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Genetic Mechanisms of Early Neurogenesis in *Drosophila melanogaster*

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Abstract

The neurogenic ectoderm of *Drosophila melanogaster* consists of the ventral neuroectoderm and the procephalic neuroectoderm. It is hypothesized that epidermal and central neural progenitor cells separate from each other in three steps: conference on the neuroectodermal cells the capability of producing neural or epidermal progenies, separation of the two classes of progenitor cells, and specification of particular types of neuroblasts and epidermoblasts. Separation of neuroblasts and epidermoblasts is controlled by proneural and neurogenic genes. *Delta* and *Notch* serve as mediators of direct protein–protein interactions. E(SPL)-C inhibits neurogenesis, creating epidermal cells. The achaete-scute complex (AS-C) controls the commitment of nonoverlapping populations of neuroblasts and leads the development of neuroectodermal cells as neuroblasts.

Index Entries: *Drosophila melanogaster; Delta; Notch;* E(SPL)-C; AS-C.

Introduction

In insects, the cells of the central nervous system (CNS) are generated by the proliferation of progenitor cells called neuroblasts, which develop from a special neurogenic region of the ectoderm. In *Drosophila melanogaster*, the neurogenic ectoderm consists of two parts, the ventral neuroectoderm, from which the ventral cord and the subesophageal ganglion will develop, and the procephalic neuroectoderm, from which the brain hemispheres emerge. Both regions give rise to neural progenitor cells; however, whereas the cells of the ventral neuroectoderm have to decide between developing either as neuroblasts or as epidermoblasts (progenitor cells of the epidermis), there is no clear picture as to how the procephalic neuroectoderm is organized and how neuroblasts develop from this region (Hartenstein and Campos-Ortega, 1984; Technau and Campos-Ortega, 1985; Jürgens et al., 1986; Stüttem and Campos-Ortega, 1991). Accordingly, this review will deal with the ventral neuroectoderm only.

In insects, the sensory organs develop from special cells called sensory organ mother cells, which originate within the epidermis in a pro-

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cess analogous to that of the segregation of the neuroblasts. A hypothesis has been formulated to account for the development of the sensory organ mother cells in the epidermis of *Drosophila* (Ghysen and Dambly-Chaudière, 1989; ref. to Ghysen et al., 1993 for a recent review). Appropriately modified, this hypothesis can also be applied to the separation of neuroblasts and epidermoblasts in the neuroectoderm. The hypothesis proposes a sequence of three steps to explain how epidermal and central neural progenitor cells separate from each other. In the first step, all cells of the neuroectoderm acquire the capability to develop as neuroblasts, whereby contiguous groups of about four to five cells, so-called proneural clusters, are each enabled to give rise to a particular type of neuroblast. In the second step, one cell in each group is singled out by intervening neuralizing signals and segregates into the space between ectoderm and mesoderm to develop as a particular type of neuroblast. In the third step, the neuroblast sends signals to the surrounding cells preventing them from following a neural fate and permitting them to assume an epidermal fate. Therefore, three operations are included in this scheme; one confers on the neuroectodermal cells the capability to produce neural or epidermal progenies, another permits the separation of the two classes of progenitor cells, and the third specifies particular types of neuroblasts and epidermoblasts.

Genetics of Early Neurogenesis

The correct separation of neuroblasts and epidermoblasts is controlled by two groups of genes, the neurogenic and the proneural genes, the products of which form a complex genetic network (Table 1). Poulson (1937) called *Notch* a "neurogenic" gene following the convention in *Drosophila* genetics of naming a gene according to the phenotype of the mutation that leads to its discovery. Accordingly, other genes that cause the same embryonic phenotype as *Notch* have also been called neurogenic (Lehmann et al., 1981, 1983; Jiménez and Campos-Ortega, 1982). However, the functions of neurogenic genes promote epidermal development. Contrarily, "proneural" are genes whose functions promote neural development (Ghysen and Dambly-Chaudière, 1989, 1990; Romani et al., 1989). With respect to CNS development, the members of the achaete–scute complex (AS-C) and ventral nervous system condensation defective *(vnd)*, and probably other as yet unidentified genes (Jiménez and Campos-Ortega, 1979, 1987, 1990; White, 1980; White et al., 1983) are proneural genes.

Evidence from various kinds of genetic analyses indicates that the neurogenic loci are linked in a chain of epistatic relationships, in which the E(SPL)-C is the last link (Vässin et al., 1985; de la Concha et al., 1988; Shepard et al.,1989; Brand and Campos-Ortega, 1990). Hence, the function of each of these genes is dependent on that of another member of the group and, consequently, the function of the entire chain is perturbed if any of the links is missing. Loss-of-function of any of the neurogenic genes causes most ectodermal cells to develop as neuroblasts. Neuralization of the ectoderm of neurogenic mutants follows the pattern of neuroblast segregation in the wild-type and proceeds in pulses (Campos-Ortega and Haenlin, 1992). In the mutants, all the neuroectoderm cells from which neuroblasts normally segregate at each pulse take on neural fate until, in mid-stage 11, all cells in the neuroectoderm have adopted neural fate. Regions from which larval sensory organs develop also exhibit a high proportion of neural cells (Hartenstein and Campos-Ortega, 1986; Ghysen and Dambly-Chaudière, 1990; Goriely et al., 1991). Therefore, the wild-type functions of the neurogenic genes are formally required to suppress neural development of a large fraction of ectodermal cells and allow them to develop as epidermoblasts. The basis for this is a signal chain, the links of which are encoded by the neurogenic genes.

Embryos homozygous for loss-of-function mutations in the proneural genes exhibit a highly hypoplasic CNS and severe defects in the PNS (Jiménez and Campos-Ortega, 1979,

Table 1
Genes Involved in Neurogenesis in *Drosophila melanogaster*

Genes	Loss-of-function phenotype	Gene product	Possible function
Proneural genes			
AS-C	Neural hypoplasia	bHLH	Regulation of transcription
vnd	Neural hypoplasia	?	?
daughterless	Neural hypoplasia	bHLH	Regulation of transcription
Neurogenic genes			
Notch	Neural hyperplasia	Transmembrane EGF-like repeats	Adhesion signal receptor
Delta	Neural hyperplasia	Transmembrane EGF-like repeats	Adhesion signal source
E(SPL)-C	Neural hyperplasia	bHLH	Regulation of transcription
master mind	Neural hyperplasia	Nuclear protein	?
neuralized	Neural hyperplasia	Homeobox Zn finger	Regulation of transcription?
big brain	Neural hyperplasia	Transmembrane	Transporter?
shaggy	Neural hyperplasia	Serine-threonine kinase	?
groucho	Neural hyperplasia	Nuclear protein	Regulation of transcription?

