} H^{2} \text{ neu}^{+}, \text{neu}^{+} H^{2}, Dp E(spl)^{+}$ and $Dp^{I} H^{2} E(spl)^{+}$ were obtained by recombinant and established as balanced stocks. Consequently, genomes with 3–4 copies of one NG gene and an amorphic mutation of another NG gene or the $H^{2}$ mutation were obtained without ambiguities. In other cases, however, we had to combine in the same genome two different chromosomes to obtain the desired genotype. Since the phenotypes were scored on embryos, and the available embryonic cuticle markers (Nüsslein-Volhard, Wieschaus and Kluding 1984; Jürgens et al. 1984; Wieschaus, Nüsslein-Volhard and Jürgens 1984; Gergen and Wieschaus 1985, 1986) are expressed chiefly on the ventral epidermis (which is generally missing in the NG mutants), marker mutations cannot be used to verify the various genotypes. Under such conditions, the main evidence for the diagnosis of a given genotype had a statistical foundation, i.e., the number of embryos exhibiting a phenotype in the proportion expected for the corresponding segregants. This number was determined in each case and used to establish the genotypes (data not shown).

Crosses were carried out according to one of the following schemes. Concerning the relations of the $X$-chromosomal $N$ locus to autosomal loci, $Dp(1;1)Co$ carried by the males in the cross $+/+; \text{mut Bal} \times Dp N^{+}/Y; +/- (\text{mut} \text{ stands for a mutation of an autosomal NG gene and Bal or balancer})$, F1 females of genotype $Dp N^{+}+/+; \text{mut Bal}$ were crossed with $+/+$ males. Approximately 25% of the entire progeny were $\text{mut Bal}$, and half of them should also carry the $Dp N^{+}$ chromosome. The same scheme applies for crosses involving $Dp amx^{+}$ (almond). Concerning duplications of autosomal loci ($Dp aut^{+}$ stands for a duplication of an autosomal NG gene) and their relations to the $X$ chromosomal $N$ locus, the scheme in principle was the same. Approximately 25% of all the embryos from the cross $N^{+} X +/-; \text{mut Bal} \times Dp aut^{+}/Y; +/-$ are $N^{+} Y ;$ half of them should carry $Dp aut^{+}$. To study relationships between loci segregating with different autosomes, flies of genotype $\text{mut Bal}+ / +; Dp aut^{+}$ were crossed with flies of genotype $\text{mut Bal}+ / +; Dp aut^{+}$. Approximately 25% of the embryos died as $\text{mut Bal}$; half of them also carried $Dp aut^{+}$. The same scheme was used also for crosses involving $H^{2}$.

Homozgyosity for $N^{+} H^{2}$ in the germ line was obtained following the techniques described by Jimenez and Campos-Ortega (1982). Irradiated females were crossed with males carrying either of the chromosomes $Dp \text{ neu}^{+}$, $H^{2}$ and $Dp E(spl)^{+}$.

Preparation of embryos: Embryos were staged according to Campos-Ortega and Hartenstein (1985). Stage 14–16 embryos were stained with an anti-horseradish peroxidase antibody (anti-HRP, purchased from Sigma) using a HRP-conjugated second antibody, following the protocol of Jan and Jan (1982) as modified by Jimenez and Campos-Ortega (1987). The cuticle was prepared from 40-h-old dead embryos, according to Van der Meer (1977).

**RESULTS**

**Effects of increasing gene dosage**: The phenotype of NG mutations has been described repeatedly (Lehmann et al. 1981, 1983; Campos-Ortega 1985; Hartenstein and Campos-Ortega 1986). In short, loss of a NG gene function leads to neural hyperplasia and concomitant lack of almost the entire epidermal sheath in the fully developed embryo. This phenotype develops because all cells of the NG ectoderm

