Distribution and function of the lethal of scute gene product during early neurogenesis in Drosophila

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Summary

Genes of the achaete-scute complex (ASC) participate in the formation of the central nervous system in the Drosophila embryo. Previous genetic analyses have indicated that lethal of scute (l'sc) is the most important gene of the complex in that process. We have obtained antibodies against the l'sc protein to study the expression of the gene during early neurogenesis. The protein is found in groups of embryonic neuroectodermal cells, analogous to the proneural clusters that precede the appearance of precursors of peripheral sensory organs in imaginal epithelia. The groups appear in different regions of the neuroectoderm, accompanying the three successive waves of neuroblast segregation. Most neuroblasts delaminate from these clusters and express position-specific levels of l'sc protein. No significant differences have been found between the distribution of l'sc RNA and protein. Phenotypic analysis of a l'sc deficiency has shown that the gene is required for neuroblast commitment, although this requirement is less widespread than the domain of l'sc expression, suggesting a high degree of redundancy in the function of genes that participate in the process of neuroblast segregation. The ASC genes have been postulated to play a role in the control of NB identity, revealed by the generation of a defined lineage of identifiable neurons. However, our study in l'sc mutants of the expression of fushi tarazu, engrailed, and even-skipped, used as markers of neuronal identity, has not provided evidence to support this hypothesis.

Key words: Drosophila, lethal of scute gene, antibodies, neuroblast segregation, neuroblast fate, functional redundancy.

Introduction

Genetic and molecular evidence indicates that the development of the central (CNS) and peripheral (PNS) nervous systems in Drosophila are analogous processes that involve the participation of a number of common genes. A working hypothesis of how peripheral sense organs are generated envisions a series of steps mediated by different families of genes (Ghysen and Dambly-Chaudière, 1989; Jan and Jan, 1990). An early and crucial step in this process is the appearance of groups of ectodermal cells that have acquired a neural potential. From these groups, the proneural clusters, a sensory mother cell will arise through a competition process mediated by cell interactions. There is genetic evidence for the existence of proneural clusters (Simpson, 1990a), which are revealed by the expression of genes of the achaete-scute complex (ASC) (Romani et al. 1989; Cubas et al. 1991; Skeath and Carroll, 1991). Indeed, genetic studies have demonstrated a requirement of the ASC for sense organ development, both in the larva and in the adult, around the time when sensory precursors become committed to the neural fate (García-Bellido and Santamaria, 1978; Ghysen and O’Kane, 1989; Bodmer et al. 1989; Cubas et al. 1991).

Cell ablation experiments performed during the early stages of neurogenesis in locust embryos suggest that the process of segregation of CNS precursor cells, the neuroblasts (NBs), also occurs through the commitment of single cells from proneural clusters in the ectoderm (Doe and Goodman, 1985). The existence of mutations in Drosophila in which the entire neuroectoderm adopts a neural fate (Lehmann et al. 1983) tends to support this view. The similarity in the mechanisms of generation of neural precursors in the PNS and CNS is paralleled by similarities in the expression of the ASC in both systems. Thus, transcription of ASC genes in the embryo closely correlates with the early phases of CNS development (Cabrera et al. 1987; Romani et al. 1987), suggesting that the ASC genes play a role in the segregation of NBs. Furthermore, analysis of the
transcription patterns has led to the proposal that the ASC participates in the establishment of NB identity (Cabrera et al. 1987). The identity of a NB (i.e. the genes it will express and the progeny of neurons it will produce) depends on its position of segregation from the neuroectoderm (Doe and Goodman, 1985). Genetic studies have also demonstrated a requirement for theASC in CNS development. A deletion of the ASC results in a highly hypoplastic embryonic CNS (Jiménez and Campos-Ortega, 1979), due in part to the commitment of fewer ectodermal cells to a NB fate than in the wild-type (Cabrera et al. 1987; Jiménez and Campos-Ortega, 1990). Phenotypic analysis of late embryos with partial deletions of the ASC points to the lethal of scute (l'sc) gene being the key element in central neurogenesis, although it interacts synergistically with the other three genes of the complex, achaete (ac), scute (sc), and asense (ase), that perform qualitatively similar functions (Jiménez and Campos-Ortega, 1987). The functional similarity between the ASC genes correlates with the structural similarity of the proteins they encode. The four proteins contain the basic-helix-loop-helix domain characteristic of a family of transcriptional regulators that are able to form functional heterodimers in vitro (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; González et al. 1989; Murte et al. 1989).