1987, 1990; White, 1980; Dambly-Chaudière and Ghysen, 1987; Brand and Campos-Ortega, 1988; Ghysen and Dambly-Chaudière, 1988; Caudy et al., 1988a). Embryos lacking the AS-C or *vnd* initiate neurogenesis with less than the normal complement of neuroblasts: 20–25% of all neuroblasts are missing (Jiménez and Campos-Ortega, 1990); mutants lacking both the AS-C and *vnd* lack up to 50% of all neuroblasts. In addition, during later stages, large numbers of cells degenerate within the neural primordium of all these mutants (Jiménez and Campos-Ortega, 1979,1990; White, 1980; Brand and Campos-Ortega, 1988). However, the CNS of these mutants still contains a significant number of neurons, indicating that still other genes are necessary for neuroblast commitment. Brand and Campos-Ortega (1988) found that the epidermal sheath of neurogenic mutants is larger when they also lack the AS-C; this suggests that at least some of the cells that fail to develop as neuroblasts in the mutants develop as epidermoblasts instead.

Physical Interactions of *Notch* and *Delta*

Notch and *Delta* encode transmembrane proteins with EGF-like repeats in their extracellular domains (Wharton et al., 1985; Kidd et al., 1986; Vässin et al., 1987; Kopczynski et al., 1988; Haenlin et al., 1990). The *Notch* protein is ubiquitously distributed during early developmental stages, although there are quantitative differences between cells suggesting a role for

Notch in epidermogenesis (Johansen et al., 1989; Kidd et al., 1989; Fehon et al., 1991; *see also* Hoppe and Greenspan, 1986, 1990). Transcription of *Delta* is spatially regulated; however, during neuroblast segregation *Delta* RNA, as well as protein, are present in all ventral neuroectodermal cells in apparently equal amounts (Vässin et al., 1987; Kopczynski and Muskavitch, 1989; Haenlin et al., 1990; Kooh et al., 1993).

Various pieces of experimental evidence support the view that *Delta* and *Notch* are essential parts of a cell communication pathway by mediating direct protein–protein interactions; *Notch* acts as multifunctional receptor. First, mutations at the *Notch* locus with different phenotypes, such as *split* and several *Abruptex* alleles, each differ from the *Notch* wild-type protein by single amino acid exchanges in specific, distantly separated EGF-like repeats, i.e., 14th with respect to *split*, and 24th, 25th, 27th, and 29th with respect to *Abruptex* alleles (Hartley et al., 1987; Kelley et al., 1987). Two alleles of *Delta* suppress the phenotype of the *split* mutation but do not modify the phenotype of *Abruptex* alleles (Brand and Campos-Ortega, 1990). Both *Delta* alleles are associated with single amino acid exchanges in the 4th and 9th EGF-like repeat, respectively (Lieber et al., 1992). Biochemical evidence provides support for a direct association of *Notch* and *Delta* and shows that, indeed, both proteins may mediate cell adhesion (Fehon et al., 1990; Rebay et al., 1991; Lieber et al., 1992). *Notch*-mediated cell adhesion has been found to depend on EGF-like repeats 11 and 12 (Rebay et al., 1991); no EGF-like repeat of *Delta* is required for adhesivity (Shepard and Muskavitch, as quoted by Lieber et al., 1992). Yet, the region of interaction between both proteins appears to be much larger. Schneider cells expressing either *split* or an *Abruptex* variant (Ax^{E2}) are still capable of adhering to cells expressing one of the *Delta* proteins that suppress *split* ($Delta^{sup5}$) with the same kinetics as cells expressing wild-type forms of *Notch* and *Delta*. However, the mutant proteins cannot compete with the corresponding wild-type proteins in cell aggregation assays, suggesting that their adhesivity is somehow impaired (Lieber et al., 1992). Suppression of *split* by $Delta^{sup5}$ is not compatible with a mechanism based on cell adhesion in vivo, since no reversion of the diminished binding activity of *split* is observed after aggregation with cells expressing the *Delta* suppressor protein. This result suggests that intracellular signaling by *split* relevant to compound eye development is mediated by specific parts of the *Notch* protein, different from those required to mediate cell adhesion.

One possible mode of direct interaction between *Notch* and *Delta* at the cell membrane involves a receptor–ligand relationship. The available evidence points to *Notch* as a receptor and *Delta* as the source of regulatory signals required for epidermogenesis (*see* Dietrich and Campos-Ortega, 1984; Hoppe and Greenspan, 1986,1990; Technau and Campos-Ortega, 1987; de Celis et al., 1991; Heitzler and Simpson, 1991, 1993; Lieber et al., 1993; Struhl et al., 1993).

The Epidermal Decision Is Controlled by the E(SPL)-C

The genetic analysis shows that the functions encoded by the E(SPL)-C are epistatic over those of the other neurogenic genes. This is compatible with the assumption that this locus is responsible for the epidermalizing function, i.e., the inhibition of neurogenesis, which is ascribed to the whole group of neurogenic genes (Vässin et al., 1985; Knust et al., 1987b; de la Concha et al., 1988). The E(SPL)-C comprises seven partially redundant, ancestrally related genes, called *HLH-mδ, HLH-mγ, HLH-mβ, HLH-m3, HLH-m5, HLH-m7,* and *E(spl)* (Knust et al., 1987b, 1992; Klämbt et al., 1989; Delidakis and Artavanis-Tsakonas, 1992; Schrons et al., 1992). The functional redundancy of these genes is owing to at least two factors.

First, immediately before and during SI and SII neuroblast segregation, transcripts from all genes of the complex but *HLH-m3* exhibit virtually identical spatial distributions, matching

remarkably well the regions of the neuroectoderm from which the neuroblasts segregate (Knust et al., 1987b, 1992). Second, sequencing of genomic and cDNA clones has shown a high degree of sequence similarity in the proteins encoded by the seven genes of the E(SPL)-C, which are all seven members of the bHLH family of transcriptional regulators (Klämbt et al., 1989; Knust et al., 1992; Tietze et al., 1992; Oellers et al., 1994).

The Neural Decision Is Controlled by the Proneural Genes

The AS-C includes four genes; *achaete, scute, lethal of scute,* and *asense,* the names being derived from the phenotypic effects of their mutations on bristle development and viability (Muller, 1935; García-Bellido, 1979; Ghysen and Dambly-Chaudière, 1988). The spatial distribution of *achaete, scute,* and *lethal of scute* transcripts in the neuroectoderm is similar for all three and shows a high degree of correlation with the process of neuroblast segregation (Cabrera et al., 1987; Romani et al., 1987; Brand and Campos-Ortega, 1988; Cabrera, 1990; Martin-Bermudo et al., 1991; Skeath and Carroll 1992; Ruiz Gómez and Ghysen, 1993). During early neurogenesis, the three transcripts are expressed in clusters of cells within the VNE. One or two cells from among these clusters will delaminate as neuroblasts. After the segregation has occurred, RNA of these genes remains detectable in the neuroblasts for some time. Cabrera (1990) and Martin-Bermudo et al. (1991) have raised antibodies against *lethal of scute,* and Skeath and Carroll (1991, 1992) against *achaete* and *scute* and they find a similar correlation between accumulation of protein and neuroblast segregation. This pattern of expression is suggestive of a role for the AS-C genes in neuroblast commitment.