### TABLE 1

<table>
<thead>
<tr>
<th>Variant</th>
<th>Cytology</th>
<th>Ploidy type of mutation</th>
<th>Reference</th>
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<tbody>
<tr>
<td>$Dp(1;1)Co$</td>
<td>SC4–5;3D6–F1</td>
<td>$Dp N^{+}$</td>
<td>a</td>
</tr>
<tr>
<td>$Dp(2;2)92$</td>
<td>30B;34A</td>
<td>$Dp \text{ amx}^{+}$</td>
<td>b</td>
</tr>
<tr>
<td>$T(2;3)dp^{R2}$</td>
<td>24F–47;32B1–2</td>
<td>$Dp \text{ bib den}^{+}$</td>
<td>c</td>
</tr>
<tr>
<td>$Dp(3;3)\text{Ant}^{+}R2$</td>
<td>84D;86A</td>
<td>$Dp \text{ neu}^{+}$</td>
<td>e</td>
</tr>
<tr>
<td>$Dp(3;3)\text{hed}^{+}10$</td>
<td>89E;92A–2–3</td>
<td>$Dp \text{ DI}^{+}$</td>
<td>f</td>
</tr>
<tr>
<td>$Dp(3;3)\text{MKRS-D2}$</td>
<td>91C–5;92E</td>
<td>$Dp \text{ DI}^{+}$</td>
<td>g</td>
</tr>
<tr>
<td>$Dp(3;3)Su^{d}$</td>
<td>96A;96E–F</td>
<td>$Dp \text{ E(spl)}^{+}$</td>
<td>h</td>
</tr>
<tr>
<td>$Dp(3;3)\text{SuM(3)M}^{d}$</td>
<td>94D;99E</td>
<td>$Dp \text{ E(spl)}^{+}$</td>
<td>f</td>
</tr>
<tr>
<td>$Dp(3;3)SuH^{2}$</td>
<td>92E;96A</td>
<td>$Dp H^{2}$</td>
<td>i</td>
</tr>
</tbody>
</table>

* LINDSEY and GRELL (1968); b, BENDER (1967); c, G. REUTER (personal communication); d, SEMSHIN and SIDONIA (1985); e, KEMPHEUS, RAPF and KAUFMAN (1983); f, VASIN, VIELMETTER and CAMPOS-ORTEGA (1985); g, W. GELBART (personal communication); h, P. RIPOLL (personal communication); i, LEHMANN et al. (1983); j, WIEGEL (1986); k, M. BRAND and J. A. CAMPOS-ORTEGA (personal communication); l, BUSSON et al. (1988).

**work are the following. $T(2;3)\text{dp}^{R2}$ was a gift of J. ZEIDONYA, and was described by SEEMSHIN and SIDONIA (1985); it translocates 24F–47;32B1–2 to the third chromosome. $Dp(2;2)92$ is a tandem duplication of 30B;34A, kindly provided by G. REUTER. Other aberrations include big brain ($\text{bib}^{+}$), $Dp(3;3)\text{Ant}^{+}R2$ is a tandem duplication of 84D;86A, including the neutralized ($\text{neu}^{+}$) locus (A. DE LA CONCHA and J. A. CAMPOS-ORTEGA, unpublished data), as described previously by KEMPHEUS, RAPF and KAUFMAN (1983), kindly provided by T. KAUFMAN. The chromosome $Dp(3;3)\text{MKRS-D2}$ is duplicated for 91C–5;92E (W. GELBART, personal communication) and includes the Delta ($Dl$) locus, being therefore $Dp DI^{+}$, it was kindly provided by W. GELBART. $Dp(3;3)Su^{d}$ is a tandem duplication of 96A;96E–F (P. RIPOLL, personal communication), including Enhancer of split ($E(spl)^{+}$); it was kindly provided by P. RIPOLL. Generally, amorphic loss-of-function mutations of NG genes, that produce extreme neural hyperplasia of the homozygous embryos, and $H^{2}$, an allelomorph of Hairless ($H^{2}$) that leads to a phenotype comparable to that of $H$ deletions, were used. In a few cases hypomorphic alleles had to be used to detect phenotypic modifications. All variants used are homozygous lethal.

