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Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*

(cell commitment/cell communication)

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ABSTRACT **In embryos of *Drosophila melanogaster* the development of a pluripotent cell in the neurogenic ectoderm as a member of either a neural or an epidermal lineage depends on its interactions with neighboring cells. Certain genes, designated neurogenic, participate in this process in that there is a deficiency of epidermal histotypes in mutant embryos lacking neurogenic gene functions. To test the cell autonomy of expression of the neurogenic phenotype, individual cells were transplanted from the neurogenic ectoderm of mutant donor embryos into wild-type host embryos. Cells transplanted from donors homozygous for any of several mutant alleles of the neurogenic genes *amx, N, bib, mam, neu,* and *Dl* were found to give rise to clones exhibiting a distribution of neural and epidermal histotypes similar to that of the wild type. By contrast, cells transplanted from donors homozygous for loss of the neurogenic *E(spl)* gene gave rise exclusively to clones of neural histotypes. Thus, only the expression of *E(spl)* is autonomous, with that of all of the other tested neurogenic genes being nonautonomous. These results are consistent with the inference that the nonautonomous genes provide a source and the autonomous gene provides a receptor of a hypothetical intercellular regulatory signal that is necessary for cell commitment to an epidermal rather than neural fate.**

Pluripotent ectodermal cells of *Drosophila melanogaster* segregate at two different stages of embryonic development to take on one of two different fates. During early stages of embryogenesis, 25% of the potentially neurogenic (NG) ectodermal cells develop as neuroblasts, whereas the remainder develops as epidermoblasts (1–3). A second segregation of developmental fate occurs at a later stage, after germ band shortening, when cells of the epidermal primordium differentiate into either sensory organs or nonsensory epidermis (4, 5). The earlier and the later segregation proceed in a continuum of cells, which, in spite of their similar environments, take on fundamentally different fates. Experimental evidence provided by cell transplantation experiments has shown that in *Drosophila* cell–cell interactions play a role in the commitment of NG ectodermal cells to alternative fates (6). Similar interactions have also been described for the corresponding process in grasshoppers (7, 8).

The function of several genes is known to be required for the normal segregation pattern of ectodermal cell fates. Some of these genes are generically designated as NG (9) because in embryos that carry loss-of-function alleles of any of them, most of the cells of the ectoderm develop the neural rather than the epidermal fate; this failure to develop the epidermal fate leads to strongly hyperplasic central and peripheral nervous systems (CNS and PNS, respectively) and to a lack of almost the entire epidermis (5, 9, 10). By contrast, in embryos that carry gain-of-function alleles, or multiple copies of the wild-type allele, of the NG gene Enhancer of split [*E(spl)*], CNS and PNS are both hypoplasic (11). Apparently the NG genes mediate a signal required for the commitment of cells to development of an epidermal rather than neural fate.

Since mutations in different NG genes produce identical phenotypic defects, it seems likely that these genes are functionally related. Indeed, the results of genetic analyses are consistent with the NG genes forming a chain of epistatic relationships (ref. 12; unpublished). Further support for such a relationship is provided by molecular analyses. Some NG genes have been cloned (13–16), and structural data about their products are becoming available. Structural homology has been found between the proteins encoded by *N* and *Dl* (16, 17) that may be the basis for their functional relationship. Homology of these products to epidermal growth factor (EGF) suggests a possible involvement in intercellular communication. Since the elements of cell communication processes generally belong to either of two categories, signal source and signal receptor, the fate of a cell transplanted from the NG ectoderm of a NG mutant donor to that of a wild-type host would differ depending on whether the mutation interferes with the source or the receptor function. In view of the number of different NG genes that have been identified, it seemed possible that some of their available mutants belong to the source and some belong to the receptor category.

In this report we present evidence supporting the existence of these two categories. Most of the NG genes whose mutants we have tested appear to belong to the signal source category, since ectodermal cells of such mutants do not express the mutant phenotype autonomously when transplanted to the neurogenic region of a wild-type host. However, in one case—namely, NG mutants lacking *E(spl)* gene function—the mutant phenotype is expressed autonomously, indicating that this gene may belong to the signal receptor category.

