The generation of cell diversity during early neurogenesis in Drosophila

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Introduction

During animal development a complex network of highly specialised cells is generated in perfect coordination with other body structures, and consolidated with an outstanding degree of specificity in what we know as the nervous system. Finding how this intricate cellular network is generated and assembled is the aim of the current research in neurogenesis.

Understanding how the nervous system is built requires us to address questions concerning the mechanisms of pattern formation and of cell differentiation, signalling and recognition. In this review, I shall consider exclusively those aspects of neurogenesis regarding the orderly production of neuronal cell diversity in the fruit fly Drosophila melanogaster.

The advantage of Drosophila as an experimental system lies in the excellences of its genetic technology. The analysis of morphogenetic mutants aims at elucidating what genes participate in a given process and what their normal role is in it. The dissection of any biological process by genetic means relies exclusively on the assessment of mutant phenotypes and, therefore, the power of resolution of the scoring process is of paramount importance. Naturally, this resolution increases in proportion with our knowledge of the normal ontogeny of the process. The balanced consideration of these data normally leads to the conception of hypothetical models that describe cellular ontogeny in terms of gene expression and function. It is hoped that the critical testing of these proposals by modern techniques of gene manipulation will ultimately result in the formulation of a consistent theory of neural development.

Ontogeny: the cellular basis of neural development

Most of what we know about the ontogeny of Drosophila's nervous system comes from comparisons with work first carried out in the grasshopper embryo. Due to its larger embryonic and cellular size, accessibility of some neurons and relatively simple ganglia, the grasshopper has been the system of choice for studies concerning the cellular basis of neural development. These studies stem from the long-standing realisation that neurons can be uniquely identified by their place and date of birth and characteristic morphology, pattern of connections, physiology and biochemistry. This set of properties led to the construction of maps of neurons which, in turn, revealed the constancy in number and other pattern characteristics of these cells. It had also been known since the times of Wheeler (1891) and Bauer (1904) that neurons arise from precursor neuroblasts by repeated asymmetric division into ganglion mother cells and subsequent division of the latter into pairs of neurons. More recently it was Bate (1976), in an effort to understand the relationship between neuroblasts and their neuronal progeny, investigated the number and organisation of these precursor cells. The maps that he obtained revealed that the neuroblasts are organised in a bilaterally symmetrical, metameric pattern, as reliable in their number and arrangement as the adult cells that they produce.

These findings posed two questions: how do the neuroblasts arise in an orderly pattern and how are neurons produced from these precursors? These two aspects were first addressed in some elegant experiments with the grasshopper embryo (Taghert et al., 1984; Taghert and Goodman, 1984; Doe and Goodman, 1985b).

The neuroblasts that give rise to the central nervous system originate from the neuroectoderm, an anteroposterior strip of ectoderm that includes and extends bilaterally from the ventral midline. The decision to become a neuroblast is taken at random amongst small groups of neuroectodermal cells by a process involving cell interactions. Indeed, ablation of a neuroblast with a laser beam triggers another neuroectodermal cell of the group to replace it. This regulative capacity is interpreted as the result of a local inhibitory signal exerted by the neuroblast over adjacent neuroectodermal cells, thus preventing them from similarly becoming neuroblasts. The rest of the cells of the group, from which the neuroblast segregates, then develop as either glia or, epidermal non-neuronal support cells, or they die (Doe and Goodman, 1985a).

As a result of the rapid division cycle of the neuroblast, clones of ganglion mother cells form a columnar array on top of each neuroblast. Subsequent division of the ganglion mother cells originates a neuronal progeny that, therefore, is related by lineage to the underlying neuroblast. The lineage of the neuroblast appears to be invariant and cell autonomous. Invariant because at each division of the neuroblast a ganglion mother cell is produced with a restricted binary neuronal fate and this fate is specified by the ganglion mother cell mitotic ancestry. The autonomy of

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*We are sad to inform readers that Carlos Cabrera died on April 10th 1992 at the early age of 41.