**Crosses**: Crosses were made in order to obtain embryos simultaneously homozygous, or hemizygous in the case of Nocha ($N$) mutations, for a loss-of-function mutation of one locus and, using a duplication of the wild-type allele, three copies of the wild-type allele of another locus. In some cases, both genetic variants to be combined (loss-of-function mutation and duplication) segregated with the same chromosome, for example a $\text{neu}^{+}$ mutation and $Dp E(spl)^{+}$.
adoption of the neural fate, as opposed to only 25% of them doing so in the wild type. The epidermal defects are easily detectable in cuticle preparations. The severity of the phenotypic defects allows us to classify the mutations in weak, intermediate and extreme according to criteria described in LEHMANN et al. (1983).

Table 2 presents the main results of our analysis. A reduction of the severity of the phenotype caused by loss of one NG gene function was frequently observed after increasing the number of copies of the wild-type allele of another NG gene. The reduction was in all cases noticeable, e.g., from an extreme to an intermediate or even to a weak degree of expression (Figure 1), although in no case the wild-type phenotype was restored. In a few instances, however, the severity of the phenotype of loss of one wild-type allele was increased by the concomitant triploidy of a second one (not shown). VASSIN, VIELMETTER and CAMPOS-ORTEGA (1985) described that Dp DI+ leads to an increased severity of the phenotype of loss-of-function N mutations. We confirm that the severity of the phenotype of N+/+.1, an amorph, and of N-2, a hypomorph (LEHMANN et al. 1983), is increased by either of two different Dp DI+. In addition, we found that Dp DI+ exerts upon neu- embryos a similar increase of the phenotypic severity. Also, the severity of the phenotype of homozygosity for the hypomorph Dp^{3837} (LEHMANN et al. 1983) is increased by triploidy for E(sp1)+. We should point out that the study of interactions of DI with other NG genes requires in some cases the use of hypomorphs. Since the lack of zygotic expression of the DI gene leads to the most extreme phenotype of all NG mutants, the strength of the phenotype of amorphic DI mutations cannot be increased further; however, such an increase can only be demonstrated for the weaker phenotype of hypomorphs (MULLER 1992). Therefore, both Dp^{3993} (an amorphic allele) and Dp^{3837} were used in parallel throughout our study. In the case of amorphic mutations of any of the other NG genes, the phenotype is not as strong as that of amorphic DI alleles and allows one to unambiguously detect an increase, as well as a decrease, in phenotypic severity. As an example, we used N^{55e11} and N^{2} in all combinations concerning N, and the same qualitative phenotypic modifications were found.

Phenotypic modifications became manifest only in some, but not in all combinations. The observed modifications exhibited a clear asymmetry. For example, three copies of neu+ in a genome do apparently not affect the phenotypic expression of a N- mutation in the same genome (Figure 1, A and B), whereas three copies of N+ lead to a reduction of the severity of the phenotype of homozygous neu- embryos (Figure 1, C and D). Therefore, triploidy for N+ apparently compensates in part the defects due to lack of neu+, whereas triploidy for neu- does not exert any apparent effect on the defects due to lack of N+. In a similar manner, Dp E(sp1)+ decreases the severity of the phenotype of N- or neu- embryos, and increases that of loss-of-function DI alleles, whereas neither Dp N+, nor Dp neu+, nor Dp DI+, affect the phenotype of E(sp1)- embryos. These differences indicate that the activity of some NG genes modifies the activity of others, and that these relationships are not reciprocal.

Table 2 shows that the phenotype of loss of the bib- function is not modified by increasing the number of wild-type alleles of amx, neu, N, DI and E(sp1), nor by the H2 mutation (see below). We studied the effects of increasing the ploidy of bib+ upon the phenotype of N- embryos. In order to increase the number of bib+ copies, we used either T(2;3)dp{H27}, which translocates the interval 24F4-7;32B1-2, including the locus of bib+, to the region 91D-E of the third chromosome, or Dp(2;2)92, a tandem duplication of 30B;34A (G. REUTER, personal communication). Unfortunately, both include also the locus of denervated (den-), which maps to 31BD. Loss of the den- function leads to considerable neural hypoplasia (M. BRAND and J. A. CAMPOS-ORTEGA, unpublished data), i.e., to effects opposite to the ones of loss-of-function mutations of the NG genes. Therefore, these

