INTRODUCTION

The development of a functional nervous system requires a complex network of well-coordinated mechanisms that lead to the specification of many different neural cell types. These cell types belong to two major groups, neurons and glia. Studies in the developing central nervous system (CNS) of vertebrates suggest the presence of common as well as separated progenitors for these two types of cells (reviewed in Cameron and Rakic, 1991). However, the mechanisms leading to the determination of glial versus neuronal cell types are still obscure.

The insect nervous system is relatively simple and readily amenable to experimental and genetic manipulation. Insect neurons are arranged in a stereotyped pattern and can be identified by their projection pattern and position. This well-ordered system derives from a set of neural stem cells, the neuroblasts, which delaminate from the neurogenic region of the ectoderm, according to a reproducible spatial and temporal pattern (Bate, 1976; Hartenstein and Campos-Ortega, 1984; Doe, 1992b). In Drosophila, this process is under the control of neurogenic and proneural genes (e.g. reviewed in Campos-Ortega, 1993). Ablation experiments (Doe and Goodman, 1985; Witten and Truman, 1991), studies on gene expression (Doe, 1992b) and in vitro studies (Huff et al., 1989; Lüer and Technau, 1992) suggest that insect neuroblasts give rise to characteristic lineages, containing well-defined numbers and types of ganglion cells. A considerable fraction of the ganglion cells represents glia. Many glial cell types have been described for insects (e.g. Wigglesworth, 1959; Nordlander and Edwards, 1969; Hoyle, 1986; Fredieu and Mahowald, 1989; Klämbt and Goodman, 1991; Doe et al., 1991; Smith et al., 1991). They differ from neurons not only by their morphology, but also with respect to their proliferation pattern (Nordlander and Edwards, 1969; Doe et al., 1988a; Shepherd and Bate, 1990). However, the origin of insect glia is known for only a few cases in the Drosophila embryo: midline glia cells are the progeny of mesectodermal precursors (Crews et al., 1988; Klämbt et al., 1991) and longitudinal glia cells derive from lateral glioblasts (Doe et al., 1988a; Jacobs et al., 1989). In both cases, embryonic glial cells derive from neural progenitors restricted to glial fate. Common precursors for glia and neurons have only been shown to exist among neuroectodermal cells prior to neuroblast segregation at the early gastrula stage (Fredieu and Mahowald, 1989; Becker and Technau, 1990).

Lineage studies of the CNS are of crucial importance if one is to take full advantage of the extensive knowledge of genetics available in flies to study CNS development. However, as opposed to Caenorhabditis or the Drosophila PNS, detailed knowledge about the types and lineage relationships of cells in the Drosophila CNS is still pending. In this report, we describe the complete embryonic lineage of a neuroectodermal precursor of Drosophila melanogaster (and D. virilis) which in the abdominal CNS consists of a typical set of neuronal and glial cell types. We present evidence that both cell types derive from the same segregating stem cell. Furthermore, we show that the composition of this segmentally repeated lineage exhibits significant and reproducible differences in the abdominal and thoracic neuromeres.
MATERIALS AND METHODS

Cell transplantation

Transplantations were performed as previously described (Technau, 1986; Prokop and Technau, 1993a). Single cell transplantations were achieved by sucking donor cells into the capillary and by transplanting them directly into the host (direct transplantations) or by washing the cells after withdrawing them from the donor in either a drop of Schneider’s medium (Gibco), Ringer’s medium (20 mM KCl; 20 mM NaCl; 0.3 mM CaCl2; 1 mM Tris) or KCl solution (0.2 M). This treatment separated the cells from their cellular and acellular microenvironment before they were implanted into the host (indirect transplantation). Cells were transplanted isotopically and isochronically (see Fig. 1). Single cell implantation was inspected with Nomarski optics. Hosts were covered with halocarbon oil and reared to the embryonic stage 16/17 (stages according to Campos-Ortega and Hartenstein, 1985).

Injection of HRP into ectodermal cells

Injections were carried out (according to Technau and Campos-Ortega, 1985) in the early gastrula at 0-20% ventrodorsal diameter and either at about 40-50% or 60% egg length (see Fig. 1) using a capillary (outer tip-diameter 1-1.5 µm) filled with a 10% solution of HRP in 0.2 M KCl. Injections lead to labelling of 1-3 ectodermal precursor cells (approx. 50% of the cases are 1-cell labellings). Injected embryos were reared to the embryonic stage 16/17.