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the neuroblast lineage (i.e. the unfolding of a lineage independently of its environment) is suggested by the results of two experiments. Firstly, if all the cells of one of those small ectodermal groups (distinguished by its late segregation timing and therefore surrounded by differentiated epidermal cells) are killed, the corresponding neuronal progeny is not produced, showing that (1) only neuroectodermal cells can give rise to neuroblasts and (2) neighbouring lineages do not change to compensate for the missing neuroblast and its progeny. Secondly, ablation of a neuroblast after its first division leads to the persistence and differentiation of the ganglion mother cell produced and, at the same time, segregation of a new neuroblast, which will originate another identical ganglion mother cell. This leads to delayed duplication of the neuronal progeny produced by the first ganglion mother cell, showing that even in a changed environment the lineage develops normally.

The generation of ganglion mother cells, unlike that of neuroblasts, is not subjected to regulation and, in fact, killing of a ganglion mother cell results in the absence of its neuronal progeny (Doe and Goodman, 1985b). However, the binary fate of the ganglion mother cell progeny is not fixed and ablation of one of the neurons results in the remaining one developing into either of the two alternatives (Kuwada and Goodman, 1985). These experiments showed that cell interactions again intervene, this time to regulate the fate of sibling neurons, in the last step of the neuroblast lineage.

In summary, although only a fraction of some neuroblast lineages has been analysed and no lineage tracer experiments have been undertaken, the present evidence supports the notion that neuroblasts are born with a set of instructions, or a genetic programme, to develop autonomously an invariant cell lineage.

As identified neurons can be traced back to the positions in the neuroectoderm where their precursor neuroblasts originate, it is important to ask how the fate of each of these precursor cells is specified. Three possibilities have been envisioned (Doe and Goodman, 1985b): (1) determination of individual neuroectodermal cells, (2) determination of groups of neuroectodermal cells that subsequently would resolve into one neuroblast per group by cell interactions and (3) all neuroectodermal cells are equivalent; cell interactions mediate the selection of some of these cells as neuroblasts whose fate is then assigned in correlation with the position where segregation occurs.

The neuroblast ablation experiments described above were used again to discriminate amongst these alternatives, this time seeking the origin of the ectodermal cell that substitutes for the killed neuroblast. The results of three different ablation experiments led to the suggestion that the ectodermal cell replacing the ablated neuroblast originates in an adjacent group, which normally would have resolved into a different lineage. This was interpreted as a positional translocation within the neuroectoderm, and it was taken to indicate that neuroectodermal cells are not determined to any specific neural fate, but are equivalent. On these grounds, the third alternative was favoured and consequently it was proposed that the decision to become a neuroblast is independent of the acquisition of its subsequent fate (i.e., neuronal progeny generated).

As revealing as the above experiments are, they are difficult and, as with any other approach to experimental embryology, they are subject to interpretation. An independent evaluation of these results was therefore sought and it was felt that genetic analysis could provide an alternative and complementary approach. Indeed, in such an experimental system, one could ask whether there are mutations that mimic the regulative properties manifested by the ablation of the neuroblast and neurons, or that impair the fate of the neuroblast and whether their phenotype is consistent with the models described. But first of all, how does one go about applying genetic analysis to insect neurogenesis?. Undoubtedly, Drosophila could provide such an experimental system, but would there be sufficient conservation of developmental strategies between hoppers and flies to support such comparison?

Phylogeny: hoppers and flies share a common plan for neural development

The 300 Myr separating hoppers and flies are reflected in the substantial differences in their ontogeny. For instance, hoppers are hemimetabolous insects and flies holometabolous. This means that the former advance into adulthood directly from postembryonic development, while the latter has an intermediate larval stage followed by metamorphosis. A second important difference concerns the organisation of the germ band. Grasshoppers are short-germ-band insects whereas Drosophila belongs to the long-germ-band class. This means that, in the former, the metameric germ band is generated by a budding off process from a short subterminal zone of the blastoderm. In contrast, in Drosophila, the metameric germ band arises without growth, by partition of the blastodermal space into segmental units (Anderson, 1972).