Sequence analyses have shown that *achaete, scute, lethal of scute,* and *asense* (Villares and Cabrera, 1987; Alonso and Cabrera, 1988: González et al., 1989), as well as *daughterless* (Caudy et al., 1988b; Cronmiller et al., 1989), encode proteins of the bHLH protein family. Murre et al. (1989a,b) showed that proteins with the basic domain and the HLH motif are able to form homodimers and heterodimers and bind to specific DNA sequences. These findings strongly suggest that all these proteins function in vivo as transcriptional regulators. A high degree of specificity and complexity in the regulatory functions of the corresponding genes may thus be achieved through the combination of different proteins to form heterodimers.

As mentioned earlier, AS-C$^-$ mutants have 20–25% fewer neuroblasts than the wild-type; similar findings have been made with *vnd* mutants. Since AS-C$^-$ and *vnd*$^-$ double mutants lack roughly 50% of all neuroblasts, the AS-C and *vnd* appear to control the commitment of nonoverlapping populations of neuroblasts. In addition, increasing the number of copies of the AS-C genes and of *vnd* by using duplications of the region leads to development of additional neuroectodermal cells as neuroblasts at the expense of epidermoblasts (Jiménez and Campos-Ortega, 1990). The complementary phenotypes caused by decreasing and increasing the number of copies and, presumably, the amount of product of the proneural genes, strongly support the hypothesis that these products are responsible for the development of neuroectodermal cells as neuroblasts. A similar role for these genes has been proposed with respect to the commitment of the sensory organ mother cells. It has been shown that, contrarily to the situation with the neuroblasts, the function of *achaete, scute,* and *asense* is prominent in sensory organ development of the larva and imago, whereas *lethal of scute* is apparently dispensable for this process (García-Bellido and Santamaria, 1978; García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987; Ghysen and Dambly-Chaudière, 1988, 1989; Ghysen and O'Kane, 1989; Rodríguez et al., 1990; Cubas et al., 1991).

Jarman et al. (1993) have found a new proneural gene, *atonal,* which also encodes a bHLH protein and has a function complementary to that of the AS-C. The gene *atonal* is

essential for the commitment of the mother cells of internal sensory (chordotonal) organs and of the ommatidia of the compound eye. However, its function does not seem to be required for commitment of the neuroblasts.

Vässin et al. (1994) describe that sensory organ mother cells start differentiation in *daughterless* mutants, as judged from the fact that they express *scute* and delaminate from the ectodermal layer. However, these cells fail to express later neuronal markers and most likely die (Brand and Campos-Ortega, 1988). This means that *daughterless* function appears to be required after that of the AS-C genes. Consequently, commitment of sensory organ mother cells is considered to occur in at least two steps, the first one being AS-C dependent and the second *daughterless* dependent. According to these observations, *daughterless* does not seem to behave as a proneural gene in the same manner as the genes of the AS-C.

Proneural Genes Activate Neurogenic Genes

Evidence has been obtained that the AS-C genes and *daughterless* are functionally interconnected with the neurogenic genes within the same genetic network (Brand and Campos-Ortega, 1988, 1990; Cabrera, 1990; Skeath and Carroll, 1992; Ruiz-Gómez and Ghysen, 1993; Haenlin et al., 1994; Hinz et al., 1994, Kramatschek and Campos-Ortega, 1994; Kunisch et al., 1994; Oellers et al., 1994). Thus, the severity of the neurogenic phenotype of double mutants for neurogenic genes and deletions of the AS-C or *daughterless* is considerably reduced, as compared to the phenotype of the same neurogenic mutation alone, i.e., without the concomitant presence of a AS-C$^-$ or *daughterless* mutation. AS-C$^-$ mutations were found to be epistatic over the neurogenic mutations, suggesting that the function of the AS-C genes is downstream to that of the neurogenic genes in the functional chain (Brand and Campos-Ortega, 1988; Heitzler and Simpson, 1993).

The distribution of products from the AS-C genes in neurogenic mutants suggests that at least some of the interactions between neurogenic and AS-C genes involve regulation of the transcription of these genes. Changes in the pattern of transcription of the genes *lethal of scute* and *achaete* have been observed in embryos carrying any of several neurogenic mutations (Brand and Campos-Ortega, 1988; Skeath and Carroll, 1992; Ruiz-Gómez and Ghysen, 1993). In these embryos, the early expression of *lethal of scute* and *achaete* is indistinguishable from the wild-type. However, RNA is found in larger cell clusters than in the wild-type, probably because in the mutants, neuroblasts do not segregate from the proneural clusters. In the wild-type, a restriction of *lethal of scute* and *achaete* transcription occurs from an initial group of several neuroectodermal cells to one, or a few neuroblasts, as they segregate from the epidermoblasts; in neurogenic mutants, this restriction fails to occur. Cabrera (1990) has presented similar evidence with respect to *lethal of scute*, and Skeath and Carroll (1992) with respect to *achaete* proteins. All these observations indicate that the neurogenic genes define the normal expression domains of AS-C genes in that they suppress the transcription (or the accumulation) of RNA from at least *lethal of scute* and *achaete* in some of the neuroectodermal cells.

Molecular evidence, both in vitro and in vivo, for interactions between proneural and neurogenic genes, derives from the analysis of the promoters of *Delta* (Haenlin et al., 1994; Kunisch et al., 1994) and of *HLH-m5* and *E(spl)* (Kramatschek and Campos-Ortega, 1994; Oellers et al., 1994), as well as from observations after ectopic expression of *lethal of scute* (Hinz et al., 1994). These studies have revealed that proneural proteins activate transcription of *Delta*, *HLH-m5*, and *E(spl)* by means of binding to multiple sites distributed throughout their promoters. The activation of E(SPL)-C genes by proneural genes within the neuroectoderm is an early event, initiating the transcription of these genes in this particular region; the assumed function of this activation process will be discussed later. As mentioned

earlier, *Delta* encodes the presumptive epidermalizing signal molecule. The activation of its transcription occurs by binding of proneural proteins to specific boxes and leads to an enhancement of the epidermalizing functions of the *Delta* protein. This indicates that transcriptional activation of *Delta* by proneural proteins serves the function of increasing the efficacy of lateral inhibition in the prospective neuroblast during its singling out from the proneural cluster.