To analyze the postulated roles of l'sc in the commitment of NBs and the determination of their fates, we have raised an antibody against the l'sc protein to study, at the single cell level, the wild-type expression of the gene before and during the process of NB segregation. Our results indicate that the l'sc protein accumulates in groups of neuroectodermal cells, which are equivalent to the proneural clusters in imaginal epithelia. Later, NBs delaminate from these groups, and express position-specific levels of protein. Phenotypic analysis of a l'sc deficiency indicates a requirement of the gene for the segregation of some NBs. The fact that only a small subset of the NBs that express l'sc are absent in the mutant embryos, suggests a high degree of functional redundancy among the group of genes that control the process of NB segregation. On the contrary, our results do not favour a role of l'sc in the specification of NB fates.

Materials and methods

Antibody production

To obtain anti-l'sc antibodies, a protein was generated that contains the entire l'sc polypeptide, minus the first 15 amino acids (Alonso and Cabrera, 1988), fused to glutathione S-transferase, and expressed from a pGEX vector (Smith and Johnson, 1988). The fusion protein was expressed in Escherichia coli and the crude bacterial lysate was subjected to SDS–polyacrylamide gel electrophoresis. After staining the gel, the band containing the fusion protein was excised, fragmented in a small volume of PBS, and used to immunize three rats. All injections were carried out subcutaneously with 0.2 mg of protein in complete Freund's adjuvant per animal, at intervals of three weeks. Antibody production was monitored by immunocytochemistry on whole embryos. The three rats, which gave similar antibody titers, were bled out ten days after the sixth injection, and the serum was preadsorbed on fixed wild-type embryos. The specificity of the antibody was tested by staining embryos from a stock of the Df(l'sc)¹sc⁰. This is a small deficiency that deletes l'sc (also called the T3 gene), but no other ASC genes. Only background staining was found in about one quarter of the embryos.

Immunocytochemistry

Decorionated embryos were fixed for 20 min in heptane, saturated with 4% formaldehyde in 0.1 M Hepes (pH 7.0), 2 mM EGTA, 1 mM MgSO₄. The vitelline envelopes were removed as described by Mitchison and Sedat (1983). All subsequent incubations and washes were performed in BBT (10 mM Tris (pH 6.9), 55 mM NaCl, 7 mM MgCl₂, 40 mM KCl, 5 mM CaCl₂, 20 mM glucose, 50 mM sucrose, 0.1% Tween 20, 0.1% bovine serum albumin). Embryos were incubated overnight at 4°C with a primary antibody, reacted with the appropriate biotinylated secondary antibody (Amersham) and stained using the Vectastain Elite ABC kit (Vectorlabs) and diaminobenzidine. Nickel and cobalt ions were added to this first staining reaction in cases of treatment with two different primary antibodies (Adams, 1981). In these cases, the first staining was followed by incubation with a second primary antibody and subsequent steps as above. Finally, the embryos were dehydrated and embedded in Epon.

NB maps of mutants were elaborated from staged embryos that had been stained first with anti-l'sc antibodies, to recognize mutant embryos, then with anti-engrailed antibodies, to mark segmental boundaries, and finally with mAb43B5 against neurotactin, to label cell membranes (de la Escalera et al. 1990).

Other procedures

In situ hybridization to whole embryos followed the protocol of Tautz and Pfeifle (1989), as modified by C. Oh and B. Edgar (personal communication).

Images were obtained with a Zeiss laser scan microscope (LSM) operating in the transmission mode, and photographed from the TV screen; except those of Fig. 7, which were obtained with a Zeiss Axioskop microscope.