In spite of these differences, the comparative neuronal anatomy of these two organisms manifests an outstanding degree of similarity, if in a different time frame and size scale (Thomas et al., 1984). Indeed, a subset of well-known neurons of the common, ladder-like scaffold of hoppers and flies display identical morphology, growth cone and selective fasciculation patterns. These attributes are thought to reflect the deployment of similar temporal and spatial recognition signals. It is therefore not surprising that the overall features of neural developmental also appear to follow a common strategy. The neuroblasts similarly arise from a subpopulation of an identically positioned neuroectodermal cell layer and attain the characteristic symmetric, metameric pattern described for the grasshopper (Campos-Ortega and Hartenstein, 1985; Doe et al., 1988a).

Furthermore, some identified hopper neuroblasts have been found in equivalent positions in Drosophila and shown to originate some similar neurons via comparable lineages (Doe et al., 1988a). These lineages also appear to be cell autonomous, as in vitro cultured neuroblasts divide normally to produce clones of ganglion mother cells and neurons and the proportion of the latter expressing serotonergic and dopaminergic phenotypes is similar to the number found in vivo (Huff et al., 1989).

While hopper neuroblasts complete their divisions in the
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was scored by histological methods. More recently, geneti-
cists started to apply molecular biology techniques to mon-
it gene expression patterns and these studies revealed a con-
siderable amount of genetic activity in the developing nervous system. Subsequently, ‘pattern searches’ by the enhancer trap technique (O’Kane and Gehring, 1987) yielded additional candidates. However, most of the genes selected by their pattern of expression have not yet been analysed genetically and, therefore, their role in neurogenesis remains obscure.

One reason for this lack of genetic data is the coincidence of the ectoderm as common anlage for neural and epidermal development; mutants of genes required in both pathways will be defective in neurogenesis primarily because of abnormal ectodermal patterning. Testing the requirement of such genes in neurogenesis, independently of their epidermal function, is a major challenge to the current research and one of considerable technical difficulty. The correlation between patterns of gene expression and neural ontogeny, however, has served several useful purposes: as a guideline to select genes that are likely to contribute to those aspects of neurogenesis under scrutiny, as a high resolution phenotypic trait and as an excellent histological marker to probe morphology beyond the level of resolution of standard techniques.

I will begin this account of the genetic control of neurogenesis by discussing postneuroblast gene activity followed by preneuroblast development.

Gene activity in postneuroblast development

Postneuroblast development is centred around the characteristic lineage of these precursors and their progeny. The developmental potential of the neuroblasts is reflected by the complexity of gene activity in these cells and their ganglion mother cell progeny. Two groups of genes active in these cells can be distinguished, depending on whether or not their spatial domains of expression are inherited from the underlying ectoderm.

The homeotic and segment polarity genes are first expressed and required in the ectoderm and then introduced into the nervous system by segregating neuroblasts, with precise preservation of their spatial boundaries. The domains of expression of these genes, therefore, are dictated by their ectodermal function, regardless of whether their regulation is actively or passively maintained in the nervous system.

The homeotic genes are expressed and required in multimeric portions of the ectoderm for the establishment and maintenance of specific segmental identities (Lewis, 1978; Kaufman et al., 1990). Segmental differences in neural development are most obvious between, for example, thoracic and abdominal ganglia of the larva. The production of more neuronal progeny by thoracic neuroblasts is the main mechanism responsible for this differential development (Bate, 1976; Truman and Bate, 1988; Shepherd and Bate, 1990), and the homeotic genes are the most likely candidates to regulate this aspect of neuroblast activity. This is, however, a feature of postembryonic development. During early neurogenesis, the homeotic genes have been proposed to control the small differences in the number of neuroblasts segregating in each metamere by regulating the activity of neuroblast generating genes (Doe, 1992).