**TABLE 2**

<table>
<thead>
<tr>
<th>Mut</th>
<th>Dp amx+</th>
<th>Dp neu+</th>
<th>Dp N+</th>
<th>Dp DI+</th>
<th>Dp E(sp1)+</th>
<th>Dp bib+</th>
<th>H-</th>
</tr>
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<tr>
<td>bib-</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>wt</td>
<td>ne</td>
</tr>
<tr>
<td>E(sp1)-</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>wt</td>
<td>inc</td>
<td>ne</td>
</tr>
<tr>
<td>DI-</td>
<td>ne</td>
<td>ne</td>
<td>wt</td>
<td>inc</td>
<td>red</td>
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<td>red</td>
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<td>N-</td>
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<td>red</td>
<td>inc</td>
<td>red</td>
<td>red</td>
<td>red</td>
</tr>
<tr>
<td>neu-</td>
<td>ne</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>ne</td>
<td>red</td>
</tr>
<tr>
<td>mam-</td>
<td>ne</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>red</td>
<td>ne</td>
</tr>
</tbody>
</table>

We studied the phenotype of embryos carrying loss-of-function, generally amorphic mutations of one of the NG genes (Mut), with a duplication of another NG gene, or the loss-of-function H2 mutation (Dupl). In several cases, no effect (ne) was observed. In other cases, the severity of the phenotype of loss-of-function mutation was either increased (inc) or reduced (red).
chromosomes are Dp bib\(^+\), den\(^+\). Altering the ploidy of den\(^+\) modifies the phenotype of NG mutations (M. Brand and J. A. CAMPOS-ORTEGA, personal communication). In order to account for possible effects of den\(^+\) superimposed upon the effects of bib\(^+\) on NG mutations, we introduced in these genomes a den\(^-\) mutation. We compared the phenotype of embryos with the genotype N\(^{55e11}\)Y\(;Dp(2;3)\)bib\(^+\), den\(^+\)/den\(^-\) to that of embryos with the genotype N\(^{55e11}\)Y;Dp(2;3)bib\(^+\), den\(^+\)/den\(^-\). The severity of the phenotype of N\(^\neg\) embryos with the former genotype is reduced; however, the severity of the phenotype of N\(^\neg\) embryos with the latter genotype is not affected. The reduction of phenotypic severity observed is therefore due to triploidy of den\(^-\). This means, increasing the ploidy of the bib gene does not modify the phenotype caused by the lack of N\(^\neg\). By using similar protocols, no modification of the phenotype...
of mam- (master mind), neu-, Dl- and E(spl)- mutations was observed in combinations with Dp bib+. All these observations suggest that no functional interactions exist between bib and the remaining NG genes.

We want to summarize the five major conclusions of our observations (Table 2): (1) Dp amn+ (almondex) does not modify the phenotype of lack of either of the NG genes. (2) The phenotype of mam- embryos is reduced by the concomitant triploidy for wild-type alleles of the other NG genes, with the exception of amn+ and bib+. (3) The phenotype of bib- embryos is not affected by triploidy for either of the other NG genes, nor does triploidy for bib- affect the phenotype of lack of either of the other genes tested. (4) The phenotypes of neu-, N-, and Dl- embryos are either increased or reduced by the concomitant triploidy for some NG genes, but left unaffected by the triploidy for others. (5) The phenotype of E(spl)- embryos is not affected by the triploidy for either of the other NG genes; however, three copies of E(spl)+ affects the phenotypic expression of lack of the other NG genes, with the exception of bib.