Preparation and staining of labelled embryos

Stage 16/17 embryos were fixed according to Zalokar and Erk (1977). The vitelline membrane was mechanically removed under phosphate buffer (PB). Embryos were stained in a solution of 0.5-1 mg of diaminobenzidine and 1 µl of 30% H2O2 in 1 ml PB for about 5 minutes. For double-labelling experiments, we used the enhancer-trap line P101 (Klambt and Goodman, 1991) as donors and hosts. Following diaminobenzidine treatment, P101 hosts were stained for β-galactosidase activity in X-Gal solution (adding one part of 20% X-Gal in DMSO to 99 parts of an approx. 60°C solution of 150 mM NaCl, 1 mM MgCl2, 3.3 mM K3[Fe(CN)6] in PB) at 37°C for several hours.

Specimens were dehydrated in alcohol and subsequently xylene. They were embedded in Araldite (Serva), either on slides or in glass capillaries of 0.2 mm inner diameter, which allow rotation under the compound microscope (for detailed description see Prokop and Technau, 1993a).

Dil labelling and in vivo observation of neuroectodermal precursor cells

T. B. developed a method to label with Dil individual ectodermal precursor cells and follow their fate in vivo (Bossl and Technau, 1993). 0.75-1 mg Dil (Molecular probes) was diluted in 1 ml salad oil, sonicated and spun for 15 minutes at 10,000 revs/minute. Dechorionated embryos were glued with their ventral side to a slide, dried slightly and covered with halocarbon oil. A thin capillary (outer diameter of the tip 0.5-1 µm) was moved into the space between the vitelline membrane and the outer surface of the ectoderm towards the cell to be labelled. A small drop (3-6 µm in diameter) of staining solution was released onto the outer membrane of the cell. Labelling is restricted to this cell and its progeny. Injection and observation were carried out on an inverted compound microscope (Leica) using a 100x objective. Labelled cells were inspected, using a fluorescent filter set, and documented at different developmental stages with a video camera system (Hamamatsu), a real time imaging system (Hamamatsu) and a commercial video recorder. To obtain permanent preparations, Dil-labelled clones in some cases were photoconverted according to Bartheld et al. (1991).

Immunohistochemistry

Staged embryos of the enhancer trap lines P101 and M84 (Klambt and Goodman, 1991) were stained with antibodies against β-galactosidase as described elsewhere (Schmidt-Ott and Technau, 1992). Following the diaminobenzidine reaction, embryos were washed in phosphate buffer (PB) and postfixed with 6% glutaraldehyde in PB. Specimens were dehydrated in ethanol, cleared in xylene and embedded in Araldite (Serva). Single embryos were selected and transferred to a slide with a small drop of Araldite. Ventral nerve cords were removed using a forceps and sucked into borosilicate capillaries with an outer diameter of less than 0.2 mm (Hilgenberg).

RESULTS

The lineage of an abdominal neuroectodermal precursor cell

To disclose the lineages of neuroectodermal precursor cells (nPC), we either isotopically transplanted single HRP-labelled nPC at the early gastrula stage, or we injected horseradish-peroxidase (HRP) directly into nPC at the same stage (Fig. 1). Progeny cells resulting from labelled nPC were analyzed at embryonic stage 16/17. Here, we describe one lineage that develops from a segmentally repeated nPC located on both sides of the ventral neurogenic region (vNR), at 5-10% ventrodorsal diameter (%VD). The lineage can be identified unambiguously by the typical morphology of its components. In the abdominal region, we analyzed 17 cases (Fig. 2; Table 1).

The lineage contains the sibling neurons aCC and pCC, which have been previously described in the grasshopper (Taghert et al., 1982; Goodman et al., 1982), Drosophila and other species (Thomas et al., 1984). In our preparations, the aCC motoneuron is located dorsally in the corner between the connectives and the posterior commissure, immediately anterior to the segment border. It projects ipsilaterally through the anterior root of the intersegmental nerve (see also Thomas et al., 1984) and terminates on the most dorsal internal oblique muscle 3 (see also Sink and Whitington, 1991; nomenclature of nerve roots according to Thomas et al., 1984, and of muscles according to Campos-Ortega and Hartenstein, 1985). The pCC interneuron is closely associated with the aCC, lying slightly posterior and ventral to it. It sends an axon into the medial section of the ipsilateral connective where it projects anteriorly, spanning approximately two neuromeres (at stage 16/17). The location of the aCC/pCC neurons was always according to this description. However, in one case, the aCC neuron was located in the hemineuromere contralateral to the other components of the clone, behaving like the contralateral aCC with respect to its position and projection.