Other aspects of homeotic gene function, such as their expression in embryonic neurons, remain elusive. This is a particularly intriguing problem, given the conservation in genomic cluster arrangement, protein sequence and patterns of expression of these genes in the nervous systems of such a wide variety of animals, from flies to mammals (Graham et al., 1989).

The segment polarity genes are required in the ectoderm for normal intrasegmental patterning of epidermal derivatives of the larval cuticle (Nüsslein-Volhard and Wieschaus, 1980). The expression of some segment polarity genes has been studied and shown to occur in one or two stripes per metamere (DiNardo and Heemskerk, 1990). The analysis of the nervous system of mutants for some of these genes led to the speculation that their wild-type alleles could contribute to the control of neuroblast fate (Patel et al., 1989). However, the patterns of ectodermal expression of many segment polarity genes are tightly interdependent, such that the absence of any one gene leads to complex rearrangements of the normal boundaries of the others (DiNardo and Heemskerk, 1990). In these circumstances, the phenotypes observed are, most likely, the result of a re-patterned ectoderm and thus the role of the segment polarity genes in neural development remains unclear.

In contrast to homeotic and segment polarity genes, a second group of genes is specifically activated in neuroblasts and ganglion mother cells (Table 1). Although many members of this group belong to the gap and pair-rule classes of segmentation genes, or are part of the dorsoventral pathway (Nüsslein-Volhard and Wieschaus, 1980; St. Johnston and Nüsslein-Volhard, 1992), this early requirement entails only transient expression. When redeployment of these genes occurs in neural precursors, they do so in patterns totally unrelated to their previous boundaries of expression. This suggests that these genes constitute a special group, specifically deployed in close connection with a distinct neural function.

Two genes fushi tarazu (ftz) and even-skipped (eve) are activated in subsets of ganglion mother cells and expressed subsequently in neurons. The requirement of these two genes has been tested by monitoring the differentiation of the RP1/RP2 and aCC/pCC pairs of sibling neurons. At the
non-permissive temperature, an *eve* temperature-sensitive allele, often leads to the appearance of two RPIs and two pCCs, whereas a *ftz* chimera, lacking a promoter element necessary for the expression of this gene in the nervous system, similarly produces two RPIs but leaves the CC cells unaltered (Doe et al., 1988a; 1988b).

The phenotypic analysis of *eve* and *ftz* mimics the sibling ablation experiments carried out in the grasshopper embryo (Kuwada and Goodman, 1985). This correlation indicates that the binary fate of the ganglion mother cells is under genetic control and suggests that the interactions between the two sibling neurons analysed depend on the activity of these two genes. A more recent and detailed interpretation of the genetic results above suggests that the role of *eve* and *ftz* is to confer ganglion mother cell identity; two ganglion mother cells immediately related by their ancestry would attain identical fates in the absence of either of these two genes (Doe, 1992).

Although the importance of these findings is obvious, there are aspects that will require further study. For instance, in the absence of *ftz*, most neurons appear to differentiate normally (Hiromi and Gehring, 1987); in particular, six well-studied cases showed wild-type axon morphology (Doe et al., 1988a). Although this suggests that *ftz* expression may not be required by most neuronal lineages, scoring other traits might reveal more subtle roles for this gene.

Finally, it is clear that a reasonable understanding of ganglion mother cell and sibling determination will involve the identification of many more genes; some 125 ganglion mother cells would have to be specified for the generation of the approximately 250 neurons present in each embryonic neuromere.