Conclusions

The emergence of neuroblasts and epidermoblasts from an undifferentiated anlage is the result of a rather complex set of operations arranged in three consecutive steps: acquisition of competence to assume the neural fate by neuroectodermal cells; selection of single cells from proneural clusters to take on the fate of particular neuroblasts; suppression of the primary neural fate by lateral inhibition of the remaining cells of the groups to enable their epidermal development (*see* Ghysen and Dambly-Chaudière, 1989, and Ghysen et al., 1993, with respect to sensory organ mother cell development). In analogy to the situation in the epidermis, where ectopic expression of proneural proteins confers epidermal cells the ability to give rise to sensory organs in ectopic positions (Rodríguez et al., 1990; Cubas and Modolell, 1992; Brand et al., 1993; Domínguez and Campuzano, 1993; Hinz et al., 1994), we assume that the competence to take on neural fate by the neuroectodermal cells depends on expression of proneural genes. However, for the neuroblasts this assumption is based on indirect evidence and a more stringent proof is still pending. Clusters of four to five cells are thus assumed to be enabled to give rise to a particular type of neuroblast, depending on the position of the cluster (Taghert et al., 1984; Doe and Goodman, 1985; Doe et al., 1988; Skeath et al., 1992). Neural competence and specification of neuroblasts and epidermoblasts are two different operations that may require the functions of different genes (Skeath et al., 1992; *see* Rodríguez et al., 1990, for sensory organ specification).

In the second step, one cell from each proneural cluster has to be singled out to become a neuroblast. The important element in this process seems to be the presence of a critical amount of proneural proteins in one of the cells of the clusters; however, it is unknown how this occurs, since all cells of the cluster are assumed to have initially the same amounts. Fluctuations in the content of proneural gene products, which would cause the predominance of these gene products in one cell and its entry into the neural pathway eliciting a chain of regulatory events *(see the following)*, may either occur in a cell-autonomous manner and/or be the consequence of information conveyed to the prospective neuroblast by way of its relations with its neighbors. Cubas and Modolell (1992) and Van Doren et al. (1992) have presented evidence that, within the epidermal proneural clusters, progenitor cells of the sensory organs develop in regions of minimal concentration of the product of *extramacrochetae*. Higher concentration of extramacrochetae would downregulate proneural proteins and impede neural development. Unfortunately, there is no evidence for a participation of *extramacrochetae* in neuroblast commitment. On the other hand, cell transplantation experiments suggest that signals with neuralizing character contribute to the neural decision of the neuroblast (Technau and Campos-Ortega, 1986; Stüttem and Campos-Ortega, 1991). Both mechanisms, i.e., decision-based on cell autonomy or mediated by cell interactions, are certainly not mutually exclusive and may well both be operative.

Finally, as far as the third step is concerned, there is good evidence that cell interactions are involved in development of the epidermoblasts. The decision of one cell from each proneural cluster to become a neuroblast is followed by lateral inhibition to impede neurogenesis in the remaining cells of the proneural clusters and permit in this way their epidermal commitment (Doe and Goodman,

1985; Technau and Campos-Ortega, 1986; Stüttem and Campos-Ortega, 1991). However, as with the neuroblasts, epidermogenesis may also be initiated autonomously, that is to say, by predominance of the products of the E(SPL)-C, and reinforced by lateral inhibition derived from the neuroblasts. In fact, observations of Lüer and Technau (1992) on the in vitro behavior of single cells from defined ectodermal regions indicate that epidermogenesis can in part be a result of a cell autonomous process.

There is increasing evidence that *Notch* acts in the neuroectoderm as a receptor for epidermalizing signals. Struhl et al. (1993) have recently described that truncated forms of the *Notch* protein, which contain only the intracellular domain, are constitutively active; neuroblasts initially do not develop in transgenic animals carrying these *Notch* variants. Similar variants have been made by Lieber et al. (1993), who showed that these variants require the genes of the E(SPL)-C to be effective. With respect to bristle development, de Celis et al. (1991) and, particularly, Heitzler and Simpson's results (1991, 1993) indicate that the amount of *Notch* present in a cell has an influence on its fate, less than normal leading to neural, more to epidermal development. All these observations are compatible with a function of *Notch* as a ligand activated receptor in both neuroblast and sensory organ mother cell segregation, its activation leading to epidermal development. Cell communication appears thus to be required to suppress expression of proneural genes in the cells normally developing as epidermoblasts.

The genetic analysis, the primary structure of the proteins encoded by the genes of the E(SPL)-C, and the results of cell transplantations (Technau and Campos-Ortega, 1987) are compatible with a function of the E(SPL)-C at regulating the specific genetic activities of the epidermoblasts. This regulation can be achieved at the transcription, at the posttranscriptional, or at both levels. Since *HLH-m5* and *E(spl)* are capable of binding to specific DNA sequences in vitro (Tietze et al., 1992; Oellers et al., 1994), this regulation may be mediated by direct binding to DNA; however, heterodimer formation with other bHLH proteins, impeding in this way their activity, is also conceivable. It seems improbable that proteins encoded by the E(SPL)-C activate directly transcription of the "realizator genes" (García-Bellido, 1975) of the epidermal pathway, i.e., the genes whose products eventually make the epidermal cells, since the complete deletion of the E(SPL)-C still permits epidermal development in the dorsal-most embryonic regions (Lehmann et al., 1983; Knust et al., 1987a). The data rather suggest that the E(SPL)-C regulates epidermogenesis indirectly, via the proneural genes (Fig. 1). Cell transfection assays show that addition of either *HLH-m5* or *E(spl)* to a specific DNA sequence in the *E(spl)* promoter, comprising an E-box and two N-boxes, reduces the transcriptional activation mediated by heterodimers between *lethal of scute* and daughterless bound to the E-box (Oellers et al., 1994). Therefore, the proteins encoded by the E(SPL)-C appear to act indirectly as negative regulators of proneural genes. Surprisingly enough, there is genetic and molecular evidence that the proneural proteins activate the transcription of the E(SPL)-C genes *E(spl)* and *HLH-m5*. in the neuroectoderm (Hinz et al., 1994; Kramatschek and Campos-Ortega, 1994). This activation of transcription is likely to be required to allow products of the E(SPL)-C genes to be present within the same neuroectodermal cells in which products of the proneural genes exist in order for them to interact.