aCC and pCC are associated with a characteristic cluster of four to six cells (Table 1). The cluster is located ventromedially to the aCC/pCC neurons immediately behind the neuromere border (Fig. 2). This is in accord with the description that aCC/pCC migrate anteriorly into the next neuromere after they are born (Taghert et al., 1982; Thomas et al., 1984). In all cases, the cluster forms a fascicle of interneuronal fibers projecting dorsally into the ipsilateral connective, where it turns posteriorly and extends to the posterior neuromere border. It appears as if most cells of the
Common origin of glia and neurons in *Drosophila*.

Fig. 1. Fate map of the early gastrula stage (lateral view; according to Technau and Campos-Ortega, 1985). The neurogenic regions of the head, thorax and abdomen are marked. Numbers indicate segment anlagen of the thorax and abdomen. nPC in the ventralmost area (0-20% VD) of different segmental regions were labelled at this stage: In the abdominal region, HRP injections (hatched arrow) and Dil labellings (open arrow) were carried out at about 40-50% EL, isotopic cell-transplantations (black arrow) at about 30% EL. In the thoracic region, nPC were labelled at about 50-60% EL with all three techniques. The drawing on the right side represents one half of a frontal section through the truncal region at the same stage. The arrow points towards the position about two to three cells apart from the midline cells, from where Dil-labelled NB1-1 segregated. dEpi, anlage of the dorsal epidermis; mes, mesoderm; vNR, ventral neurogenic region; % EL, % egg length (0%, posterior pole); % VD, % ventrodorsal dimension (0%, ventral midline).

Fig. 2. Example of the abdominal variant of the NB1-1 clone labelled with HRP (A,C, photomontages; B,D, camera-lucida drawings). The same preparation is shown in lateral view (A,B) and horizontal view (C,D). Anterior is to the left. Broken lines in the camera-lucida drawings (B,D) mark the outlines of the neuropil; the outline of the ventral nerve cord is marked by solid lines. The aCC/pCC neurons (shaded in B and D) are lying dorsally, slightly posterior to the posterior commissure (p). The aCC sends an ipsilateral projection through the anterior root of the intersegmental nerve (long dark arrow) and a short contralateral projection into the posterior commissure (small arrowhead). The pCC projects anteriorly through the medial section of the ipsilateral connective (small open arrows). A cluster of four cells (big open arrow) lies ventrocaudally to the aCC/pCC neurons and forms a posterior interneuronal fascicle (triangles). Two glial cells are located at the dorsal and one is located at the ventral periphery of the ventral nerve cord (short dark arrows in A,C). The anterior dG (vertically hatched in B,D) lies anterior to the aCC neuron, above the posterior commissure, and the posterior dG (diagonally hatched) lies posterior to the pCC at the segment border (big arrowheads in D point to the dorsoventral channels, which demarcate the neuromere border). The vG (horizontally hatched) lies in the ventral periphery below the cell cluster. a, anterior commissure; p, posterior commissure. Scale bar 20 µm.
cluster contribute axons to this fascicle. In some preparations, where 1 or 2 neurons of the cluster were dislocated dorsally or ventrally (see Table 1), we always detected the typical fascicle of axons.

In addition to these neurons, the lineage in all cases contained characteristic glial cells (Fig. 2; Table 1). One or two of them are lying at the dorsal surface of the CNS near the aCC/pCC neurons. We will refer to these cells as dorsal glia (dG). A further glial cell, the ventral glia (vG), is lying at the ventral surface of the vNC, in most cases close to the ventral midline, in other cases shifted to more lateral regions. The dG cells as well as the vG cells show prominent peripheral processes, which sometimes reach into the cortex. Since both are located at the surface of the vNC, they can be classified as subperineurial glia (SuPn; Hoyle, 1986).

Taken together, the embryonic lineage of the labelled abdominal nPC consists of the aCC/pCC neurons, a cluster of four to six cells with a characteristic position and interneuronal projection pattern as well as two or three glial cells (1-2 dG, 1 vG).