MATERIALS AND METHODS

Strains. Alleles of the NG genes *amx, N, bib, mam, neu, Dl,* and *E(spl)* were used for cell transplantations. The following stocks provided donor embryos: $w^{a}N^{55e11}/FM7$, $amx^{1}f/ClB$, $amx^{1}f/ClB$; $Tl/+$, $bib^{ID05}cn\ bw/CyO$, $cn\ mam^{217.4}bw/CyO$, $neu^{IF65}/TM3\ Ser$, $Df(3R)Dl^{FX3}/TM3\ Sb\ Ser$, $h\ st\ Dl^{9P39}e/TM3\ Ser$, $e\ Df(3R)E(spl)^{8D06}/TM3\ Sb\ Ser$, $e\ Df(3R)E(spl)^{B251}/TM3\ Sb\ Ser$ (see ref. 18 for balancer chromosomes and marker mutations). Host embryos were of Oregon R wild-type stock. Standard conditions of culture were used throughout this study.

Cell Transplantations and Preparation of Embryos. These procedures were carried out as described (19). In short, before cell formation the center of donor embryos was injected with 5–10 nl of a 2:1 mixture of 10% horseradish peroxidase (HRP) and 10% fluorescein isothiocyanate-dex-

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Abbreviations: NG, neurogenic; CNS, central nervous system; PNS, peripheral nervous system; EGF, epidermal growth factor; HRP, horseradish peroxidase; FITC, fluorescein isothiocyanate.

tran (FITC-dextran; $M_r \approx 20,000$) as cell lineage tracers. The injected tracers became incorporated into the embryonic cells upon cell formation and cell closure. Eight to 12 min after the onset of gastrulation cells were removed from the ventral (0–30% of the ventrodorsal axis) sector of the NG region of the donors, at about 50% egg length, and singly transplanted to the corresponding region of unlabeled wild-type hosts of the same age. FITC-dextran allowed viewing cells immediately after transplantation in living embryos using epifluorescence, to ascertain that only a single cell had been transplanted.

Whole-mount staining for HRP was carried out in host embryos upon completion of head involution. The phenotype of the donors was checked after completion of development by staining with fuchsin (20).

Germ-Line Clones. Two different experiments were performed to study the effect of maternal gene expression on the commitment of transplanted ectodermal cells. Hemizygous N^- embryos developing in the absence of maternal *N* products were obtained by using x-ray-induced mitotic recombination (21, 22). $w^aN^{55e11}/FM7$ females were crossed with $Fs(1)ovo^{D1}/Y$ males; these males carried a dominant mutation causing female sterility (22). Larvae (36 ± 12 hr) were x-irradiated at a dose of 1000 rad (1 rad = 0.01 gray). Flies were left in the same vials after eclosion, where females mated with their sibling *FM7/Y* males. All females are agametic, except those in which mitotic recombination took place to remove the *Fs* allele and to introduce the *N* allele in the same cell (22). Females laying eggs were separated to obtain donor embryos. Approximately 50% of the embryos derived from this experiment are w^aN^{55e11}/Y, which developed without maternal gene expression. They were used as donors for transplantation of ectodermal cells. Homozygous $E(spl)^-$ embryos developing in the absence of maternal *E(spl)* products were obtained by using the technique described in ref. 23. Pole cells were transplanted isochronically from donors homozygous for $Df(3R)E(spl)^{8D06}$ into female hosts of the genotype $Fs(1)ovo^{D1}/+$. The resulting females were crossed with $Df(3R)E(spl)^{8D06}/+$ males to obtain homozygous $Df(3R)E(spl)^{8D06}$ embryos. All female hosts are agametic, with the exception of those that had successfully incorporated pole cells. Homozygous mutant embryos were then used as donors of ectodermal cells for transplantations.

