New neuroblast markers and the origin of the aCC/pCC neurons in the Drosophila central nervous system

Julie Broadus, James B. Skeath, Eric P. Spana, Torsten Bossing, Gerhard Technau, Chris Q. Doe

Drosophila is an ideal system for identifying genes that control central nervous system (CNS) development. Particularly useful tools include molecular markers for subsets of neural precursors (neuroblasts) and the simple expression pattern of the even-skipped (eve) gene in a subset of neurons. Here we provide additional molecular markers for identified neuroblasts, including several with near single cell specificity. In addition, we use these new markers to trace the development of several eve+ neurons. Our results show that the eve+ aCC/pCC neurons develop from a different neuroblast than previously thought, and have led us to assign new names for several neuroblasts. These results are supported by Df cell lineage analysis of neuroblasts identified in vivo.

Keywords: Drosophila; Central nervous system; Neural precursor; Neuroblast; Ganglion mother cell; Even-skipped; Gooseberry
neuroectoderm is necessary, and sufficient when ectopically expressed, to specify the identity of row 5 NBs (Skeath et al., 1995). Finally, expression of long-lived markers, such as the β-gal product expressed by lacZ transgenes, provides a method for predicting lineage relationships between recently divided cells. However, acquisition of β-gal as a result of cell division cannot always be distinguished from de novo transcription of lacZ; therefore, the use of inheritable gene products to trace cell divisions must be used cautiously.

Specification of unique NB fates is the first step in generating cellular diversity in the CNS. Subsequently, the majority of different cell types arise as each NB produces a characteristic cell lineage of 2-20 neurons and/or glia. Within a subset of NB cell lineages, the relatively simple pattern of eve expression (Frasch et al., 1987; Patel et al., 1989) is frequently used as an assay for GMC and neuronal identity (e.g. Doe et al., 1988; Smouse et al., 1988; Patel et al., 1989; Jimenez and Campos-Ortega, 1990; Doe et al., 1991; Duffy et al., 1991; Cui and Doe, 1992; Chu-LaGraff and Doe, 1993; Yang et al., 1993; Bhat and Schedel, 1994; Spana et al., 1995; Yeo et al., 1995). eve is expressed in a small number of GMCs and neurons in each hemisegment. Despite the widespread use of eve as a marker for GMC and neuronal identity, our knowledge of how the eve pattern develops is incomplete.

A detailed description of eve expression at earlier stages is important for the interpretation of many existing mutant phenotypes.

Here we provide additional molecular markers for identified NBs. We use these new markers to trace the development of several eve+ neurons. Our results show that the aCC/pCC neurons develop from a different NB than previously thought (Doe, 1992), and have led us to assign new names to several NBs in the map. In addition, these results are supported by DiI cell lineage analysis showing that the NB at the 1-1 position generates a clone containing the eve+ aCC/pCC neurons.

2. Results

2.1. An updated and revised neuroblast map

The NB map shown in Fig. 1 is based on the map of Doe (1992) and includes the expression patterns for seven additional genes: mirror-lacZ (mir-lacZ), odd-skipped

![Fig. 1. Summary of molecular markers expressed in neuroblasts. Summary of the ‘consensus pattern’ of NBs at different developmental stages, with the expression of 15 molecular markers indicated in color. The pattern of odd is shown for S1–S3 only. achaete (ac), engrailed (en), wingless (wg), gooseberry-distal (gsb-d), and odd-skipped (odd) represent protein patterns; prospero (pros) represents nuclear protein localization; mirror-lacZ (mir-lacZ), engrailed-lacZ (en-lacZ), fushi tarazu-lacZ (ftz-lacZ), seven up-lacZ (svp-lacZ), ming-lacZ, huckebein-lacZ (hkb-lacZ), wingless-lacZ (wg-lacZ), and unplugged-lacZ (upg-lacZ) represent β-gal patterns; eagle (eag) is an RNA pattern; see text for details. (A) Early S1 NBs; late stage 8. (B) S1 NBs; early stage 9. (C) S2 NBs; stage 9. (D) S3 NBs; stage 10. (E) S4 NBs; stage 11. (F) S5 NBs; late stage 11. Anterior, top; ventral midline, dotted line; large circles, NBs; small black spots, sites of NB formation at the next stage. This map and subsequent updates can be accessed over the internet at: http://sippie.life.uiuc.edu/doelab/nbintro.html.]