Since, in the neuroectoderm, the precursor of this particular lineage cannot be recognized from the surrounding cells, labelling was applied to random nPC in a ventral sector of the vNR. Among the many other lineages that we obtained in this way, we never detected the aCC or pCC or one of the glial cells observed here. But in addition to these two or three SuPn glia cells, there are at least five other SuPn cells arranged at different positions along the surface of the hemineuromere (see below). Thus, there exist other precursors that contribute to the population of SuPn cells.

The lineage of the nPC is conserved among different Drosophila species

The same type of abdominal lineage exists in D. virilis. This has been shown by intraspecific transplantation of single HRP-labelled nPC in this species (preparations kindly provided by T. Becker; Becker and Technau, 1990). Two clones resulting from these transplantations were in complete accordance with the nPC lineage described above for D. melanogaster (Table 1). These findings suggest that the mechanisms leading to that nPC lineage are conserved among different Drosophila species.

A single neural precursor generates both neurons and glia

The nPC lineage, which we described above, contains glial cells as well as neurons. Among them are the aCC/pCC neurons, which have previously been shown to be the daughter cells of the first ganglion mother cell of NB 1-1 (Thomas et al., 1984; Doe, 1992b). Thus, the lineage of NB 1-1 is at least included in our clone. However, the question arises whether NB 1-1 is identical to the stained nPC (in this case NB 1-1 would be a neuroglioblast; Fig. 3B). Alternatively, the nPC may divide, while still in the peripheral ectoderm, during the 14th cell cycle (Hartenstein and Campos-Ortega, 1985; Foe, 1989) to produce two independently segregating neural precursor cells, one glioblast giving rise to only glia and one neuroblast (NB1-1) producing only neurons (Fig. 3A).

To distinguish between these possibilities, we labelled nPC with the fluorescent dye DiI. This method (Bossing and Technau, 1993) enabled us to mark single nPC prior to the 14th cell cycle and to follow their further development in vivo. Four of the labelled abdominal nPC segregated as SII neuroblasts (Hartenstein and Campos-Ortega, 1984) from a

Table 1. Composition of all NB1-1 clones analyzed

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Table 1. Composition of all NB1-1 clones analyzed (D. virilis)

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Table 2. Composition of all NB1-1 clones analyzed

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Table 3. Composition of all NB1-1 clones analyzed

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Table 4. Composition of all NB1-1 clones analyzed

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Numbered (No.) horizontal lines represent individual clones. Cases are arranged according to their segmental distribution (see 2nd column; T, thoracic segments, A, abdominal segments) and to the techniques applied (Transplantations, Injections, Dil labelling). Cell numbers of the various clonal components are listed in columns 3-8: aCC motoneuron (aCC), pCC interneuron (pCC), ventral glia (vG), dorsal glia (dG), cell cluster (Cl) and motoneuronal fibers labelled in addition to the aCC projection (adMN). Since in most cases the Dil-labelled thoracic clones were inspected in vivo, it was not possible to judge whether the adMN projection consists of one or two fibers (1(2)). Cases in which cells of the cluster are shifted from their normal position towards more dorsal or ventral sites are marked (*).
position about two cell diameters distant from the midline cells (Fig. 4; see also Doe, 1992b). They did not divide prior to segregation and gave rise to clones comprising aCC/pCC, a cluster of four to five cells with an ipsilateral, posteriorly directed fascicle, vG and dG cells (Table 1).

Thus, we show here for the first time that an insect neuroblast can give rise to glia and neurons. NB1-1 has to be regarded as a neuroglioblast.

The identity of glial cells in the NB1-1 lineage

The abdominal lineage of NB 1-1 generally comprises one vG and one or two dG cells. In cases with two dG, the anterior dG lies anterior to the aCC (Fig. 2). This position is typical of the previously described B-glia cell (Klämbt and Goodman, 1991). The posterior dG lies posterior to the pCC. This position is typical of the previously described A-glia cell (Klämbt and Goodman, 1991). To test whether the dG cells are identical to A- and B-glia, we used the enhancer-trap line P101 (kindly provided by C. Klämbt; Klämbt and Goodman, 1991). P101 expresses β-galactosidase (β-gal) in the A- and B-glia and in six further SuPn cells distributed between dorsolateral and ventral positions of each abdominal hemineuromere (Fig. 6).

We isotopically transplanted HRP-labelled nPC in the ventral abdominal vNR of P101 and double stained the resulting clones for β-gal and HRP. Six NB1-1 lineages resulted from these experiments (Table 2). In three cases, the anterior dG was clearly double labelled for HRP and X-gal (Fig. 5). Thus, the anterior dG represents B-glia. In three further cases, this cell only stained positive for β-gal.