Results

Expression of the lethal of scute protein before neuroblast segregation

The l'sc protein is already detectable in the cellular blastoderm (stage 5; Campos-Ortega and Hartenstein, 1985). It is found in the nuclei, as previously observed by Cabrera (1990), in agreement with its postulated function as a transcription factor (Murte et al. 1989). Its pattern of expression undergoes very dynamic changes. Six broad dorsoventral stripes of staining, resembling the expression of pair-rule genes, are first seen in the blastoderm region that will give rise to the germ band (Fig. 1A). This pattern evolves, giving rise to twelve thinner stripes that initially appear to be continuous and of alternating intensity (Fig. 1B). The expression is not uniform along the dorsoventral axis. Thus, the intensity and width of the stripes is highest in the ventral-most region of the presumptive neuroectoderm, and the presumptive mesoderm is not stained (Fig. 1C).
Fig. 1. The wild-type pattern of l'sc protein distribution before the onset of neuroblast segregation. (A) First signs of expression in the blastoderm as six broad bands, that soon (B) become twelve, alternating in intensity. (C) Ventral view of an embryo that is initiating mesoderm invagination (vf: ventral furrow). The striped expression in the presumptive ectoderm ceases abruptly as it reaches the mesoderm (ms), which is not stained. (D) The twelve stripes become interrupted in the middle by a lateral rostrocaudal band of non-stained cells. (E) Ventrolateral view during germ band extension, showing how cells from both halves of the stripes become organized as two groups which occupy the dorsal- and ventral-most territories of the neurogenic region. (F) Cells between both groups begin to express the protein, leading to the formation of characteristic clusters at the end of stage 8 (see also Fig. 2B). Anterior is to the left in all pictures. Arrows in E and F point to the midline. Scale: 50 μm.

later phase, l’sc expression ceases along a lateral rostrocaudal band and the stripes become split in two parts (Fig. 1D). During early germ band extension, both halves of each stripe appear to rearrange, giving rise to a dorsal and a ventral group of stained cells, which are irregular in shape and size (Fig. 1E). The groups are confined to the neurogenic region of the ectoderm (Hartenstein et al. 1985). As germ band extension proceeds, newly expressing cells connect the dorsal and ventral groups, leading to the formation of large clusters by the end of stage 8 (Figs 1F, 2B). These clusters are equivalent to the ‘circles’ described by Cabrera (1990). Each cluster consists of 30–40 neuroectodermal cells that display very different levels of protein.

In contrast to the observations of Cabrera (1990), we find that the l’sc protein levels increase gradually between stages 5 and 8, paralleling a progressive accumulation of l’sc RNA (not shown). Furthermore, he detected l’sc protein accumulation in only a subset of the cells expressing l’sc RNA during stage 8; however, after analyzing many embryos at this stage, we believe that the domains of l’sc RNA and protein expression, as detected with our antibody, are coincident (Fig. 2).

Protein expression during neuroblast segregation
In early stage 9, about ten NBs segregate from the neuroectoderm of each hemisegment and become arranged in three longitudinal rows (Hartenstein and Campos-Ortega, 1984). Four NBs occupy the medial and lateral rows, respectively, and about two NBs the intermediate row. We find that most of these NBs express l’sc at fairly reproducible levels, which are characteristic of the position they occupy within the NB map (Fig. 3B,C). Eight NBs, expressing intermediate to high levels of protein, have apparently delaminated from each cluster of neuroectodermal cells that express l’sc. As a rule, these NBs are more strongly stained than...
During stage 10, the patterns of I'sc expression in the neuroectoderm and in the NB layer become even more complex and have not been studied in detail. However, these patterns seem to display the same basic features as those already described, i.e. new groups of ectodermal cells begin to express the protein, and intermingle with previously expressing clusters. NBs of the third and last subpopulation delaminate from these groups and apparently accumulate characteristic levels of protein, according to their position. It is difficult to assess whether I'sc is expressed in all the NBs that segregate during this stage, since the rows of NBs are now disorganized. Protein expression ceases in the ectoderm and in the NB layer during stage 11, except in some small cell clusters, which are presumably precursors of peripheral sensory organs. In addition, protein accumulation has not been detected in the NB progeny.

**Pair-rule genes regulate the expression of lethal of scute**

The early patterns of I'sc expression display a precise periodicity along the rostrocaudal axis, which evolve from an initial pattern of six stripes in the blastoderm, to 12, and later to 24 during stage 9. Segmentation genes are therefore likely candidates to control these early patterns. We have found that the pattern in stage 9 is modified in embryos that are homozygous for all pair-rule mutations examined (ftzw20, eve Rn, prd 2 4S17, opa SH97, run h), h2). The alteration is specific for each mutant, as shown for two cases in Fig. 4. The existence of cross-regulatory interactions between pair-rule genes (Ingham and Gergen, 1988) hinders the easy determination of which of these genes may directly regulate the expression of I'sc. The analysis of mutations in several segment polarity genes (ci, gsb X62, hh gk94, nkd 7 16, ptc N168 and w g) has not revealed appreciable alterations of these early patterns (not shown).