Since the neurogenic phenotype of double mutants AS-C$^-$ and E(SPL)-C$^-$ is reduced as compared to that of the E(SPL)-C$^-$ mutants alone (Brand and Campos-Ortega, 1988), this suggests that the AS-C, as well as the other proneural genes, suppress the function of epidermal "realizator" genes: The deletion of the AS-C eliminates part of this suppressive effect and permits the development of some additional epidermis. This assumption, of course, implies that the elimination of the other, not yet discovered, proneural genes would cause the complete suppression of the neurogenic phenotype. I further assume that the proneu-

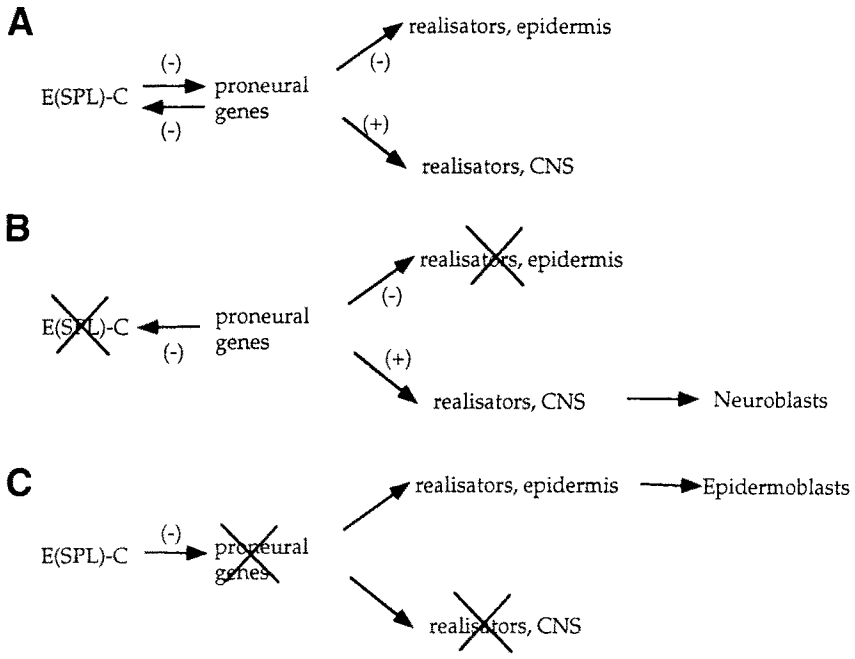

Fig. 1. A formal genetic model of the interrelationships between proneural and E(SPL)-C genes during neuroepidermal lineage segregation. **(A)** Proneural and E(SPL)-C genes are thought to functionally inactivate each other; proneural genes activate the neural and repress the epidermal "realizator" genes. If the balance between both groups of regulatory genes is perturbed, proneural **(B)** or E(SPL)-C genes **(C)** will predominate in the corresponding cell, leading to its development as a neuroblast or as an epidermoblast, respectively.

ral gene products activate in addition the neural "realizator" genes and thus permit neural development of the neuroblasts.

Cell determination in the neuroectoderm can thus be envisaged as the result of a delicate balance between the functional activity of the proteins encoded by the proneural genes and the E(SPL)-C. This balance is assumed to be modified as a result of signals derived from surrounding cells resulting in either the predominance of the proneural proteins or their functional suppression in any particular neuroectodermal cell and leading to its commitment to one of the developmental fates. In the cells destined to become neuroblasts, proneural gene products would activate the neural and suppress the epidermal "realizator" genes; in the other cells, the proneural gene products would be suppressed by the products of the E(SPL)-C. Proneural gene products in any given cell activate the synthesis of the molecular machinery to establish communication with neighboring cells (Hinz et al., 1994; Kramatschek and Campos-Ortega, 1994; Kunisch et al., 1994), whereby the efficacy of this machinery is assumed to depend on the amount of proneural products (Fig. 2). This initial communication would permit reception of neuralizing signals and thus reinforce and stabilize the neural pathway in a cell with a higher concentration of proneural gene products than the surrounding ells. A second step for the same cell on its way to neurogenesis would be the increased synthesis of the molecular apparatus for transmission of epidermalizing signals, that is to say, formation of functional *Delta* protein in order to permit lateral inhibition to occur. Since all cells of a proneural cluster initially contain comparable amounts of proneural proteins, activation of *Delta* is assumed to start equally in all cluster cells. However, activation of *Delta* is likely also to be dependent

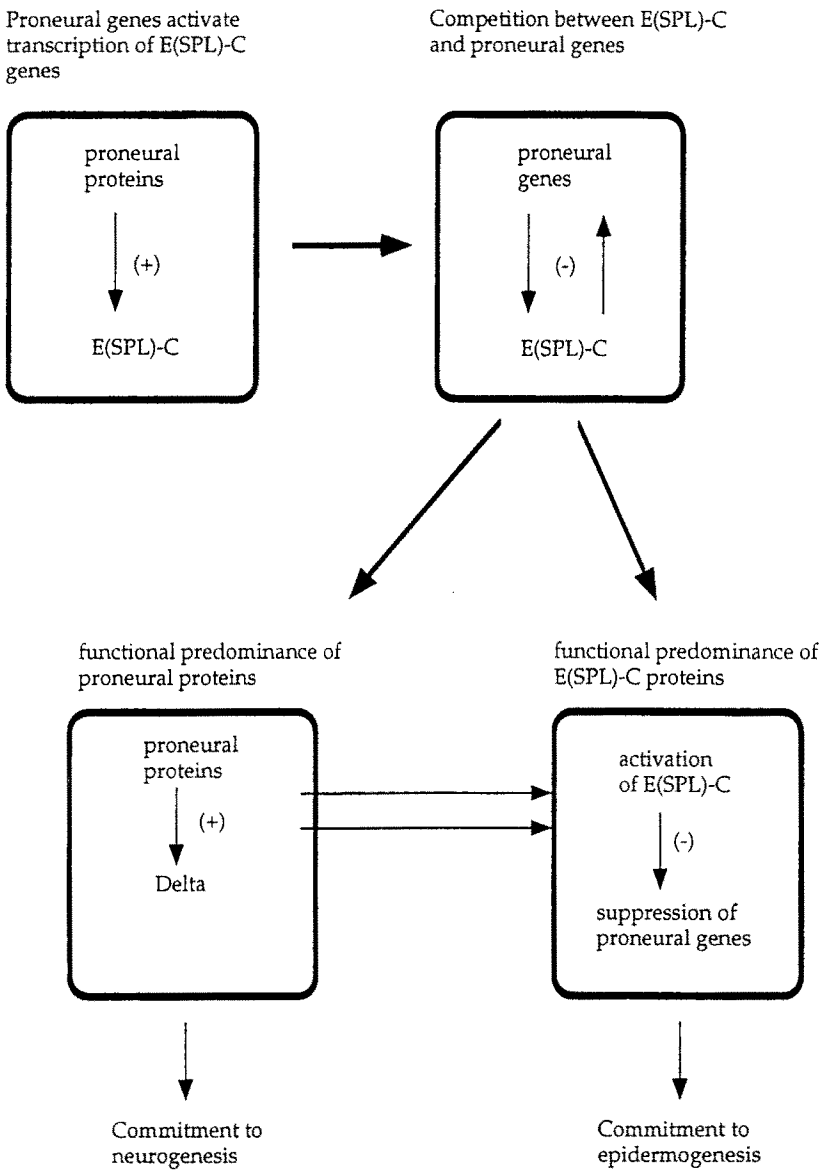

Fig. 2. Sequence of events assumed to occur during lineage segregation. The squares represent cells within a proneural cluster. Refer to text for further details.

on the concentration of proneural proteins. Hence, the amount of inhibitory signal would increase in the cell that initiates neurogenesis because of its higher content in proneural gene products; this would lead to inhibition of the surrounding cells of the cluster and to reinforcement of the neural decision in the neuroblast. Conversely, the behavior of those cells of the proneural cluster that follow the epidermal pathway is likely to reflect the functional suppression of the proneural proteins, and consequently the initiation of the constitutive developmental pathway, with increased synthesis and/or activation of the receptor for epidermalizing signals in order to reinforce and stabilize the epidermal decision.