Effect of Hairless on the expression of NG mutations: Hairless (H) is a third chromosomal gene with haplo-insufficient expression (LINDSLEY et al. 1972; VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985); flies with only one copy of H+ lack several macrochaetae (LINDSLEY and GREL 1968). If the H+ function is absent, the animals die late in embryogenesis. The overwhelming majority of these animals do not show any striking structural defects; only a few of them (2–3%) exhibit hypoplasia of the CNS as well as a lack of sensory organs in some segments (Figure 2B). A few embryos (2–3%) with additional copies of the H+ gene [supplied by means of the Dp(3;3)Su H2, st e chromosome, (VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985)] show local neural hyperplasia (Figure 2C). Despite the very low frequency of these defects we believe that they are related to the modifications of H gene activity, rather than to other background effects. No such defects appear in stainings of other mutants that are seemingly unrelated to neurogenesis (not shown). Relationships between H and N and Dl have been known for long time (LINDSLEY and GREL 1968); several functional relations between H and N, E(spl) and Dl have also been described by DIETRICH and CAMPOS-ORTEGA (1984) and VÄSSIN, VIELMETTER and CAMPOS-ORTEGA (1985).

We found that a H loss-of-function mutation (H2), apart from its known effects on N- and Dl- (VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985), also causes a reduction of the neural hyperplasia due to homozygosity for neu- or mam- alleles. We further confirm VÄSSIN, VIELMETTER and CAMPOS-ORTEGA (1985), in that H2 does not affect the phenotype of homozygous E(spl)- embryos. As discussed in VÄSSIN, VIELMETTER and CAMPOS-ORTEGA (1985), these observations suggest that the interaction of H with N, Dl, neu and mam cannot be direct, for the genes are absent in the studied mutants, but probably occurs through other gene(s) which are functionally linked with these four loci. Since H2 does not affect the phenotype of E(pl)-, the E(spl) function is a major target of H and all interactions between H and NG genes are likely to be mediated by E(spl). No effect of H2 was observed on the phenotype of homozygous bib- embryos.

Role of maternal expression on interactions of NG genes: The aim of the following experiments was to detect a possible influence of maternal expression of the NG genes on their functional interactions. With the exception of bib, there is evidence for a maternal expression for all NG genes (JIMENEZ and CAMPOS-ORTEGA 1982; DIETRICH and CAMPOS-ORTEGA 1984; VÄSSIN, VIELMETTER and CAMPOS-ORTEGA 1985; VÄSSIN and CAMPOS-ORTEGA 1987; KNUST et al. 1987). Hence, the results of our experiments might be
different after removing the maternal component of gene expression. In order to test for the influence of maternal expression, reciprocal crosses were carried out in each case, i.e., the duplication was provided by the mothers and the loss-of-function mutation by the fathers, and vice versa. With one exception, no differences were observed in the results of such reciprocal crosses. In the case of Dp Dl+ and mam−, however, a conspicuous reduction of phenotypic severity was only observed when the Dp Dl+ was provided by the mother (not shown).

A more reliable way to test the influence exerted by the maternal expression could be followed in the case of N. Zygotic and maternal components of expression were removed as described previously by Jimenez and Campos-Ortega (1982), following a method developed by Wieschaus (1980). This technique permits the generation of N− embryos derived from germ-line cells that are homozygous for a N− mutation. We studied the effects of Dp neu+*, Dp E(spl)+ and H− on the phenotype of N− embryos devoid of maternal and zygotic expression. No qualitative difference was found as compared to the phenotype of N− embryos lacking only the zygotic component (Figure 1, E and F). However, it should be noted that the degree of reduction in phenotypic severity is relatively more pronounced in N− embryos that have developed in the absence of maternal expression than in N− embryos that have developed in the presence of maternal expression. In particular, the Dp E(spl)+ causes a very conspicuous reduction of the severity of the N− phenotype in germ-line clones (Figure 1, G and H).