Fourteen genes have been found that are activated in neuroblasts (Table 1), most of which are also expressed in ganglion mother cell and neurons. The genetic requirements of three members of this group (*prospero*, *runt* and *cut*) have been analysed. *Propero* RNA is expressed by most neuroblasts and ganglion mother cells but not by neurons (Doe et al., 1991; Vässin et al., 1991). *Propero* protein, however, accumulates in ganglion mother cells only (Vässin et al., 1991). The earliest effect of *prospero* null alleles is detected in the pattern of gene expression of *eve* and *ftz*. Indeed, despite the fact that neuroblasts segregate normally and ganglion mother cell progeny are produced, the number of ganglion mother cells expressing *eve* and *ftz* is reduced. Conversely, the expression of another ganglion mother cell marker gene, *engrailed*, is widened (Doe et al., 1991). These effects appear to be elicited specifically at the ganglion mother cell level, for the expression of the neuroblast marker gene *hunchback* appears to be normal.

Consequent to the influence of *prospero* in ganglion mother cell gene expression, *prospero* embryos display aberrant neuronal fasciculation (Doe et al., 1991; Vässin et al., 1991). The *prospero* gene, therefore, is required for the normal expression of, at least, a subset of the neuroblast traits. Other aspects of the neuroblast lineage, like the number of cell divisions and characteristic neuronal progeny, can not be easily scored with the available techniques and, thus, a full account of the function of *prospero* will require further work.

*runt* is a segmentation gene also expressed by subsets of neuroblasts, ganglion mother cells and neurons (Doe et al., 1991). Its function in the nervous system has been tested with a transgenic construct that is normally expressed during segmentation but is defective, at least in part, in neural expression. It has also been examined with the aid of a temperature-sensitive allele. As some of the neurons that express *runt* co-express *eve*, the role of the former in the nervous system was assessed by scoring *eve*-expressing cells. It was shown that, indeed, the absence of *runt* produces a dramatic reduction in the number of *eve*-expressing neurons suggesting that *runt* is required for some step of cell identity downstream in the neuroblast lineage (Doe et al., 1991).

The requirement of *cut* has been analysed only in the peripheral nervous system where its absence was shown to provoke a clean transformation between two types of sensory structure: the so-called ‘external’ into ‘chordotonal’ organs (Bodmer et al., 1987). Both types of organ are generated from single precursor cells that produce distinct lineages (Bodmer et al., 1989) and thus the transformation caused by the lack of *cut* is thought to stem from mis-specification at the precursor level (Blochlinger et al., 1990). In line with this, *cut* protein is expressed in the precursors of the ‘external’ organs and subsequently in all their progeny, but is absent from the ‘chordotonal’ organ lineage (Blochlinger et al., 1990). This is consistent with a model in which *cut* would impose a new differentiation pathway to a precursor cell already specified to a more basal fate. Whether this mechanism of fate determination is a particular feature of the more simple sensory organ lineages is not yet known. The comparative distribution of genes activated in neuroblasts is suggestive of a similar combinatorial code of gene expression that would implement alternative fates to different neuroblasts.

Finally, the fact that for neuroblast-specific gene activation occurs without apparent relationship to the ongoing process of ectodermal segmentation suggests the existence of a mechanism distinctly responsible for this operation. It is clear, however, that such a mechanism can not be independent of ectodermal positional cues, because the nervous system is metamERICally organised and is generated in a precise region of the embryo in coordination with other body structures for their normal functional interaction. How this remarkable pattern of neuroblast-specific gene expression is activated concerns preneuroblast development.

**Gene activity in preneuroblast development**

The first sign of gene expression detected in the embryo in relation to neurogenesis is that of the *achaete-scute* gene complex (ASC) (Cabrera et al., 1987; Romani et al., 1987; Alonso and Cabrera, 1988; Cabrera, 1990; Martín-Bermudo et al., 1991). The ASC and adjacent proximal chromosomal regions constitute a complex locus of qualitatively similar genetic functions. The histological analysis of individuals carrying progressively larger deficiencies of this region showed correspondingly increasing levels of neural hypotrophy. Duplication of the ASC region proved capable of rescuing the largest deficiency to levels quantitatively comparable to the deletion of the ASC alone, suggesting some degree of functional redundancy (Jiménez and
Campos-Ortega, 1979). Finding that the ASC comprises four homologous genes [achaete, scute, lethal of scute and asense (Villares and Cabrera, 1987; Alonso and Cabrera, 1988)], together with the genetic results just described, suggested the existence of a clustered gene family (henceforth referred to as the AS-Group), of which the ASC is a subset. Different members of this family appear to contribute in different extents to a common function in neurogenesis. What is this function?  