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References

Alonso M. C. and Cabrera C. V. (1988) The *achaete–scute* gene complex of *Drosophila melanogaster* comprises four homologous genes. *EMBO J.* **7,** 2585–2591.

Brand M. and Campos-Ortega J. A. (1988) Two groups of interrelated genes regulate early neurogenesis in *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **197,** 457–470.

Brand M. and Camposrtega J. A. (1990) Second site modifiers of the *split* mutation of *Notch* define genes involved in neurogenesis in *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **198,** 275–285.

Brand M., Jarmm A. P., Jan L. Y., and Jan Y. N. (1993) *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. *Development* **119,** 1–17.

Cabrera C. V. (1990) Lateral inhibition and cell fate during neurogenesis in *Drosophila:* the interactions between *scute, Notch,* and *Delta. Development* **109,** 733–742.

Cabrera C. V., Martinez-Arias A., and Bate M. (1987) The expression of three members of the *achaete–scute* gene complex correlates with neuroblast segregation in *Drosophila. Cell* **50,** 425–433.

Campos-Ortega J. A. and Haenlin M. (1992) Regulatory signals and signal molecules in early neurogenesis of *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **201,** 1–11.

Caudy M., Grell E. H., Dambly-Chaudière C., Ghysen A., Jan L. Y., and Jan Y. N. (1988a) The maternal sex determination gene *daughterless* has zygotic activity necessary for the formation of peripheral neurons in *Drosophila. Genes Dev.* **2,** 843–852.

Caudy M., Vässin H., Brand M., Tuma R., Jan L. Y., and Jan Y. N. (1988b) *daughterless,* a gene essential for both neurogenesis and sex determination in *Drosophila,* has sequence similarities to *myc* and the *achaete–scute* complex. *Cell* **55,** 1061–1067.

Cronmiller C., Schedl P., and Cline T. W. (1989) Molecular characterization of *daughterless, a Drosophila* sex determination gene with multiple roles in development. *Genes Dev.* **2,** 1666–1676.

Cubas P., de Celis J.-F., Campuzano S., and Modolell J. (1991) Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev.* **5,** 996–1008.

Cubas P. and Modolell J. (1992) The *extramacrochetae* gene provides information for sensory organ patterning. *EMBO J.* **11,** 3385–3393.

Dambly-Chaudière C. and Ghysen A. (1987) Independent subpatterns of sense organs require independent genes of the *achaete–scute* complex in *Drosophila* larvae. *Genes Dev.* **1,** 297–306.

de Celis J., Marí-Beffa M., and García-Bellido A. (1991) Cell autonomous role of *Notch,* an epidermal growth factor homolog, in sensory organ differentiation in *Drosophila. Proc. Natl. Acad. Sci. USA* **88,** 632–636.

de la Concha A., Dietrich U., Weigel D., and Campos-Ortega J. A. (1988) Functional interactions of neurogenic genes of *Drosophila melanogaster. Genetics* **118,** 499–508.

Delidakis C. and Artavanis-Tsakonas S. (1992) The *Enhancer of split [E(spl)]* locus of *Drosophila* encodes seven independent helix-loop-helix proteins. *Proc. Natl. Acad. Sci. USA* **89,** 8731–8735.

Dietrich U. and Campos-Ortega J. A. (1984) The expression of neurogenic loci in imaginal epidermal cells of *Drosophila melanogaster. J. Neurogen.* **1,** 315–332.

Doe C. Q. and Goodman C. S. (1985) Early events in insect neurogenesis. II. The role of cell interactions and cell lineages in the determination of neuronal precursor cells. *Dev. Biol.* **111,** 206–219.

Doe C. Q., Hiromi Y., Gehring W. J., and Goodman C. S. (1988) Expression and function of the segmentation gene *fushi-tarazu* during *Drosophila* neurogenesis. *Science* **239,** 170–175.

Domínguez M. and Campuzano S. (1993) *asense,* a member of the *Drosophila achaete–scute* complex, is a proneural and a neural differentiation gene. *EMBO J.* **12,** 2049–2060.

Fehon R. G., Johansen K., Rebay I., and Artavanis-Tsakonas S. (1991) Complex cellular and subcellular regulation of *Notch* expression during

embryonic and imaginal development of *Drosophila*: implications for *Notch* function. *J. Cell. Biol.* **113,** 657–669.

Fehon R. G., Kooh P. J., Rebay I., Regan C. L., Xu T., Muskavitch M. A. T., and Artavanis-Tsakonas S. (1990) Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. *Cell* **62,** 523–533.

García-Bellido A. (1975) Genetic control of wing disc development in *Drosophila*, in *Cell Patterning*, Ciba Foundation Symposium 29 (Brenner S., ed.), Elsevier, Excerpta Medica, North Holland Assoc., Amsterdam, Oxford, New York, pp. 161–178.

García-Bellido A. (1979) Genetic analysis of the *achaete–scute* system of *Drosophila* melanogaster. *Genetics* **91,** 491–520.

García-Bellido A. and Santamaria P. (1978) Developmental analysis of the *achaete–scute* system of *Drosophila melanogaster*. *Genetics* **88,** 469–486.

Ghysen A. and Dambly-Chaudière C. (1988) From DNA to form: the *achaete–scute* complex. *Genes Dev.* **2,** 495–501.

Ghysen A. and Dambly-Chaudière C. (1989) Genesis of the *Drosophila* peripheral nervous system. *Trend Genet.* **5,** 251–255.

Ghysen A. and Dambly-Chaudière C. (1990) Early events in the development of *Drosophila* peripheral nervous system. *J. Physiol. (Paris)* **84,** 11–20.

Ghysen A., Dambly-Chaudière C., Jan L. Y., and Jan Y.-N. (1993) Cell interactions and gene interactions in peripheral neurogenesis. *Genes Dev.* **7,** 723–733.

Ghysen A. and O'Kane C. (1989) Detection of enhancer-like elements in the genome of *Drosophila*. *Development* **105,** 35–52.

González F., Romani S., Cubas P., Modolell J., and Campuzano S. (1989) Molecular analysis of *asense*, a member of the *achaete–scute* complex of *Drosophila melanogaster*, and its novel role in optic lobe development. *EMBO J.* **8,** 3553–3562.