RESULTS

Developmental Potential of Transplanted Wild-Type Cells. Cells from the NG ectoderm of wild-type donors isotopically transplanted into wild-type hosts gave rise to three main classes of histotypes. Morphological and topological criteria were used to classify the histotypes (ref. 6; see Fig. 2). Clones found within the CNS were classified of neural histotype if they exhibited processes growing out of a cluster of HRP-labeled cells (see Fig. 2*a*). Additional criteria were size and shape of the cells forming the processes and number of labeled cells. Neural cells have small, round perikarya; clones of neural cells may comprise >20 cells. Several clones were found within the CNS that did not exhibit processes, although they probably were neural. All of these cells were weakly stained, so that their failure to show characteristic processes was most likely due to their low HRP content. Only clones of cells of epidermal histotype were found to be integrated in the epidermis; they comprised as many as 8 flat cells, predominantly oriented in a dorsoventral direction (see Fig. 2*c*). In some cases epidermal clones comprised sensory organs. We also found a relatively high number of clones of mixed histotypes, comprising neural cells within the CNS and epidermal cells within the epidermis. Finally, several clones developed muscle histotypes (ref. 6; see control clones in Table 1). Mesodermal cells may often inadvertently be removed together with the ectodermal cells, due to the ventromedial level of origin of the transplanted cells. Since mesodermal cells are known to be already committed to mesodermal fates at this early stage of development (24), it is very likely that mesodermal cells gave rise to these muscle cell clones.

Developmental Potential of Transplanted Mutant Cells. Donor embryos homozygous for NG mutations were used in a second series of experiments otherwise identical to the first. These experiments were performed to test the cell autonomy of expression of NG genes. Two conditions must be met for the correct interpretation of the transplantation experiments to be presented here: (*i*) only single donor cells are actually transplanted; (*ii*) the transplanted donor cells are actually of the alleged genotype. The first condition was met by observing the transplantation directly under differential interference-contrast optics. Since the transplanted cells contained FITC, in addition to HRP, the inferred number of transplanted cells was confirmed by fluorescence microscopy. The second condition was met by studying the phenotype of the fully developed donor embryos. All embryos derived from *amx* parents are appropriate donors since *amx* is a maternal effect mutant (9, 25). Mutants of the other NG genes, however, have predominantly zygotic expression; since the NG phenotype is recessive, only 25% of the embryos derived from heterozygotes over balancer chromosomes are appropriate donors. Appropriate donor embryos cannot be distinguished from their inappropriate siblings at the time of transplantation. However, the neural hyperplasia developed by the 25% of appropriate donor embryos can be distinguished without ambiguity in the fully differentiated, fuchsin-stained embryos from the remaining 75% of inappropriate embryos that develop the normal phenotype. Since the latter carry, in all cases, wild-type alleles of the NG genes, the cell clones derived from nonneuralized donors served as wild-type controls (see above). The distribution of histotypes derived from these cells was identical to that of an additional set of wild-type control clones, available from previously studied material (6).

Table 1 and Fig. 1 present the distribution of histotypes. With the exception of experiments 9 and 10 (Table 1), donor mutant cells were found to yield epidermal progeny cells upon transplantation into wild-type hosts. Each of the various histotypes was found in proportions comparable to the ones of wild-type controls—i.e., the majority of clones developed the neural fate; the epidermal fate developed in roughly one-third to one-fourth of the cases. Some clones had cells in the epidermis and in the CNS; a few clones had cells forming part of sensory organs, together with other epidermal cells. A number of clones developed muscle histotypes. The size of clones derived from homozygous mutant cells was comparable to that of clones derived from wild-type cells (data not shown)—that is, neural cell clones within the CNS (Fig. 2*b*) comprised between 1 and 28 cells; epidermal cell clones comprised up to 8 cells (Fig. 2 *d* and *e*).