Embryonic stages are according to Campos-Ortega and Hartenstein (1985); NB stages (S1–S5) are according to Doe (1992). In addition, the NB map can be accessed via the internet (the URL is http://sippie.life.uiuc.edu/doelab/nbintro.html). This ‘hyper-NB map’ contains cross-referenced information on all of the genes described in this paper including expression pattern, mutant phenotype (when known), and references; in addition, cell lineage information will be updated for each NB as it becomes available (e.g. the identified neurons produced from a NB, or the DiI clone of neurons produced from each NB).

New information on the aCC/pCC lineage (see below) and new gene expression data in both Drosophila and Schistocerca (grasshopper) have led us to change the names of several NBS in the map. Drosophila NBS were originally named according to the best estimate of their positional homologue in Schistocerca, but positional similarity does not necessarily mean molecular or lineage homology. Although full cell lineage analysis is required to determine the relationship between NBS in the two embryos, the changes made here optimize known homologies between Drosophila and Schistocerca. Specifically, the NB identified previously as NB 2-2 (Doe, 1992) is renamed NB l-1, to reflect the fact that this NB makes the aCC/pCC lineage in both Drosophila and Schistocerca (see below); the NB identified previously as NB 1-1 (Doe, 1992) is renamed NB 1-2, because in both Drosophila and Schistocerca NB 1-2 is the only en+ anterior row NB (Doe, 1992; Condron et al., 1994); and NBS identified previously as NBS 2-3 and 1-2 (Doe, 1992) have been renamed NBS 2-2 and 2-3, respectively, to keep the names of row 2 NBS consistent with the position of NBS in Schistocerca (Doe and Goodman, 1985). We use the revised NB names throughout this paper.

mir-lacZ is a P-element insertion in the mirror gene (H. McNeill and M. Simon, pers. commun.). At stage 9, the anterior row of S1 NBS (1-1, 3-2, 2-5) are mir-lacZ+ (Fig. 2A). At stage 10, the S2 NBS 1-2 and 2-2 express mir-lacZ. During stages 10-11, the NBS 1-1, 3-4, the glial precursor, the median NB, and all row 2 NBS express mir-lacZ. Thus, mir-lacZ expression is observed in a row of NBS per segment, including all row 2 NBS and several NBS that flank row 2. All mir-lacZ+ NBS delaminate from mir-lacZ+ ectoderm. mir-lacZ+ NBS and the overlying ectoderm show reproducible differences in the levels of β-gal protein, with the most medial NBS (1-1, 2-2) and all row 2 NBS showing the highest level of expression. mir-lacZ expression in the CNS is observed throughout embryogenesis; the position of later-forming mir-lacZ+ neurons and glia suggests they derive from mir-lacZ+ NBS (data not shown).

odd is expressed in pair rule stripes prior to CNS expression (E. Ward and D. Coulter, pers. commun.). At the S1 stage of the NB pattern, we observe transient odd expression in NBS 1-1 and 2-5 (Fig. 2B). The first born GMCs of each NB also transiently express odd. odd expression in the NB 1-1 lineage is gone by stage 10 (S3 NB pattern), shortly before the first eve expression in the CNS. Late in stage 10, odd is expressed in the MP2 precursor; MP2 divides to produce a pair of post-mitotic neurons at the end of stage 10, and the protein can be detected in the larger dMP2 progeny until mid-stage 16 and in the smaller vMP2 progeny until late stage 11 (Spana et al., 1995).

Some stages of gsb-d expression in NBS have been described previously (Doe and Technau, 1993; Zhang et al., 1994; Skeath et al., 1995). The gsb-d gene is first ex-
pressed in row 5 NBs (5-2, 5-3, 5-6) and in NB 7-1 at late stage 8 (early S1 NB pattern). As more NBs form, gsb-d is expressed in all NBs in rows 5 and 6, as well as in a single row 7 NB (7-1). gsb-d is expressed strongly in NBs and GMCs, whereas the adjacent gsb-proximal (gsb-p) gene shows most intense expression in neurons.

The pros gene encodes a protein with nuclear localization in the MP2 precursor (Spana and Doe, 1995), as well as most if not all GMCS (Vaessin et al., 1991; Matsuizaki et al., 1992; Spana and Doe, 1995). The en-lacZ gene (Hama et al., 1990) is expressed in exactly the same NBs as the native en gene (Doe, 1992). Similarly, the wg-lacZ gene is expressed in the same NBs as the native wg gene (Doe, 1992). The RNA expression pattern of eaq in the late-forming NBs 6-4, 2-4, 3-3, and 7-3 is included in the current NB map based on data of S. Higashijima and H. Okano (pers. commun.). Based on their results, the names of NBs 3-3 and 3-4 have been switched at the S5 stage.