None of the clones allowed us to colocalize the markers in the posterior dG. In five cases, the posterior dG only stained positive for HRP (except one questionable case of double labelling; see Table 1), although an X-gal-labelled A-glia cell was detected in the corresponding position of the contralateral control hemi-neuromere (Fig. 5). Conversely, in one case, we found a X-gal-labelled A-glia but no HRP signal in that position. This suggests that, if colocalized in one cell, the stronger marker may mask the weaker one. Our failure in most of the cases to detect both signals simultaneously in the dG is therefore most likely due to methodological problems inherent with the double-labelling technique.

Although we thus have only indirect proof for the posterior dG, our results strongly suggest the anterior dG to be the B-glia and the posterior dG to be the A-glia cell.
The segmental pattern of A- and B-glia in the vNC
The neuromeres of the vNC of *Drosophila* show segment-specific differences (reviewed by Doe and Scott, 1988). To test for possible variations of the NB1-1 lineage, we analyzed the pattern of A- and B-glia cells throughout the vNC. We stained embryos of the enhancer-trap lines *P101* and *M84* (Klämbt and Goodman, 1991) and embryos carrying both P-element insertions for β-gal expression (Fig. 5). In *P101*, as well as in *M84*, the most posterior cells of the A- and B-type are the A-glia cells in the anterior part of the terminal neuromere A8/9. Thus, the most posterior A- and B-glia containing NB1-1 lineage seems to arise in A8, with its B-glia cell migrating anteriorly into A7. In *P101* and *M84*, the most anterior B-glia cells lie in the posterior part of neuromere T3, obviously deriving from the NB1-1 in A1. *M84*, but not *P101*, labels one A/B-like glia cell near the anterior border of each thoracic hemineuromere (Fig. 6). This A/B-like glia cell occupies an intermediate position between the typical locations of abdominal A- and B-cells. The fact that *P101* does not label these cells and that there is only one A/B-like glia cell labelled in each thoracic hemineuromere of *M84* suggested that the NB1-1 lineage might differ between the thorax and the abdomen.

**Table 2. Double-labelling experiments with embryos of the enhancer-trap line *P101***

<table>
<thead>
<tr>
<th>No</th>
<th>B-glia</th>
<th>ant dG</th>
<th>A-glia</th>
<th>post dG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbered (No.) horizontal lines represent individual preparations. The ant dG/B-glia, and the post dG/A-glia, were inspected for HRP- and/or X-Gal staining. Direct evidence that ant dG is identical with B-glia is presented by double staining in three cases and one likely case for the post dG/A-glia (shaded). ant dG, anterior dorsal glia; post dG, posterior dorsal glia; +, staining; −, no staining.

The NB1-1 lineage differs in thoracic and abdominal segments
To test this hypothesis, we labelled nPC in the presumptive thoracic region at 0-20%VD and about 60%EL (Fig. 1) by injecting HRP, by isotopically transplanting HRP-labelled nPC, or by applying Dil. Dil application and subsequent in vivo observation revealed that, like in the abdomen, the presumptive thoracic NB1-1 do not divide before they segregate (approx. 220-235 minutes after egg laying; early SII-NB) from a position 1-2 cell diameters apart from the midline cells. These findings are in accordance with other data on NB1-1 (Doe, 1992b). The resulting lineages labelled with either HRP or Dil were analyzed at stage 16/17 (25 cases; Table 1).

Each clone contained the aCC neuron and pCC neuron and a cluster of cells (Fig. 7). The cluster occupied a position comparable to the abdominal cell cluster and also formed a posteriorly directed fascicle. However, in contrast to the abdomen, the size of thoracic clusters varied between 8 and 14 cells (in one exceptional case 6-8 and in another 16-18). In addition to the interneuronal fascicle, the thoracic cell cluster formed a motoprojection, which separated from the ipsilateral interneuronal fascicle to project through the segmental nerve. In four preparations, this projection could be traced only to the border of the vNC; in 13 preparations, it reached the region where the pleural external oblique muscles overlap the dorsalmost ventral external oblique muscles. The exact target muscle could not be resolved. In some cases, the projection seemed to carry two closely associated fibers. Thus, there are one or two motoneurons among the cells of the thoracic cluster that do not present in the abdominal lineage. A further difference compared to the abdomen is the absence of dG cells as well as vG cells in the thoracic NB1-1 lineage. Thus, the thoracic A/B-like glia cell labelled in *M84* does not originate from NB1-1. Instead we detected this cell in a clone that significantly differs from the NB1-1 lineage and which derives from a nPC close to the midline (unpublished observation).