Experiment 4, in which the donor cells were derived from amx^1/amx^1; *Tl*/+ females, is noteworthy. *Tl* is a dominant maternal effect mutation that causes ventralization of all eggs produced by heterozygous females. When such females are homozygous for the NG mutation amx^1 as well, none of the offspring embryos develops any epidermis, since their ectoderm becomes completely neuralized (26). However, upon transplantation into wild-type hosts, the ectodermal cells of such embryos give rise to epidermal histotypes to the same extent as other NG mutants or as the wild-type controls. Upon transplantation of *bib* homozygous cells (experiment 5), no mixed neural-epidermal clones were found. Yet, the number of these clones found in the other experiments was highly variable; thus, it is difficult to assess the significance of this particular result. Upon transplantation of $E(spl)^-$ cells (experiment 9), the overwhelming majority of

Table 1. Distribution of clones according to histotype

	Genotype		Histotype*						
			CNS						
Exp.	Donor	Host	Proc.	No Proc.	Epid.	Epid. + CNS	Epid. + SO	Muscle	None
1	N^{55e11}	*wt*	24 (53)	3	6 (13)	12 (27)	3 (7)	—	26
	Control†	*wt*	24 (47)	8	12 (23)	12 (23)	3 (6)	2	56
2§	N^{55e11}	*wt*	23 (66)	4	8 (23)	3 (8)	1 (3)	11	37
	Control	*wt*	17 (63)	9	6 (22)	4 (15)	—	8	24
3	amx^{1}	*wt*	19 (57)	11	7 (21)	7 (21)	—	29	120
4	amx^{1}-*Tl*	*wt*	27 (66)	6	11 (27)	3 (7)	—	3	80
5	bib^{ID05}	*wt*	24 (75)	8	7 (22)	—	1 (3)	5	55
	Control	*wt*	42 (63)	8	13 (19)	11 (16)	1 (2)	5	107
6	$mam^{217.4}$	*wt*	17 (53)	4	7 (22)	7 (22)	1 (3)	5	53
	Control	*wt*	35 (55)	5	18 (28)	10 (16)	1 (1)	2	74
7	neu^{IF65}	*wt*	10 (55)	—	6 (33)	2 (11)	—	—	8
	Control	*wt*	24 (43)	8	20 (36)	12 (21)	—	1	51
8‡	Dl^{9P39}	*wt*	5 (42)	4	5 (42)	2 (16)	—	2	21
	Control	*wt*	14 (61)	7	4 (17)	4 (17)	1	8	45
	Dl^{FX3}	*wt*	6 (46)	3	4 (31)	3 (23)	—	4	34
	Control	*wt*	14 (42)	10	14 (42)	4 (12)	1 (3)	11	48
9‡	$E(spl)^{8D06}$	*wt*	38 (95)	9	1 (2.5)	1 (2.5)	—	5	49
	Control	*wt*	43 (49)	19	22 (25)	21 (24)	2 (2)	4	116
	$E(spl)^{B251}$	*wt*	15 (100)	3	—	—	—	—	20
	Control	*wt*	6 (35)	5	6 (35)	4 (23)	1 (6)	—	30
10§	$E(spl)^{8D06}$	*wt*	52 (100)	5	—	—	—	1	62
	Control	*wt*	47 (52)	4	30 (33)	14 (15)	—	3	106

CNS, clones within the CNS; Epid., epidermis; None, no differentiation; Proc., neural processes; SO, sensory organs.
*Percentages are given in parentheses.
†Control clones were provided by the nonmutant siblings of each case.
‡Two different *Dl* and *E(spl)* alleles were used.
§Donor embryos developed in the absence of maternal expression of the respective gene from homozygous mutant germ-line cells.

clones developed neural histotypes; only 1 epidermal and 1 mixed epidermal-neural clone among 55 clones with ectodermal derivatives were found. This number of non-neural clones is very low compared to the other experiments