2.2. Origin of the aCC/pCC neurons

2.2.1. NB 1-1 produces the eve+ GMC 1-la

To determine the origin of the aCC/pCC neurons, we used molecular markers to identify which NBs produce the two earliest born eve+ GMCs. Subsequently, these eve+ GMCs can be followed through development to determine which develops into the aCC/pCC neurons. In this section, we provide evidence that the first eve+ GMCs originate from NBs 1-1 and 7-1, and are named GMC 1-la and GMC 7-la, respectively. In the next section, we present evidence that GMC 1-la produces the aCC/pCC neurons.

eve expression is first observed in two GCMs per hemisegment that are medially positioned just anterior and just posterior to the segmental border. NBs 1-1, 1-2, 2-2, and 7-1 occupy this position and are therefore candidate progenitors of the eve+ GMCs. The expression of en-lacZ, hkb-lacZ, and mir-lacZ can be used to identify each of these NBs uniquely: NB 1-1 is en-lacZ+, hkb-lacZ-, mir-lacZ+; NB 1-2 is en-lacZ+, hkb-lacZ+, mir-lacZ+; NB 2-2 is en-lacZ-, hkb-lacZ+, mir-lacZ+; NB 7-1 is en-lacZ-, hkb-lacZ-, mir-lacZ+. The β-gal product expressed under the control of the en, hkb, and mir promoters is stably inherited by young GCMs following NB division. Therefore, the expression of these three markers can be used to distinguish the GCMs produced by the first divisions of NBs 1-1, 1-2, 2-2, and 7-1. Newly born GCMs which lack β-gal expression are not derived from a β-gal+ NB; this result is definitive because sufficient time for degradation of the β-gal protein has not elapsed. Conversely, β-gal expression in newly born GCMs suggests, although does not prove, their origin from a β-gal+ NB.

To determine the identity of the first two eve+ GCMs, stage 10 embryos expressing en-lacZ, hkb-lacZ, or mir-lacZ were double-labeled for β-gal and eve (Fig. 3). The eve+ GMC that lies in the anterior segmental compartment (the posterior GMC of the pair) is en-lacZ+, hkb-lacZ+, mir-lacZ+, which is identical to the expression observed in NB 1-1. Lack of en-lacZ and hkb-lacZ expression in this GMC clearly shows that it is not derived from en-lacZ+ NBs 1-2 and 7-1 nor hkb-lacZ+ NB 2-2. Therefore, we identify this GMC as GMC 1-la. The eve+ GMC lying in the posterior segmental compartment (the anterior GMC of the pair) is en-lacZ+, hkb-lacZ+, mir-lacZ+, which is identical to the expression observed in NB 7-1. Therefore, we identify this GMC as GMC 7-la.

2.2.2. GMC 1-la generates the aCC/pCC neurons

To follow the fate of GMC 1-la and GMC 7-la, we used expression of gsb to distinguish the identities of closely positioned eve+ neurons. Labeled NB clones generated using FLP recombinase technology show lineage-specific expression of gsb (Buezenow and Holmgren, 1995). GMC 1-la is eve+, gsb+, whereas GMC 7-la is eve+, gsb+ (Fig. 4A). By early stage 11, GMC 1-la and GMC 7-la have each divided (Fig. 4B). eve+ GCMs 1-la1 and 1-la2 are produced by the division of GMC 1-la (nomenclature from Doe, 1992). The division of GMC 7-la produces eve+, gsb+ neurons 7-la1 and 7-la2. A second eve+, gsb+ GMC is observed near neurons 7-la1 and 7-la2, but its parent NB is unknown. At this stage of development eve expression is also seen in GMC 4-2a (Fig. 4B).

By late stage 11, neurons 1-la1 and 1-la2 have migrated anteriorly into the posterior part of the next segment (Figs. 4C,C'). They are juxtaposed to the four eve+, gsb+ neurons, forming a 6-cell cluster. Within the cluster, neurons 1-la1 and 1-la2 are unambiguously identifiable by their lack of gsb expression. GMC 4-2a is positioned apart from other eve+ cells such that its fate is easily followed. GMC 4-2a divides to produce the RP2 neuron and the RP2sib (Patel et al., 1989; Doe, 1992), both of which are eve+, gsb+. Shortly after its birth, the RP2sib begins to shrink in size (Figs. 4C,C').