Taken together, thoracic NB1-1 segregate from similar positions along the VD axis and at about the same time as abdominal NB1-1. Thoracic NB1-1 lineages comprise elements that are homologous to the abdominal lineage (aCC/pCC; location and interneuronal projection of the cell cluster) as well as significant differences (no vG and dG; a higher number of cells in the cell cluster; additional motoneuron(s)). Thus, the segmentally repeated NB1-1 lineage expresses segment-specific differences at the cellular level.
DISCUSSION

The technical approach
We have used three different techniques to trace the development of an nPC: transplantation of single HRP-labelled nPC, injection of HRP into nPC and DiI labelling of individual nPC. These techniques have advantages and disadvantages, which complement each other. The first two techniques allow analysis in detail of the differentiated clones in permanent preparations of late embryos. DiI labelling was used to inspect aspects of the development in vivo. Whereas the transplantation as well as DiI-labelling techniques allow tracing of the fate of individual precursor cells, the injections may lead to labelling of more than one precursor. For transplantation (although isotopic transplantation was attempted), cells have to be removed from their original microenvironment; this is not the case for the other two techniques. However, the fact that the composition of the clone was confirmed by all of these approaches can be taken as an indication that none of them causes significant artefacts.

The abdominal NB1-1 is a neuroglioblast
The existence of nPC that give rise to glia as well as neurons has been previously reported (Becker and Technau, 1990; for in vitro studies see also Fredieu and Mahowald, 1989). However, whether these nPC delaminate from the ectoderm as common precursors for both neural cell types or whether they produce further nPC, which then independently segregate as glioblasts and neuroblasts, was not known. Here we show for the first time that, like in vertebrates (reviewed in Cameron and Rakic, 1991), neurons and glia in the CNS of *Drosophila* can share a common neural precursor. Firstly, we described the lineage of a nPC from abdominal ventral positions comprising identifiable glial as well as neuronal cell types. Secondly, we demonstrated that this nPC does not divide prior to delamination. Thus, the delaminating progenitor gives rise to neurons and glia, and has to be regarded as a neuroglioblast. So far, for the embryo, only neural progenitors restricted to either glial or neuronal fate have been described: some mesectodermal precursors solely give rise to midline glia (Crews et al., 1988; Klämbt and Goodman, 1991; Klämbt et al., 1991) and a lateral glioblast exclusively produces longitudinal glia cells (Doe et al., 1988a; Jacobs et al., 1989). In contrast, some midline precursors (Thomas et al., 1984) and a significant fraction of individually transplanted early gastrula nPC exclusively produce neurons (Technau and Campos-Ortega, 1986; Becker and Technau, 1990; our own observations).

The fact that NB1-1 delaminates from the ectoderm as a neuroglioblast and taking into account that its first two progeny (aCC and pCC; Doe, 1992b) are neurones, we conclude that glia-determining mechanisms do not only act in the neurogenic ectoderm but also at later stages during lineage development.

Independent markers for the glia in the abdominal NB1-1 lineage
To test for the identity of the glia in the abdominal lineage, we generated clones by cell transplantations in the enhancer-trap line *P101*, which labels A- and B-glia amongst other subperineurial (SuPn) cells. As the anterior dG stained positive for β-gal and HRP in three cases, it is identical with the B-glia cell. The position of the posterior dG as well as its complementation with the pattern of β-gal-labelled A-glia cells strongly suggest that the posterior dG is an A-glia
cell. Thus, besides independent markers for aCC and pCC (Doe et al. 1988a,b), the enhancer-trap lines M84 and P101 provide independent markers for a further (segment-specific) element of the NB1-1 lineage (dG) that will be useful for the analysis of the mechanisms involved in the specification of this lineage (Prokop and Technau, 1993b).

The complementary pattern of dG cells and A- and B-glia cells also shows that the implantation of an additional nPC into the vNR is regulated in a way that it replaces a local nPC as presumptive NB1-1. Thus, the implant does not independently produce an additional NB1-1 lineage but becomes integrated to participate in the interactions among the nPC of the host.