DISCUSSION

The major aim of our research was to test whether the NG genes are in some way functionally linked. Some of their functional interrelationships were already known (Campos-Ortega et al. 1984; Dietrich and Campos-Ortega 1984; Vassin, Vielmetter and Campos-Ortega, 1985). However, until now we had been unable to study several of the pertinent genotypes, due to the lack of appropriate chromosomal aberrations and, thus, no synthesis of these somewhat fragmentary observations had yet been possible. In addition, previous studies on the same problem chiefly relied upon heterozygous adult animals, whereas in the present study all observations were performed on homozygous mutant embryos. When loss-of-function mutations of the genes N, mam, DI and E(spl) are homozygous with the wild-type alleles, various complex patterns of imaginal defects develop (Lindsley and Grell 1968; Lehmann et al. 1983; Vassin, Vielmetter and Campos-Ortega 1985; Vassin and Campos-Ortega 1987). Vassin, Vielmetter and Campos-Ortega (1985) assessed modifications of these imaginal defects in heterozygous flies, the genotype of which could be unambiguously verified because of additional marker mutations. However, some of these results were rather difficult to interpret, probably due to the heterozygous condition of the mutants (see below). In the present work, in order to avoid such difficulties, we were exclusively concerned with homozygous mutant embryos. The material presented circumvents most of the previous difficulties. Our experiments allow us to propose a model which incorporates most of the observations and permits predictions about the function of some of the NG genes. Our results are best explained by assuming that the NG genes act as consecutive links of an epistatic gene series that leads to epidermogenic commitment of ectodermal cells. Hence, the phenotype of a mutation in any of the NG genes would actually manifest the functional deterioration of the last link.

For the purposes of our discussion, we assume that an increase of the number of wild-type alleles leads to an increase of the corresponding gene product (see below). Two NG genes are assumed to be functionally linked, if increasing the product of one of them affects the expression of lack of the other one. Moreover, the effects of our crosses were asymmetric: mutation of one gene and duplication of another gene in the same genome led to a given effect, whilst the duplication of the former gene did not have any effect upon the phenotype of a mutation of the latter gene. This asymmetry indicates a direction in the relationship, i.e., the activity of one gene modifies the activity of another gene, but not vice versa. This may be interpreted to mean that one gene product follows the other gene product in an ordered pathway with a common output: epidermogenesis. For example, Dp N+ leads to the reduction of the neu− phenotype, whereas Dp neu+ does not modify the phenotype of N−. Since the degree of neural hyperplasia is reduced, the increment of N product allows in fact the development of more epidermis in an embryo that lacks neu; it makes it more like wild-type. On the other hand, the increment of the amount of neu+ product does not improve the defective epidermogenesis due to absence of N. Such a situation could be explained by assuming that a normal function of neu contributes to a normal function of N, while neu function itself is independent of N. More neu+ product might well be available in N− animals carrying Dp neu+; however, since N is absent, no change is expected in its activity. The consequence of the lack of N would be that the activity of the genes that might follow N in an epistatic series would remain unmodified. In the opposite case, when neu is absent and the amount of N+ product is increased, a reduction of the phenotype produced by the absence of neu should be observed. This would occur
because the genes following $N$ would increase their activity due to the increment of $N^+$ and, therefore, the functional (epidermogenic) efficacy of the system as a whole would improve.

An indispensable prerequisite of this model is a basal level of activity of each one of the NG genes in the absence of any of the previously acting links. By itself, this basal activity would be insufficient to carry out the normal function; otherwise it would compensate the defects due to lack of one of the previous links of the epistatic series. However, in our model, such basal activity is necessary in order to explain why the duplication of a NG gene has a functional effect when the gene that influences the activity of the duplicated gene is absent. We assume that such a basal activity of a NG gene can be provided by other genes that, like $H$ (see below), participate in supporting the function of the NG genes.

Following the same lines of reasoning, we have attempted to construct a sequence of relations by means of studying all possible genotypic combinations. Indeed, our results allow the arrangement of six of the NG genes to a sort of epistatic series (Figure 3). $bib$ behaves as functionally independent from the other NG genes (see below). Among the genes studied, $amx$ is the only predominantly maternal one (Shannon 1972; Lehmann et al. 1983). $Dp$ $amx^+$ is unable to compensate for the phenotype of lack of any of the other NG genes (Campos-Ortega et al. 1984; present results), suggesting that the maternal information provided by $amx$ participates in the first step(s) of the functional chain. Since the phenotype of $E(spl)^-$ is not modified by a duplication of any other NG gene, while $Dp$ $E(spl)^+$ modifies the phenotype of any NG mutant, with the exception of $bib$ mutants, $E(spl)$ behaves as the last member of the epistatic series of genes. Hence, the phenotype of homozygous NG mutants reflects the disturbance of the $E(spl)$ function. When the number of copies of the wild-type allele of a NG gene is increased in the genome of a homozygous NG mutant, the $E(spl)$ function will be modified if the triploid NG gene is closer in the epistatic series to $E(spl)$ than the homozygous mutant NG gene. This modification of the $E(spl)$ function would then lead to a modification of the phenotype, whereas no modification is expected in the opposite case, i.e., when the homozygous mutant NG gene is closer to $E(spl)$ than the triploid one.