**Requirements of lethal of scute for neural development**

It has been reported that the ASC is required for the commitment of ectodermal cells to NBs (Cabrera et al. 1987; Jiménez and Campos-Ortega, 1990). However, these studies involved the analysis of chromosomal deficiencies that delete more than one gene of the complex, preventing assessment of the particular role of each gene. Furthermore, it has been suggested that the provision of specific quantities of ASC gene products might contribute to elements of NB identity (Cabrera et al. 1987). In this context, it is interesting that each NB, identified by its position in the NB map, accumulates characteristic amounts of I'sc protein. To evaluate the functional significance of the expression pattern of I'sc, we have analyzed the consequences that alterations in the protein levels, or the removal of the gene, have for NB segregation and establishment of NB identity.

Several mutations affecting the expression of the sc gene are associated with chromosome rearrangements that map close to the I'sc transcription unit (Campuzano et al. 1985; Balcells et al. 1988). We have analyzed I'sc expression in some of these mutations and have focused...
Fig. 3. The wild-type pattern of l'sc protein distribution during neuroblast segregation. (A) Detail of the neuroectoderm in early stage 9, 40 min (at 25°C) after the beginning of germ band extension. Stained cells are still organized in large clusters as in stage 8 (Fig. 2B), although the number of cells located closest to the midline (thin arrow) has slightly decreased. Vertical brackets on the right margin of the picture mark the extent of the clusters. In a deeper plane of focus (B), most NBs of the first subpopulation can be seen, arranged in three rows: medial (m), intermediate (i) and lateral (l). NBs differ in the amount of protein they accumulate. In particular, one NB per hemisegment (arrow heads) does not seem to express l'sc. Analysis of several embryos leads to the scheme shown in C, representing the neuroblast map and the relative intensities of staining, although these are not exactly reproducible in all embryos. Black indicates strong staining, hatched intermediate staining, and white no staining, as observed in most embryos. Combinations of this code in a circle indicate that a certain degree of variation in the staining of the NB is frequently found. Vertical brackets on the right margin of the scheme indicate the group of NBs that delaminate from each large cluster midline: ml. (D) 20 min later (mid-late stage 9), the pattern of expression has changed in the neuroectoderm. From a continuous rostrocaudal band of expressing cells (white arrow) new NBs have delaminated, occupying positions in the medial row, as seen in a deeper plane of focus (E). In addition, one new cluster of stained cells is seen in the midline of each segment (open arrow heads). The scheme in F shows the relative levels of protein accumulation in NBs at this stage. Arrows in C and F indicate the segmental borders, as determined by simultaneous staining with engrailed antibodies. Anterior is to the top. Scale: 20 μm.
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Fig. 4. Patterns of l'sc protein distribution in stage 9 wild-type and pair-rule mutant embryos. The double-stripe pattern observed in a lateral view of a wild-type embryo (A) is modified in eve^{R13}(B) and opt^{RSP} (C) embryos. Note that eve embryos do not extend the germ band completely. Scale: 50 \(\mu\)m.

Fig. 5. l'sc protein distribution in In(l)sc\(^{260,14}\) embryos. (A) A detail of the NB layer of a mutant embryo, 1 hour after the beginning of germ band extension (the same stage as in Fig. 3D-F). Medial (m), intermediate (i), and lateral (l) rows are indicated, although the latter is out of focus. Note the reduced levels of expression, as compared with a sibling wild-type embryo (B) that has been processed and photographed under exactly the same conditions. Arrows point to the midline. Scale: 20 \(\mu\)m.

Fig. 6. Neuroblast segregation in a deletion of l'sc. The diagram in A shows the NB map and the relative levels of protein accumulation in a wild-type thoracic hemisegment, 1 hour after the beginning of germ band extension (see Fig. 3 for symbols). (B) A scheme resulting from the detailed analysis of NB maps from four Df(1)sc\(^{24,9R}\) embryos of the same age. Three thoracic hemisegments were analysed per mutant embryo. The number within each circle indicates the number of cases in which the particular NB is missing. Note that there is no strict correlation between the levels of protein in the wild-type and the positions affected in the mutant.

preferentially affected (Fig. 6), similar to the effect of deleting the whole ASC (Jiménez and Campos-Ortega, 1990).