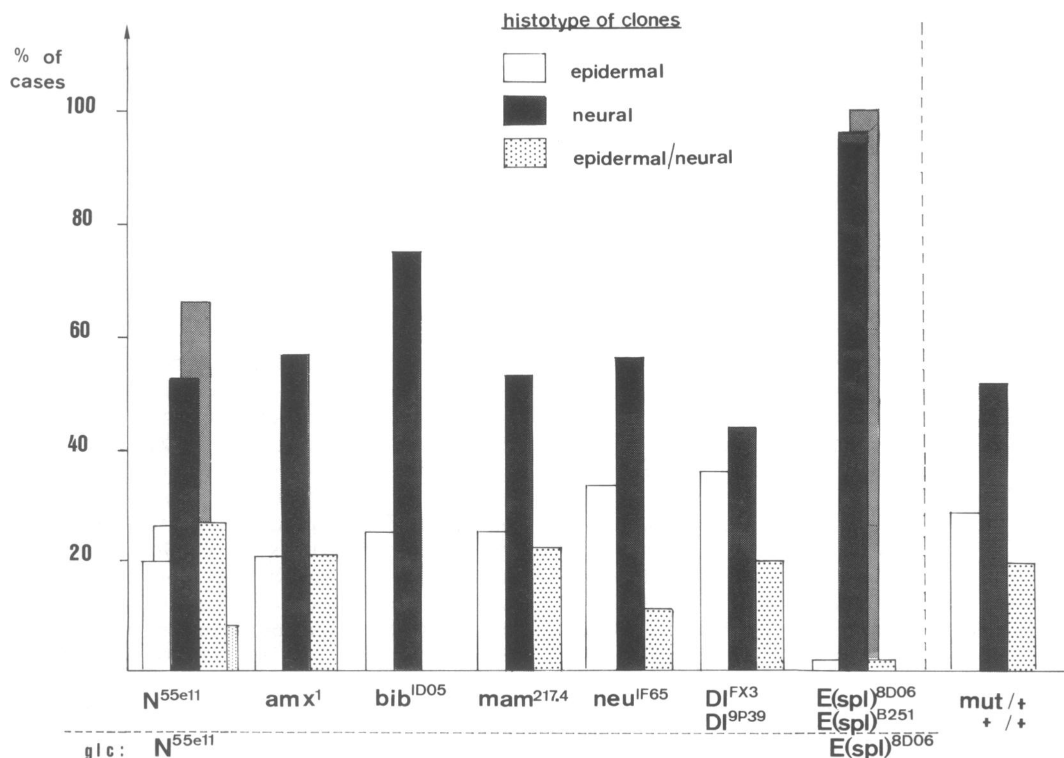

FIG. 1. Percentage (ordinate) of cell clones of epidermal, neural, and mixed epidermal/neural histotype (compare to Table 1). Genotypes of donor embryos are indicated on the abscissa. Control clones derived from cells of wild-type genotype (+/+) or cells heterozygous for any of the mutant alleles (*mut*/+). Columns in the background indicate the distribution of histotypes following transplantation of cells homozygous for either of the NG mutations, N^{55e11} or $E(spl)^{8D06}$, from donors having developed in the absence of maternal gene expression (germ-line clones, *glc*).