Positive or negative signs in our model (Figure 3) reflect the kind of observed modifications, that is, a decrease or an increase, respectively, of the severity of the phenotype, and therefore the kind of influence that is thought to be exerted by one gene upon the function of the following one. This means, we assume a positive influence of one gene (for example, $neu$) upon the next (for example, $N$) when the severity of the phenotype of a mutation in the former is decreased by the duplication of the latter. In $neu^-$, the function of $N$, and of the following genes, is insufficient; epidermogenic commitment is defective. Increasing $N^+$ leads to an improvement of epidermogenesis. We assume a negative influence if the phenotypic severity is increased. $DI$ is thought to repress the function of $E(spl)$, and both $N$ and $neu$ to repress that of $DI$. Granted such a situation, the lack of either $N$ or $neu$ would be followed by derepression of $DI$ and, therefore, by an increase of $DI^+$ function in general. The consequence would be to increase the functional repression exerted by $DI$ upon $E(spl)$, followed by a defective epidermogenic commitment. If a $N$, or a $neu$, mutant carries a $Dp$ $DI^+$, the repression of $E(spl)^+$ would even be stronger and, therefore, the phenotype more severe. The same logic applies to the other cases.

Our present results suggest that $N$ and $DI$ exert opposite effects on $E(spl)$, whereas Vassin, Vielmetter and Campos-Ortega (1985) had proposed like effects for both $N$ and $DI$. The latter authors observed that modifications of the phenotype of $E(spl)^-$ flies heterozygous with the wild-type allele were caused by either $Dp$ $N^+$ or $Dp$ $DI^+$, whereas in our present material none of these two duplications modifies the phenotype of homozygous $E(spl)^-$ embryos. Since the only genotypic difference between heterozygous and homozygous animals is the presence of one copy of the $E(spl)$ wild-type allele in the former, the different phenotypic behaviour is most probably due to the existence of $E(spl)$ gene product in the heterozygotes. In fact, the previous results (Vassin, Vielmetter and Campos-Ortega 1985) did not permit to define a hierarchy of action among the three genes. This led to postulate reciprocal relationships between them, i.e., of $N$ and $DI$ on $E(spl)$ and vice versa, whereas the present results are compatible with unidirectional relationships.

$bib$ acts as functionally independent from the remaining loci, for the phenotype of loss of $bib$ function is not modified by changing the dosage of any of the other studied NG genes, nor does the increment of the number of $bib^-$ copies modify the phenotype due...
to lack of any of the other NG genes. Consequently, we propose that *bib* is located outside the pathway formed by the other genes, and that its function is required in parallel to the function of the remaining NG genes.

All known NG genes, with the exception of *bib*, are expressed maternally and zygotically (Jiménez and Campos-Ortega 1982; Dietrich and Campos-Ortega 1984; Perrimon, Engstrom and Mahowald 1984; Labonne and Mahowald 1985; Vässin, Vielmetter and Campos-Ortega 1985; Knust et al. 1987). Our work has been mainly concerned with the zygotic component of NG gene expression. However, we found that the maternal expression of *N* does not seem to qualitatively affect its relationships to the other NG genes. Since the results of reciprocal crosses have not shown any difference, we assume that the same applies to the maternal expression of the other genes. Our present data do not allow to satisfactorily explain why the phenotypic severity of *N* embryos is more strongly reduced by *Dp E(spl)* when both maternal and zygotic *N* function are completely removed, as compared to when only the zygotic expression is missing. Such an effect may perhaps reflect interrelationships of the maternal products of these two genes.