Goriely A., Dumont N., Dambly-Chaudière C., and Ghysen A. (1991) The determination of sense organs in *Drosophila*, effect of the neurogenic mutations in the embryo. *Development* **113,** 1395–1404.

Haenlin M., Kramatschek B., and Campos-Ortega J. A. (1990) The pattern of transcription of the neurogenic gene *Delta* of *Drosophila melanogaster*. *Development* **110,** 905–914.

Haenlin M., Kunisch M., Kramatschek B., and Campos-Ortega J. A. (1994) Genomic regions regulating expression of the *Drosophila* neurogenic gene *Delta*. *Mech. Dev.* **47,** 99–110.

Hartenstein V. and Campos-Ortega J. A. (1984) Early neurogenesis in wildtype *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193,** 308–325.

Hartenstein V. and Campos-Ortega J. A. (1986) The peripheral nervous system of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **195,** 210–221.

Hartley D. A., Xu T., and Artavanis-Tsakonas S. (1987) The embryonic expression of the *Notch* locus of *Drosophila melanogaster* and the implications of point mutations in the extracellular EGF-like domain of the predicted protein. *EMBO J.* **6,** 3407–3417.

Heitzler P. and Simpson P. (1991) The choice of cell fate in the epidermis of *Drosophila*. *Cell* **64,** 1083–1092.

Heitzler P. and Simpson P. (1993) Altered epidermal growth factor-like sequences provide evidence for a role of *Notch* as a receptor in cell fate decisions. *Development* **117,** 1113–1123.

Hinz U., Giebel B., and Campos-Ortega J. A. (1994) The basic-helix-loop-helix domain of the *Drosophila lethal of scute* protein is sufficient for proneural function and activates neurogenic genes. *Cell* **46,** 73–85.

Hoppe P. E. and Greenspan R. J. (1986) Local function of the *Notch* gene for embryonic ectodermal choice in *Drosophila*. *Cell* **46,** 773–783.

Hoppe P. E. and Greenspan R. J. (1990) The *Notch* locus of *Drosophila* is required in epidermal cells for epidermal development. *Development* **109,** 875–885.

Jarman A. P., Grau Y., Jan L. Y., and Jan Y. N. (1993) *atonal* is a proneural gene that directs chrodotonal organ formation in the *Drosophila* peripheral nervous system. *Cell* **73,** 1307–1321.

Jiménez F. and Campos-Ortega J. A. (1979) A region of the *Drosophila* genome necessary for CNS development. *Nature* **282,** 310–312.

Jiménez F. and Campos-Ortega J. A. (1982) Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. *Roux's Arch. Dev. Biol.* **191,** 191–201.

Jiménez F. and Campos-Ortega J. A. (1987) Genes in subdivision 1B of the *Drosophila melanogaster* X-chromosome and their influence on neural development. *J. Neurogen.* **4,** 179–200.

Jiménez F. and Campos-Ortega J. A. (1990) Defective neuroblast commitment in mutants of the

achaete-scute complex and adjacent genes of *Drosophila melanogaster*. *Neuron* **5,** 81–99.

Johansen K. M., Fehon R. G., and Artavanis-Tsakonas S. (1989) The *Notch* gene product is a glycoprotein expressed on the cell surface of both epidermal and neuronal precursor cells during *Drosophila* development. *J. Cell. Biol.* **109,** 2427–2440.

Jürgens G., Lehmann R., Schardin M., and Nüsslein-Volhard C. (1986) Segmental organisation of the head in the embryo of *Drosophila melanogaster*. A blastoderm fate map of the cuticle structures of the larval head. *Roux's Arch. Dev. Biol.* **195,** 359–377.

Kelley M. R., Kidd S., Deutsch W. A., and Young M. W. (1987) Mutations altering the structure of epidermal growth factor-like coding sequences at the *Drosophila Notch* locus. *Cell* **51,** 539–548.

Kidd S., Kelley M. R., and Young M. W. (1986) Sequence of the *Notch* locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. *Mol. Cell. Biol.* **6,** 3094–3108.

Kidd S., Baylies M. K., Gasic G. P., and Young M. W. (1989) Structure and distribution of the *Notch* protein in developing *Drosophila*. *Genes Dev.* **3,** 1113–1129.

Klämbt C., Knust E., Tietze K., and Campos-Ortega J. A. (1989) Closely related transcripts encoded by the neurogenic gene complex *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **8,** 203–210.

Knust E., Bremer K. A., Vässin H., Ziemer A., Tepass U., and Campos-Ortega J. A. (1987a) The *Enhancer of split* locus and neurogenesis in *Drosophila melanogaster*. *Dev. Biol.* **122,** 262–273.

Knust E., Tietze K., and Campos-Ortega J. A. (1987b) Molecular analysis of the neurogenic locus *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **6,** 4113–4123.

Knust E., Schrons H., Grawe F., and Campos-Ortega J. A. (1992) Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* **132,** 505–518.

Kooh P. J., Fehon R., and Muskavitch M. A. T. (1993) Implications of dynamic patterns of *Delta* and *Notch* expression for cellular interactions during a *Drosophila* development. *Development* **117,** 493–507.

Kopczynski C. C., Alton A. K., Fechtel K., Kooh P. J., and Muskavitch M. A. T. (1988) *Delta*, a *Drosophila* neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. *Genes Dev.* **2,** 1723–1735.

Kopczynski C. C. and Muskavitch M. A. T. (1989) Complex spatio-temporal accumulation of alternative transcripts from the neurogenic gene *Delta* during *Drosophila* embryogenesis. *Development* **107,** 623–636.

Kramatschek B. and Campos-Ortega J. A. (1994) Neuroectodermal transcription of *Drosophila* neurogenic genes *E(spl)* and *HLH-m5* is regulated by proneural proteins. *Development* **120,** 815–826.

Kunisch M., Haenlin M., and Campos-Ortega J. A. (1994) Lateral inhibition mediated by the *Drosophila* neurogenic gene *Delta* is activated by proteins encoded by proneural genes. *Proc. Natl. Acad. Sci.* **91,** 10,139–10,143.

Lehmann R., Dietrich U., Jiménez F., and Campos-Ortega J. A. (1981) Mutations of early neurogenesis in *Drosophila*. *Roux's Arch. Dev. Biol.* **190,** 226–229.

Lehmann R., Jiménez F., Dietrich U., and Campos-Ortega J. A. (1983) On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **192,** 62–74.

Lieber T., Alcamo E., Hassel B., Krane J. F., Campos-Ortega J. A., and Young M. W. (1992) Single amino acid substitutions in EGF-like elements of the *Notch* and *Delta* proteins modify *Drosophila* development and depress cell adhesion in vitro. *Neuron* **9,** 847–859.