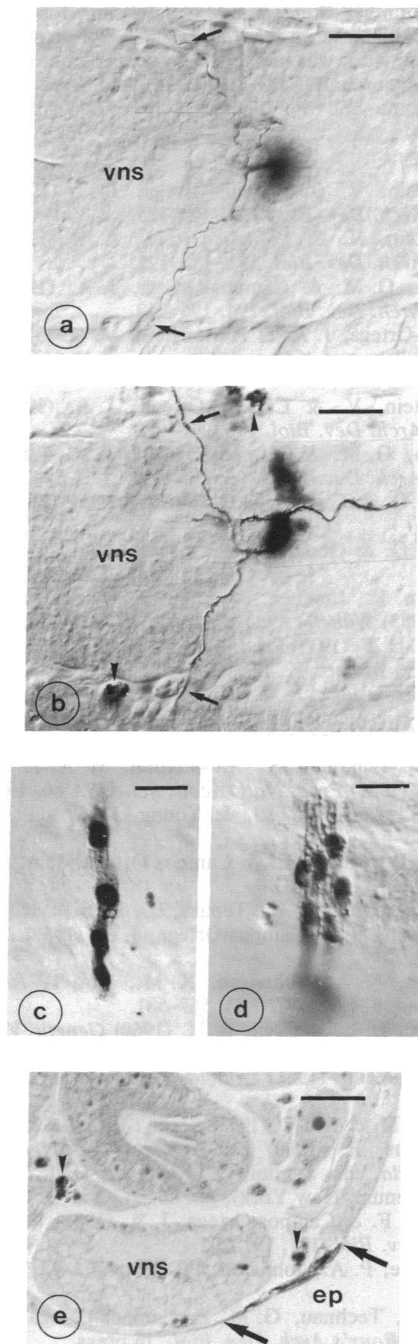

FIG. 2. Examples of HRP-labeled cell clones. (*a–d*) Whole mounts photographed with Nomarski interference optics. (*e*) A 2-μm-thick transverse section. The progenitors of these cell clones were taken from the ventral NG region of labeled donor embryos of different genotypes at the early gastrula stage; individual cells were isotopically and isochronically transplanted into unlabeled wild-type hosts. (*a* and *b*) Neural clones within the ventral cord, both including motor neurons projecting through segmental nerves (arrows). The clone in *a* developed from a wild-type progenitor cell; the clone in *b* developed from a cell hemizygous for the mutation N^{55e11}. The characteristics of both clones are comparable. (*c–e*) Clones of epidermal cells located ventrally. The clone in *c* contains four cells derived from a wild-type NG ectodermal cell. The clone in *d* contains eight cells (two of them are out of focus) derived from a cell homozygous for the mutation neu^{IF65}. Only two epidermal cells (arrows), of a clone of 6, are visible in the section shown in *e*, which derive from a cell homozygous for the mutation Dl^{FX3}. Arrowheads in *b* and *e* point to macrophages, presumably becoming labeled due to phagocytosis of extracellular HRP. ep, Epidermis. vns, Ventral cord. (Bars in *a*, *b*, and *e* = 20 μm; bars in *c* and *d* = 10 μm.)

and leads to the conclusion that cells homozygous for $E(spl)^-$ mutations can only rarely take on the epidermal fate.

Effect of Maternal Expression of NG Genes: Germ-Line Clones. NG genes are expressed during oogenesis (12, 22, 27, 28). In experiments 1 and 2, cells hemizygous for an N^- allele were transplanted. Experiment 2 was designed to test the role played by maternal N^+ gene products in the development of epidermal progenies. For this experiment, donors were used that were derived from eggs that developed in the absence of maternal gene expression—i.e., from homozygous N^-/N^- germ-line cells following induction of mitotic recombination (22). No influence of maternal N^+ products could be found: N^- cells devoid of maternal gene expression gave rise to epidermal histotypes at a frequency comparable to that of N^- cells with maternal expression. In experiment 10, $E(spl)^-$ embryos were used as donors that had developed in the absence of maternal gene expression—i.e., that were derived from pole cells homozygous for an $E(spl)^-$ mutation—following the transplantation of these pole cells into female sterile hosts (see *Materials and Methods*). Upon their transplantation into wild-type hosts, cells from donor embryos devoid of maternal expression gave rise to a total of 52 neural cell clones; no epidermal cell clone was found.

DISCUSSION

An essential assumption underlying the interpretation of our transplantation experiments is that their results reflect a normal development of the transplanted cells. At present, we cannot envisage any empirical proof of the validity of this assumption. However, it is possible to compare the fate of the transplanted cells with the known course of normal *Drosophila* embryogenesis (4). These comparisons (6, 19, 24, 29) provide no reason to believe that the transplantation process itself would give rise to abnormal developmental potencies of the transplanted cells. In all cases the transplanted cells followed fairly closely the known course of normal development.