In the case of the vG, the interpretation is more difficult. The vG is located in the ventral periphery, its sheath-like extensions are closely associated with the surface of the vNC, and its nucleus appears in most cases rather big. This classifies the vG as SuPn glia (Hoyle, 1986). M84 and P101 stain the same set of six SuPn cells beneath the A-glia and B-glia in each abdominal hemineuromere. This is in accordance with descriptions using further glial markers (Urban et al., 1993). Thus, these six cells, together with the A- and B-glia seem to represent the complete SuPn population. This suggests that the vG is included in the SuPn set labelled by P101 or M84, although the double-labelling experiment failed to prove this.

**How variable is the NB1-1 lineage?**

For the grasshopper, it has been shown by laser ablation experiments that the first ganglion mother cell of NB1-1 always gives rise to aCC and pCC, even if its birth is delayed with respect to its local environment (Doe and Goodman, 1985). Investigations in *Drosophila* have shown a cascade of gene expression in cells of the early NB1-1 lineage that seems to be necessary for correct development of the aCC/pCC-neurons (Doe et al., 1988a,b, 1991; Treacy et al., 1991; Vässin et al., 1991). Furthermore, work in the grasshopper has shown that cell interactions between the aCC and pCC neurons, rather than with other cells, are necessary for their correct development (Kuwada and Goodman, 1985). Preliminary results suggest the presence of a gene in *Drosophila* that can interfere with these interactions of the aCC/pCC-neurons (Goodman and Doe, 1993). The second ganglion mother cell of NB1-1 has been shown to express the gene *ming* reproducibly (Cui and Doe, 1992). Thus, there are a number of arguments suggesting an autonomous, invariable development of the NB1-1 lineage. From these and other studies, it has been proposed that neural precursors, once they have been specified, give rise to invariant lineages (for reviews, see Cabrera, 1992; Doe, 1992a,b; Goodman and Doe, 1993).

However, since only the early components of the NB1-1 lineage (aCC/pCC) have been identified so far, it was not possible to test for variability amongst components of the clone, which arise at later stages. The approaches that we used allowed us to uncover the complete embryonic lineage of NB1-1.

On the one hand, our results confirm that NB1-1 invariably gives rise to aCC and pCC. In addition to this, we found other invariable pattern elements of the clone. These are the SuPn glia cells (in the abdomen), additional motoneuron(s) (in the thorax) projecting to a specific ventrolateral site and a cell cluster forming a typical posteriorly directed interneuronal fascicle. This fascicle is a peculiarity of the clone, as it remains ipsilateral. This is in contrast to most neural

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**Fig. 7.** Example of the thoracic variant of the NB1-1 clone. The precursor was labelled with DiI, and a permanent preparation of the fully differentiated clone was obtained following photomontage. (A) Horizontal view with aCC and pCC in focus (photomontage); (B, horizontal view; C, lateral view) camera lucida drawings of the same clone. The use of symbols is as in Fig. 2. Note that the cell cluster (big open arrow) contains more cells than the cluster in the abdominal lineage, a motorprojection (flat arrowhead) separates from the interneuronal fascicle (triangles) of the cluster and the SuPn glia cells are missing. Whereas the aCC projection (long dark arrow) runs through the anterior root of the intersegmental nerve, the additional motorprojection (flat arrowhead) takes the segmental nerve. Scale bar 20 µm.
lineages that we have found so far, which develop prominent contralateral projections (data not shown; see also Goodman and Doe, 1993). Thus, the NB1-1 lineage is characterized by a number of invariable pattern elements. These elements are conserved among different Drosophila species: in D. virilis, the composition of the clone is essentially the same.

On the other hand, with respect to cell numbers, we found some variations among components of the clone, which arise later than aCC and pCC: In the abdomen, the size of the cluster varied between four and six cells corresponding to a difference of one NB division. The abdominal NB1-1 lineages also revealed variations in the number of dG cells. This is in contrast to the A- and B-glia pattern in P101 and M84, where in general both cells are present in each abdominal hemineuromere. However, this pattern is often irregular (see Fig. 6), in that A- or B-cells are displaced up to several cell diameters from the normal position in the dorsal periphery, or shifted between the aCC/pCC neurons. Especially the latter case may be a possible reason for the detection of only one dG in many NB1-1 lineages.

In the thoracic lineages, the number of cells in the cluster varied between 8 and 14, corresponding to a difference of up to three neuroblast divisions. Although we cannot exclude mistakes in counting among closely associated cells, or failure in detecting weakly labelled cells, we think this variability to be significant.