Certainly, one functional aspect of the AS-Group is the generation of the neuroblasts: the levels of neural hypertrophy elicited by progressively larger deficiencies are correlated with an escalating absence of neuroblasts (Cabrera et al., 1987; Jiménez and Campos-Ortega, 1990; Martín-Bermudo et al., 1991). However, the extent of neural hypertrophy always exceeds the levels expected from the number of precursors missing and, in fact, an additional cause for the loss of nervous system is the increased amount of cell death during postneuroblast development (Jiménez and Campos-Ortega, 1990).

These data indicate that the requirements for the AS-Group span pre- and postneuroblast development; hence, either the generation of neuroblasts is linked to their subsequent fate or these genes are utilised at two different times during neurogenesis. As expression of the three best known members of the AS-Group (the achaete, scute and lethal of scute genes) occurs continuously from their onset in the ectoderm until the neuroblasts have segregated, when they are switched off, the former seems to be the correct alternative (Cabrera et al., 1987; Alonso and Cabrera, 1988; Cabrera, 1990 and unpublished data).

The most obvious regulatory role of the AS-Group in the fate of the neuroblasts is the onset of these precursor’s specific patterns of gene expression (Alonso and Cabrera, 1988; Cabrera and Alonso, 1991). Indeed, the ASC products form DNA-binding heterodimers with the product of the daughterless gene, these heterodimers bind to specific DNA sequences (Murre et al., 1989; Cabrera and Alonso, 1991; van Doren et al., 1991) and they function as transcriptional activators in a yeast assay system (Cabrera and Alonso, 1991). A motif for ASC/DA binding has been found in the promoter of the hunchback gene (see Table 1) and shown to mediate transcriptional activation in the yeast system (Cabrera and Alonso, 1991). In line with this, embryos deficient for the lethal of scute gene fail to activate hunchback in a third of the neuroblasts generated during the first wave of segregation, suggesting an ASC-dependent activation of hunchback in the neuroblasts (Cabrera and Alonso, 1991). In addition, the three resulting ASC/DA heterodimers display characteristic affinities for a given sequence, suggesting that each ASC gene might be responsible for an unique aspect of this pattern of gene activation (Cabrera and Alonso, 1991). The technical difficulties inherent in the detailed analysis of neuroblast lineages, however, have precluded the stringent assessment of the contribution of each of the components of the locus. Nonetheless, it is conceivable that, if the different members of the AS-Group were expressed in partially overlapping patterns [as is the case between achaete and scute whose expression is totally overlapping and interdependent (Cabrera unpublished data)], the cumulative result of the deletion of several of its components would be reflected in the aberrant specification of those cells still expressing some of these functions. If, however, the full complement of genes expressed in a given cell had been deleted, specification would not occur at all. In the former case the lineage would be abnormal and cell death might ensue; in the latter neuroblasts would fail to segregate.

Other authors, however, have argued against the involvement of the AS-Group of genes in neuroblast fate determination (Jiménez and Campos-Ortega, 1990; Martín-Bermudo et al., 1991; Doe, 1992). Their view is that the AS-Group is composed of redundant functions with only partial requirements in the segregation process, providing ectodermal cells with an unspecified neuroblast state. The lack of nervous system arising from single gene deficiencies that do not perturb neuroblast segregation is thought to be due to physiological weakness of the resulting precursors (Jiménez and Campos-Ortega, 1990).