We have closely examined differential gene expression in NB lineages, which is expected to be altered...
if NB identity is not correctly determined. In particular, we have analyzed the late expression of engrailed (en), fushi-tarazu (ftz), and even-skipped (eve) in subsets of neurons, by staining with specific antibodies against the corresponding proteins. No noticeable differences between Hw^{9sc} and wild-type embryos have been observed in any of the three cases (not shown). In Df(1)sc^{9sc} embryos, the expression patterns of the three genes remain basically unaffected, although the number of stained cells is slightly lower than normal (Fig. 7). These mild defects can be attributed to the lower number of NBs that delaminate, as described above, and by the occurrence of cell death within the neural primordium of mutant embryos, as reported previously (Jiménez and Campos-Ortega, 1979).

**Discussion**

We have used a polyclonal antibody to study the expression of the l'sc protein during embryogenesis. Our analysis has focused on the expression before and during the first waves of NB segregation. Presumably, we have detected all putative posttranslational variants of the protein, since the antibody was raised against a fusion protein that contains most of the l'sc amino acid sequence.

**Protein expression and the process of NB segregation**

The initial striped expression of l'sc in the blastoderm becomes progressively modified and refined, and leads to the accumulation of the protein in a fraction of neuroectodermal cells. These cells are arranged in periodic clusters by the end of stage 8, shortly before the first NBs segregate. What is the significance of this expression pattern? It has been proposed that the commitment of sensory mother cells (SMCs) in the imaginal discs is initiated when specific groups of epidermal cells acquire a proneural state (Ghysen and Dambly-Chaudière, 1989; Jan and Jan, 1990). These groups of cells, or proneural clusters, become defined by the expression of two members of the ASC, the ac and sc genes. The activity of these genes is thought to confer the ability to become SMCs to cells of the cluster (Romani et al. 1989; Cubas et al. 1991; Skeath and Carroll, 1991). Subsequent cell interactions lead to the singling out of one cell as the SMC and the inhibition of the neural fate in the remaining cells of the cluster. Experimental and genetic evidence (Doe and Goodman, 1985; Lehmann et al. 1983) allows the extension of this hypothesis to the process of NB commitment in the embryo. Thus, every large cluster of ectodermal cells expressing l'sc in stage 8 can be envisaged as a series of adjacent smaller groups of cells that have acquired neurogenic competence. Early in stage 9, each of these small proneural clusters gives rise to one NB that accumulates the protein in higher amounts than the remaining cells of the group. Similarly, in imaginal discs, SMCs also exhibit higher levels of ac and sc expression than the surrounding cells of the proneural clusters (Cubas et al. 1991; Skeath and Carroll, 1991). Interestingly, from the ten NBs that segregate per hemisegment in the first wave, one of them and the underlying ectodermal cluster, do not seem to stain with the antibody. A second NB, which usually shows low levels of protein, has a cluster underneath that is apparently devoid of staining. Preliminary observations indicate that, at this stage, ac and sc are precisely expressed only in the four NBs, and associated proneural clusters, that display lower or no staining for l'sc (M. Ruiz, personal communication, and our own observations). Thus, all proneural clusters that give rise to the first NB subpopulation would express one or several ASC genes.

In mid-late stage 9, when previous expression in neuroectoderm and NBs still persists, new groups of cells begin to accumulate l'sc protein in areas of the neuroectoderm previously devoid of staining. A recent model for the mechanism of segregation of neural precursors in imaginal epithelia (Simpson, 1990b) proposes that, even in areas that will become densely populated with sensory organs, only a fraction of the cells have neural potential at any point in time. Assuming that l'sc (and ac/sc) expression is defining the proneural state of cells, the aforementioned results indicate that the same model may apply to the...
segregation of NBs in the embryonic neuroectoderm. Initially, not all cells of the neural region would have neural potential, although most or all of them may eventually acquire it in the course of the successive pulses of expression of the ASC genes.

Our description of the wild-type patterns of protein expression differs in several aspects from that of Cabrera (1990). As already mentioned, we find that the relative levels and domains of RNA and protein expression always appear to coincide, thus indicating that posttranscriptional regulation, if it exists, does not play a major role in establishing the pattern of protein accumulation. Cabrera also observed that all cells that accumulate the l'sc protein segregate as NBs, whereas we have found that most expressing cells of the proneural clusters remain in the ectoderm, once a small fraction of them have delaminated. It is possible that these discrepancies are due to the different immunogens and the different staining techniques employed in both studies. In particular, it should be noted that the antibody used by Cabrera might recognize only an unphosphorylated variant of the protein (Cabrera, 1990).