We would like to point out that there is no direct evidence concerning the molecular level at which the interactions proposed above actually take place. The conclusions and various hypotheses derived from our observations are to be understood as exclusively based on formal arguments of transmission genetics. It remains for a molecular approach to this problem to work out the actual basis of the interactions. However, the structure of the putative proteins encoded by the genes *N* (Wharton et al. 1985) and *Di* (Vässin et al. 1987), both of which are presumably located in the cell membrane and contain EGF-like repeats, strongly suggests that at least some of the functional interactions between NG gene products take place within the membrane of the ectodermal cells. Several data from experimental embryology support this notion. Apparently, all cells of the Drosophila NG ectoderm initially follow neural development (Hartenstein and Campos-Ortega 1984) the switch of 75% of these cells into epidermogenesis being mediated by cell-cell interactions (Technau and Campos-Ortega 1986). Our present hypothesis to account for such interactions requires that the cells continuing neurogenesis send a signal that is received by their neighboring cells. After transduction, the signal would then be transmitted to the genome of the receiving cell to regulate its genetic activity in order to repress the neural fate. The products of the NG genes may provide the material basis for this signal. The spatial pattern of *Di* gene expression, restricted to territories with NG abilities, indicates that this gene provides the specificity required for the source of the regulatory signal (Vässin et al. 1987). *E(spl)*, as the last member of the epistatic series, might thus receive the regulatory signal and act as the regulator that switches the cells into the epidermal fate—or it might also be the step before (an)other, still unknown gene(s), which would then prolong the chain to the genome. In support of this hypothesis, Technau and Campos-Ortega (1987) found that most NG genes are not cell autonomous in the expression of their phenotype, suggesting that they are involved in sending the hypothetical signal, whereas *E(spl)* is cell autonomous in the expression of its phenotype, its function, therefore, being most probably located on the receptor side. These results are in accord with the proposed function of *N* and *Di*: to interact directly with *E(spl)*, the last link of the chain.

Apart from the lack of a firm molecular evidence concerning the level at which these functional interactions take place, the following weak points and inconsistencies of the present model should also be emphasized. (1) There is no direct support for the assumption that triploidy for a NG gene leads to an increase of the amount of the corresponding product, for we are as yet unable to measure directly the amount of NG gene products. Yet, there is a large body of positive evidence concerning the structural genes of many different enzymes, for which a dosage dependent expression has been demonstrated (reviewed by O'Brien and MacIntyre 1978). (2) The maternal effect of *am*1 makes in some cases difficult its pertinent combination with duplications of the other NG genes; the evidence for the proposed location of *am* relies only on the results with *Dp am*1. (3) In a similar way, the location proposed for *mam* is supported only by the results with a *mam* mutation, because a *Dp mam* was not available to us. (4) We believe that, together with other NG genes, *bib* acts in sending the signal. In transplantations of *bib* cells, this gene behaves as not cell-autonomous (Technau and Campos-Ortega 1987). However, our results point to *bib* as acting separately from the flow proposed for the hypothetical signal. This suggests that *bib* function either follows another pathway, with an independent, as yet unknown receptor gene, or it points to inconsistencies in our approach. (5) Our model presupposes that the absence of *H* product leads to a removal of the repression of *E(spl)* function, assumedly exerted by *H*, and consequently to an increase of the *E(spl)* function; it also presupposes that additional copies of *H* should lead to insufficiency of *E(spl)* (see also Vässin, Vielmetter and Campos-Ortega, 1985). Granted that *E(spl)* acts as the last link in the chain leading to epidermogenic commitment, the increment of its activity should lead to neural deficits and its repression to neural hyperplasia. However, the phenotype of most loss of function *H* mutants or animals carrying duplications of *H* is normal. We assume that this is due to "buffering" in
the regulation of $E(spl)$ activity, probably because of other still unknown genes with a role similar to that of $H$.

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