The laser ablation experiments carried out with the grasshopper embryo first suggested that the decision to become a neuroblast is independent of the acquisition of its subsequent neural fate (Doe and Goodman, 1985b). The data discussed above suggest that, on the contrary, the generation of the neuroblasts is linked to the acquisition of genetic instructions that influence their subsequent fate (see Fig. 1). The AS-Group appears to be responsible for the acquisition of these fates (Cabrera et al., 1987; Alonso and Cabrera, 1988; Cabrera, 1990; Cabrera and Alonso, 1991). How is the activity of the ASC genes implemented?

The onset of ASC RNA expression germaine to neurogenesis occurs in the cellular blastoderm in the form of one dorsoventrally split stripe per merameric unit (Cabrera et al., 1987; Cabrera, 1990 and unpublished data). These stripes extend from the boundary of the mesodermal anlage to the dorsal midline and undoubtedly must result from the positional specification laid down by both the anteroposterior and dorsoventral axis forming systems (St. Johnston, D. and Nüsslein-Volhard, 1992). As the germ band extends, the blastoderm stripes of expression are transformed into discrete groups of ASC RNA-expressing cells positioned at the ventral neuroectoderm. Some of these cells will segregate as neuroblasts (see below) and those remaining on the ectoderm cease expressing ASC RNA quickly (Cabrera et al., 1987; Cabrera, 1990). Subsequent ASC expression reappears in a complex pattern, both in the central, and later in the peripheral, nervous systems (unpublished data). How this expression is implemented is as yet unknown for, despite a clear dorsoventral component in the arrangement of the peripheral nervous system (Dambly-Chaudière and Ghysen, 1986), the expression of the pair-rule genes has already faded away. This second round of ASC expression labels the precursors of the second and third wave of central neuroblasts and subsequently the peripheral precursors. It is at this stage that an as yet ill-defined ectodermal patterning system, possibly based on cellular interactions, may influence the outcome of neuroblast identity by regulating ASC expression.

In summary, according to our current working model, a combinatorial code of the AS-Group expression determines neuroblast lineage specification. The outcome of this code would entail the activation of different sets of neuroblast-
The segregation of the neuroblasts

Laser ablation experiments showed that neuroblast segregation is a stochastic process, governed by interactions amongst groups of equivalent ectodermal cells (Taghert et al., 1984; Taghert and Goodman, 1984; Doe and Goodman, 1985b). Genes of the neurogenic class were first proposed to mediate this process because the phenotype of their lack-of-function alleles mimics the laser ablations (Doe and Goodman, 1985b); that is, their mutations elicit the segregation of supernumerary neuroblasts (Poulsen, 1937; Lehmann et al., 1983; Bourouis et al., 1989; Cabrera, 1990; Hoppe and Greenspan, 1990). This is in contrast to the phenotype shown by some ASC mutants in which neuroblasts fail to segregate (Cabrera et al., 1987). Subsequently, by using an antibody specific for the lethal of scute protein, it was shown that a fraction of the RNA-producing cells accumulate the protein and that these latter cells correspond to the neuroblasts (Cabrera, 1990; similar results have since been obtained with an achaete-specific antibody (unpublished data)).

In embryos mutant for neurogenic genes the domains of lethal of scute RNA remain normal, but every RNA-containing cell in the mutant accumulates protein and the number of cells segregating as neuroblasts increases. This observation suggests that the lethal of scute is regulated post-transcriptionally (Cabrera, 1990).

Antibodies have been generated against two forms of the lethal of scute protein. One of them recognises an epitope in a potential protein kinase phosphorylation site (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Cabrera, 1990). The second one was raised against the whole protein (Martín-Bermudo et al., 1991). The former antibody recognises a protein that is produced only in a subset of the cells in which the lethal of scute RNA is detected and labels the segregating neuroblasts (Cabrera, 1990). The second antibody detects a product that is present in every cell that accumulates the lethal of scute RNA and therefore labels the segregating neuroblasts as well as those cells failing to segregate (Martín-Bermudo et al., 1991). These observations suggest the following working hypothesis: there are two forms of the lethal of scute protein; (1) an unphosphorylated or active form detected with the former antibody, which shows a restricted domain of accumulation that is likely the result of post-transcriptional regulation, and (2) a phosphorylated form, present in every cell that contains the lethal of scute RNA and inactive.