Granted this essential assumption, it can be concluded from the present study that the mutant phenotype of all NG genes, except *E(spl)*, is expressed nonautonomously. When mutant donor cells are transplanted into a wild-type host, the surrounding host cells supply the mutant donor cells with whatever signal they lack in their original environment, thus enabling them to take on the epidermal fate. In previous studies, adult flies carrying genetic mosaics of heterozygous and homozygous cells for various mutant alleles of *N, bib, mam, neu, Dl*, and *E(spl)* had been investigated (27). Morphogenetic abnormalities or cell lethality were found associated with homozygosity for all mutant alleles, except for *bib* mutant alleles. These findings were interpreted to mean that the products of these genes, except of *bib*, are unable to diffuse over long distances, being compatible with a cell autonomous expression of the mutant phenotypes of these genes. The study of embryos that carried mosaics of hemizygous N^- and heterozygous N^+/N^- cells has recently led to the same conclusions (30). However, the abnormalities described in these earlier reports affected whole organs rather than single cells. Hence, these results did not allow one to decide whether or not phenotypic expression is autonomous at the level of single cells. By contrast, the transplantation experiments presented here permit the resolution at the level of single cells. Our results indicate that the mutant genotype of the transplanted cell can be complemented by the surrounding wild-type tissue—i.e., the expression of the mutant phenotype is *not* cell autonomous. However, in view of the earlier observations, the present results indicate that immediate contact between wild-type and NG mutant cells is necessary for expression of wild phenotype of mutant genotype cells. P. E. Hoppe and R. J. Greenspan (personal communication) have recently confirmed the nonautono-

mous phenotypic expression of the *N* gene. They found that clones of one or two cells homozygous for N^- mutations, which are surrounded by wild-type cells, may differentiate as epidermal cell clones at ventral levels of the embryo. A similar observation had already been made by Wieschaus and Gehring on "*in vivo*" cultures of a mixture of N^- homozygous and wild-type cells (31).

The present results are compatible with the conclusion that some NG genes function in providing a source, and others function in providing a receptor for the signal that leads to commitment to the epidermal fate of the NG ectodermal cells. Upon transplantation into wild-type tissues, mutant cells lacking the source function of the signal, but with a normal receptor function, should still be able to take on an epidermal fate in response to a signal provided by the surrounding wild-type cells. Mutant cells lacking the function of any of the six NG genes *amx, N, bib, mam, neu*, and *Dl* were found to act in this way. By contrast, mutant cells lacking the receptor function mechanisms should not be able to respond to the wild-type environment and they should therefore be unable to take on an epidermal fate. Except for two clones, mutant cells lacking the function of the NG gene *E(spl)* were found to act in this latter way.

These two exceptional $E(spl)^-$ clones could be the result of an experimental error, for example, in diagnosing the genotype of the donor embryos, or, alternatively, they could be due to the maternal component known to be associated with the expression of *E(spl)* (11, 12). Since the NG mutations are normally maintained in heterozygosity, maternal gene products contained in the eggs may have led to the formation of epidermal progenies in some cases. N^- cells derived from homozygous germ-line cells that are completely devoid of the corresponding gene product were found to produce epidermal progenies as frequently as N^- cells derived from heterozygous germ-line cells (Fig. 1). Since our transplantation experiment is designed to test the abilities of mutant cells to react to the wild-type environment, no difference due to maternal expression should be expected for N^- cells if these mutant cells lacked the signal source function but not the signal receptor function. However, the situation is different for a gene such as *E(spl)*, which we infer to participate in the signal receptor function. Here, small amounts of gene product in the egg may already be sufficient for some mutant cells to take on the epidermal fate upon transplanting them into a wild-type environment. Cells transplanted from donors having developed in the absence of maternal *E(spl)* products gave rise exclusively to neural clones. This suggests that the two epidermal clones observed as progeny of $E(spl)^-$ cells may have arisen through rescue by the maternal component.

Two NG genes, *N* (17, 32) and *Dl* (16), have been found to encode proteins containing several peptide sequence repeats homologous to EGF and other proteins of mammals. Although the function of the EGF-like repeats is unknown, Wharton *et al.* (17) note that the structure of the *N* gene product suggests a molecular basis for processes of cell communication. The study of genetic mosaics, discussed above (27, 30), indicates that the products of the NG genes, with the exception of *bib*, do not diffuse over long distances. This argues against processing of the EGF-like repeats into diffusible peptides, as is the case for the EGF precursor of mammals. Our present results are compatible with the proposed extracellular location of the EGF-like repeats on the membrane of ectodermal cells (17), where they may interact with neighboring cells.

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