Taken together, the NB1-1 lineage is composed of invariable pattern elements. But there seems to be some variability in the number of cells constituting certain of these elements, and this variability is likely to be expressed rather late by the developing lineage. We do not know whether variable clone sizes are conserved during further development or whether cell numbers will be adjusted at later stages, for example by cell death (e.g. Abrams et al., 1993).

The NB1-1 lineage shows segment-specific differences

The vNC of Drosophila shows neuromere-specific morphological differences especially at postembryonic stages (e.g. Hertweck, 1931; Teugels and Ghysen, 1983; Thomas and Wyman, 1984; Ghysen et al., 1985; Truman and Bate, 1988). However, in the embryo, the pattern of neuroblasts seems to be the same in different segments of the vNC (Doe, 1992b) and segmental differences have not been described so far on the cellular level during embryonic neurogenesis. The only indication is the segment-specific expression of several genes and markers (e.g. Jiménez and Campos-Ortega, 1981; White and Wilcox, 1985; Bray et al., 1989; Gould et al., 1990; Bourgouin et al., 1992; Cohen et al., 1992; Graba et al., 1992; Nose et al., 1992; Mellerick et al., 1992; Cui and Doe, 1992).

We now demonstrate that, in Drosophila, a segmentally repeated embryonic neuroblast gives rise to segment-specific lineage variants (for the grasshopper, see also Bate et al., 1981). Preparations of NB1-1 lineages in most truncal neuromeres (Table 1) suggest the existence of the same lineage variant in all thoracic neuromeres and one abdominal variant for most of the abdominal neuromeres. Correspondingly, the pattern of the A- and B-glia in enhancer-trap lines P101 and M84 is also significantly different between thorax and abdomen. The A/B-like glia cell in the thorax of M84 may have similar functions as A- and B-cells in the abdomen; however, it does not originate from NB1-1.

The thoracic and abdominal NB1-1 lineages also differ in their cell numbers. The thoracic lineage comprises significantly more cells than the abdominal lineage. This is in accord with the fact that proliferation patterns in the late embryonic vNC differ between thorax and abdomen (Prokop and Technau, 1991; Prokop and Technau, 1993c). Among the additional cells in the thoracic lineage are one or two motoneurons. These neurons are located in the cell cluster. In the corresponding positions of abdominal neuromeres, ipsilaterally projecting motoneurons do not seem to exist (Sink and Whiting, 1991). Also the pattern of somatic muscles expresses segmental differences (Campos-Ortega and Hartenstein, 1985; Hooper, 1986; Bate, 1993). Furthermore, considering the pattern of postembryonic neuroblasts (Truman and Bate, 1988), it is likely that in the thoracic neuromeres, as opposed to most of the abdominal neuromeres, one of the cells in the cluster represents a presumptive postembryonic neuroblast (Prokop and Technau, 1991), although, at this stage, such a cell is not identifiable by morphological criteria.

We hypothesize that common features of the thoracic and abdominal lineage are generated at early stages. This is the case for aCC and pCC (Thomas et al., 1984) and might be also true for part of the cluster. Subsequently, both lineages diverge to produce SuPn glia in the abdomen or further neural cells as part of the cluster in the thorax. Accordingly, preliminary results from BrDU injections suggest that A- and B-glia are born rather late (unpublished results). The decision between thoracic and abdominal pathways of NB1-1 is under the control of homeotic selector genes (Prokop and Technau, 1993b).

Prospects

The NB1-1 clone is the first cell lineage in the Drosophila CNS described in full. Knowing the composition of the NB1-1 clone, its segment-specific variants, and independent markers for cells of this lineage enable an experimental approach to the mechanisms involved in the specification of this precursor and its lineage (Udolph et al., 1993; Prokop and Technau, 1993b). Unlike the nematode or the Drosophila PNS, where the combination of lineage analysis and genetics prompted rapid progress, genetic and molecular analysis of the Drosophila CNS has been hindered by the lack of wild-type lineage information. The techniques available now make the CNS of Drosophila accessible for a detailed lineage analysis. Once the lineages are known, investigating the consequences of transplantation or of mutant and ectopic gene expression on the phenotypes of individual lineages will provide insight into the mechanisms leading to cell diversity in the CNS.

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Common origin of glia and neurons in *Drosophila*.


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