It is conceivable that some of the neurogenic genes encoding membrane-bound products (Notch and Delta; Wharton et al., 1985; Kidd et al., 1986; Knast et al., 1987; Kopczynski et al., 1988) function as receptors of a signal that is involved in the orderly process of neuroblast segre-
The signal would reach the lethai of scute protein in the nuclei by a process of signal transduction. It is noteworthy that the product of another neurogenic gene, shaggy, encodes a potential kinase (Bourdakis et al., 1989 and 1990; Siegfried et al., 1990) and it is, therefore, a candidate for involvement in the signal transduction process leading to the phosphorylation/inactivation of ASC proteins.

The neurogenic genes, therefore, provide a device for pattern refinement which has been classically known as ‘lateral inhibition’ (Wigglesworth, 1940). The post-transcriptional regulation of the lethai of scute gene provides a means to visualise this process. That is, a group of cells all of which express lethai of scute is capable of regulating its subsequent fate by means of controlling which cells accumulate a specific form of the protein. Those cells potentially capable of taking upon a given fate are called ‘equivalence alones’ and were first described during vulva development in the nematode C. elegans (Kimble, 1981; Kimble and White, 1981). This mechanism of cell diversification appears to be a prevalent one and, in addition, has been shown to occur during bristle development in Drosophila (Stern, 1968) and algae (Wilcox et al., 1973).

This process may respond to the lack of precision of the underlying molecular machinery to select a single cell out of a complex population and therefore the alternative strategy is used: a group is selected and competition amongst the cells in the group, a different subroutine, resolves the problem.

That the extra neuroblasts segregated in neurogenic mutants correspond to equivalence groups is supported by two lines of evidence. Firstly, neural precursor markers like ftz display the corresponding enlarged domain of expression in neurogenic mutant embryos (unpublished data). Secondly, the number of differentiated cell types in peripheral sensory organs is increased in these mutants, again indicating that multiple, identical lineages have been generated (Hartenstein and Campos-Ortega, 1986; see Fig. 2). Given that most neuroectodermal cells segregate in neurogenic mutants, the fact that correct lineages are still generated roblasts while still in the ectoderm (see Fig. 1).

Certainly the main theme of neurogenic gene function discussed here is not their exclusive activity. For one thing only a few components of this group have been mentioned (see Lehmann et al., 1983). In addition, requirements for these genes have been demonstrated in mesodermal development (Corbin et al., 1991) and neurogenic gene function is required as well in epidermal cells for their viability (Hoppe and Greenspan, 1990).

Neuroblast segregation is a regularly regulated process in which the timing and number of cells involved is controlled (Bate, 1976; Doe and Goodman, 1985a; Campos-Ortega and Hartenstein, 1985; Doe et al., 1988a; Shepherd and Bate, 1990). What triggers neuroblast segregation and therefore the initiation of neurogenesis? Inductive processes similar to those described in vertebrates have been ruled out (Leptin, 1991; Kosman et al., 1991; Rao et al., 1991). The nature of the signal in Drosophila is unknown. However, the discovery that the membrane-bound Notch and Delta proteins can interact heterotypically in a fashion characteristic of cell adhesion molecules has opened new horizons (Fehon et al., 1990). Indeed, cellular properties such as cell sorting have been shown to result from differences in the amount and the degree of specificity of interaction of the mammalian cell adhesion molecules expressed in tissue culture (Friedlander et al., 1989). Similar modulations could take place during the expression of Notch and Delta. It is conceivable that a signal may be generated in this way and that transduction of this signal to the nuclei activates the ASC proteins, thus triggering neurogenesis.

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