We have shown that pair-rule genes regulate l'sc expression, and the same is probably true of the genes ac and sc, which are also expressed in periodical patterns. This regulation might serve to allocate differentially the ASC gene products in the neuroectoderm, where they could play a role in the establishment of NB identities (Cabrera et al. 1987) (however, see below). Alternatively, if the function of the ASC genes is restricted to confer on ectodermal cells a general neural character, their regulation by pair-rule genes could be a way of ensuring a registry between the metamerism of the epidermis and the metamerism of the CNS. This registry would facilitate the participation of the same genes in the establishment of cell identities in both tissues. In this regard, it is worth mentioning that segment polarity genes, which contribute to establishing cell identities in the epidermis of every segment (DiNardo and Heemskerk, 1990), do probably play a similar role in the NB layer (Patel et al. 1989). Likewise, homoeotic genes, which determine the segmental identities of the epidermis (Morata et al. 1990), probably also act to establish segment-specific NB patterns (Hartenstein and Campos-Ortega, 1984).

**Functional redundancy**

Phenotypic analysis of a deletion of l'sc has revealed defects in the NB map, demonstrating a requirement of the gene for NB segregation. However, the defects are very mild, in contrast to the extensive expression of the gene in most proneural clusters and NBs of the wild-type embryo. For example, NBs of the intermediate row, which accumulate large amounts of protein in the wild-type, seem to segregate normally in l'sc− embryos. Mutants with a deficiency of the entire ASC lack a higher proportion of NBs than l'sc− embryos (Jiménez and Campos-Ortega, 1990), even though the individual deletions of either ac, sc, or ase do not seem to affect NB segregation (unpublished observations); i.e. the defects in the NB map of ASC− embryos are stronger than the sum of defects produced by the mutation in every single gene of the complex. These observations highlight the cooperation and functional redundancy of the ASC genes in the process of NB segregation, which could be mediated by their similar gene products. The four ASC proteins belong to the family of HLH transcriptional regulators, and proteins of this class are able to form functional heterodimers (Murro et al. 1989). Thus, this property provides a molecular basis for the cooperation and redundancy of the ASC genes.

It is clear that the position within the neuroectoderm from which a NB segregates determines its fate, i.e. the characteristic neuronal lineage it will produce (Doe and Goodman, 1985; Doe and Smouse, 1990). Several indirect lines of evidence suggest that the ASC genes could be involved in specifying NB identities. First, neuronal cell death in ASC mutants (Jiménez and Campos-Ortega, 1979) may result from defective determination of NB fates, for the ASC genes are not expressed in the NB progeny. Second, as already mentioned, genes of the ASC are expressed in partially overlapping periodic patterns (Cabrera et al. 1987; Romani et al. 1987) that may contribute to establish a set of spatial coordinates in the neuroectoderm, which are used to assign NB identity. Furthermore, we have shown that NBs accumulate distinct amounts of l'sc protein, a situation analogous to the accumulation of ftz protein in a set of neurons, where it functions to establish neuronal identity (Doe et al. 1988a). We have therefore asked whether changes in the levels of l'sc protein affect NB identity. These levels are substantially reduced in In(1)sc26014 and Hwsc mutants, yet they develop normally, suggesting that profound changes in NB fates have probably not occurred. Expression of en, ftz and eve in neurons, a reliable indicator of NB lineages, is normal in those mutants. Similarly, expression of these three genes is essentially normal in a l'sc deficiency, even though the lack of some NBs, and cell death in the progeny of others, have precluded a more accurate analysis. Therefore, the available data do not suggest that l'sc functions in the positional specification of the unique identities of NBs. To obtain conclusive results, other neural markers should be employed (i.e. genes expressed in specific NBs, before the onset of cell death), or different experimental approaches should be attempted (i.e. ectopic expression of the ASC genes). It is also true that, if l'sc indeed belongs to the group of genes that control NB identity and their functions are partially redundant, then the perturbation of only one of these genes may not always result in substantial modifications of wild-type patterns. In this regard, it is worth mentioning that the lack of ftz or eve function produces appreciable identity changes in only a small fraction of the neurons expressing these genes (Doe et al. 1988a; Doe et al. 1988b). These results have been explained as the consequence of functional redundancy and compensatory mechanisms between regulatory genes.
In summary, our results have shown that lsc is expressed in most proneural clusters in the neuroectoderm and in the NBs that delaminate from these clusters. They also have demonstrated the partial requirement of lsc for the commitment of ectodermal cells to NBs, but do not provide evidence of a role for lsc in the specification of particular NB fates.

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