Lieber T., Kidd S., Alcamo E., Corbin V., and Young M. W. (1993) Antineurogenic phenotypes induced by truncated *Notch* proteins indicate a role in signal transduction and may point to a novel function for *Notch* in nuclei. *Genes Dev.* **7,** 1949–1965.

Lüer K. and Technau G. M. (1992) Primary culture of single ectodermal precursors of *Drosophila* reveals a dorsoventral prepattern of intrinsic neurogenic and epidermogenic capabilities at the early gastrula stage. *Development* **116,** 377–385.

Martin-Bermudo M. D., Martínez C., and Jiménez F. (1991) Distribution and function of the *lethal of scute* gene product during early neurogenesis in *Drosophila*. *Development* **113,** 445–454.

Muller H. J. (1935) The origination of chromatin deficiencies as minute deletions subject to insertion elsewhere. *Genetica* **17,** 237–252.

Murre C., Schonleber McCaw P., and Baltimore D. (1989a) The amphipathic helix-loop-helix: a new

DNA-binding and dimerization motif in immunoglobulin enhancer binding *daughterless*, MyoD and myc proteins. *Cell* **56,** 777-783.

Murre C., Schonleber McCaw P., Vässin H., Caudy M., Jan L. Y., Jan Y. N., Cabrera C. V., Buskin J. N., Hauschka S. D., Lassar A. B., Weintraub H., and Baltimore D. (1989b) Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58,** 537–544.

Oellers N., Dehio M., and Knust E. (1994) Basic-helix-loop-helix proteins of the *Enhancer of split* complex of *Drosophila* negatively interfere with transcriptional activation by proneural proteins. Submitted.

Poulson D. F. (1937) Chromosomal deficiencies and embryonic development of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **23,** 133–137.

Rebay I., Fleming R. J., Fehon R. G., Cherbas L., Cherbas P., and Artavanis-Tsakonas S. (1991) Specific repeats of *Notch* mediate interactions with *Delta* and *Serrate*: implications for *Notch* as a multifunctional receptor. *Cell* **67,** 687–699.

Rodríguez I., Hernández R., Modolell J., and Ruiz-Gómez M. (1990) Competence to develop sensory organs is temporally and spatially regulated in *Drosophila* epidermal primordia. *EMBO J.* **9,** 3583–3592.

Romani S., Campuzano S., and Modolell J. (1987) The *achaete–scute* complex is expressed in neurogenic regions of *Drosophila* embryos. *EMBO J.* **6,** 2085–2092.

Romani S., Campuzano S., Macagno E. R., and Modolell J. (1989) Expression of *achaete* and *scute* genes in *Drosophila* imaginal discs and their function in sensory organ development. *Genes Dev.* **3,** 997–1007.

Ruiz-Gómez M. and Ghysen A. (1993) The expression and role of a proneural gene, *achaete*, in the development of the larval nervous system of *Drosophila*. *EMBO J.* **12,** 1121–1130.

Schrons H., Knust E., and Campos-Ortega J. A. (1992) The *Enhancer of split* complex and adjacent genes in the 96F region of *Drosophila melanogaster* are required for segregation of neural and epidermal progenitor cells. *Genetics* **132,** 481–503.

Shepard S. B., Broverman S. A., and Muskavitch M. A. T. (1989) A tripartite interaction among alleles of *Notch*, *Delta*, and *Enhancer of split* during imaginal development of *Drosophila melanogaster*. *Genetics* **122,** 429–438.

Skeath J. B. and Carroll S. B. (1991) Regulation of *achaete–scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5,** 984–995.

Skeath J. B. and Carroll S. B. (1992) Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. *Development* **114,** 939–946.

Skeath J. B., Panganiban G., Selegue J., and Carroll S. B. (1992) Gene regulation in two dimensions: the proneural *achaete* and *scute* genes are controlled by combinations of axis-patterning genes through a common intergenic control region. *Genes Dev.* **6,** 2606–2619.

Struhl G., Fitzgerald K., and Greenwald I. (1993) Intrinsic activity of the *Lin-12* and *Notch* intracellular domains in vivo. *Cell* **74,** 331–345.

Stüttem I. and Campos-Ortega J. A. (1991) Cell commitment and cell interactions in the ectoderm of *Drosophila melanogaster*. *Development* **Suppl. 2,** 39–46.

Taghert P. H., Doe C. Q., and Goodman C. S. (1984) Cell determination and regulation during development of neuroblasts and neurons in grasshopper embryos. *Nature* **307,** 163–165.

Technau G. M. and Campos-Ortega J. A. (1985) Fate mapping in wildtype *Drosophila melanogaster*. II. Injections of horseradish peroxidase in cells of the early gastrula stage. *Roux's Arch. Dev. Biol.* **194,** 196–212.

Technau G. M. and Campos-Ortega J. A. (1986) Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. II. Commitment and proliferative capabilities of neural and epidermal cell progenitors. *Roux's Arch. Dev. Biol.* **195,** 445–454.

Technau G. M. and Campos-Ortega J. A. (1987) Cell autonomy of expression of neurogenic gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **84,** 4500–4504.

Tietze K., Oellers N., and Knust E. (1992) *Enhancer of split*^{D}, a dominant mutation of *Drosophila* and its use in the study of functional domains of a helix-loop-helix protein. *Proc. Natl. Acad. Sci. USA* **89,** 6152–6156.

Van Doren M., Powell P. A., Pasternak D., Singson A., and Posakony J. W. (1992) Spatial regulation of proneural gene activity: auto and cross-activation of *achaete* is antagonized by *extramacrochatae*. *Genes Dev.* **6,** 2592–2605.

Vässin H., Brand M., Jan L. Y., and Jan Y.-N. (1994) *daughterless* is essential for neuronal precursor differentiation but not for initiation of neuronal precursor formation in *Drosophila* embryos. *Development* **120,** 935–945.

Vässin H., Bremer K. A., Knust E., and Campos-Ortega J. A. (1987) The neurogenic locus *Delta* of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6,** 3431–3440.

Vässin H., Vielmetter J., and Campos-Ortega J. A. (1985) Genetic interactions in early neurogenesis of *Drosophila melanogaster*. *J. Neurogen.* **2,** 291–308.

Villares R. and Cabrera C. V. (1987) The *achaete-scute* gene complex of *Drosophila melanogaster*: conserved domains in a subset of genes required for neurogenesis and their homology to *myc*. *Cell* **50,** 415–424.

Wharton K. A., Johansen K. M., Xu T., and Artavanis-Tsakonas S. (1985) Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43,** 567–581.

White K. (1980) Defective neural development in *Drosophila melanogaster* embryos deficient for the tip of the X-chromosome. *Dev. Biol.* **80,** 322–344.

White K., DeCelles N. L., and Enlow T. C. (1983) Genetic and developmental analysis of the locus *vnd* in *Drosophila melanogaster*. *Genetics* **104,** 433–488.