During germ band retraction at stage 12, the RP2 neuron and the smaller RP2sib are positioned just anterior to the 6-cell cluster containing neurons 1-la1, 1-la2, 7-la1, 7-la2, and two additional eve+, gsb+ neurons. The first cells of the eve+ lateral (EL) cluster of neurons are observed when germ band retraction is nearly complete at late stage 12; gsb expression is absent from the EL cluster. At this stage, it is unclear whether the eve+ EL cells are GCMs or neurons.

At stage 13, the eve+ 6-cell cluster has grown to 7 cells by addition of a third eve+, gsb- neuron (data not shown). Medial eve+ neurons assume different dorsal-ventral positions. The eve+, gsb- neurons 1-la1 and 1-la2 lie at the dorsal surface of the CNS; their expression of eve and characteristic position relative to the axon scaffold allows us to identify these neurons unambiguously as the aCC and pCC. Thus, NB 1-la produces GMC 1-la which generates the aCC/pCC neurons. One pair of eve+, gsb- neu-
rons lie in a medial dorsal-ventral position, and the second pair of eve+, gsb+ neurons lie in a ventral position; the position of these four neurons indicates that they are the CQ neurons (Patel et al., 1989). The RP2sib is eve−; whether it turns off eve or dies is unknown. The EL cluster has grown to 4–6 neurons.

The mature embryonic eve pattern observed at stages 16/17 includes one additional CQ neuron (origin unknown) and an increase of the EL cluster to a total of 8–12 neurons (Figs. 4D,D'). The final pattern of eve+ neurons is highly stereotyped (Fig. 5). The aCC/pCC neurons lie at the dorsal surface near the junction of posterior commissure and longitudinal connective. The fpCC neuron that lies just ventral to aCC/pCC has previously been identified by its position and characteristic axon projection (Jacobs and Goodman, 1989; Goodman and Doe, 1993); we observe an eve+, gsb− neuron at this position which we designate 'candidate fpCC' based on its position. The RP2 is also dorsal and positioned near the junction of the anterior commissure and longitudinal connective. Two CQ neurons lie ventral to candidate fpCC; three additional CQ neurons lie most ventral and have migrated laterally. The EL neurons lie in a ventral and lateral position in the anterior segmental compartment. eve expression is maintained at high levels in all neurons throughout embryogenesis, while gsb expression decreases in CQ neurons after stage 13.

2.3. Dil lineage tracing of NB 1-1

To confirm that NB 1-1 produces the aCC/pCC cell lineage, we labeled individual NBs with the lipophilic tracer Dil (Bossing and Technau, 1994) and determined

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Fig. 3. NB 1-1 produces the eve+ GMC 1-la. The differential expression of en-lacZ, hkb-lacZ, and mir-lacZ identifies the progenitor NBs of the first eve+ GMCs. The posterior GMC of the pair is identified as GMC 1-la (arrow), and the anterior GMC of the pair is identified as GMC 7-la (arrowhead) (see text for details). The segmental boundary lies between the two eve+ GMCs. Confocal images of embryos double-labeled for eve (green) and en-lacZ (A), hkb-lacZ (B) or mir-lacZ (C,D) (red); co-expression appears yellow. (A) GMC 1-la is eve+, en-lacZ+ (arrow), whereas GMC 7-la is eve+, en-lacZ+ (arrowhead). (B) Both GMC 1-la (arrow) and GMC 7-la (arrowhead) are hkb-lacZ+. Asterisk marks the hkb-lacZ+ NB 2-2; double arrowhead marks hkb-lacZ+ NB 4-2. (C) GMC 1-la is eve+, mir-lacZ+ (arrow), whereas GMC 7-la is eve+, mir-lacZ+ (arrowhead). (D) Dorsal focal plane of a stage 16 embryo. aCC/pCC are eve+, mir-lacZ+ (arrows), as observed for GMC 1-la. The eve+ RP2 neuron (asterisk), as well as all other eve+ neurons (data not shown), do not express mir-lacZ. A single hemisegment is shown in (A–C); ventral midline, left. Arrowhead at top marks ventral midline in (D). Anterior, top for all panels.
Fig. 4. GMC 1-1a generates the aCC/pCC neurons. gsb expression distinguishes the identities of closely positioned eve* neurons such that aCC/pCC are uniquely identifiable throughout neurogenesis and their origins can be traced back to GMC 1-1a. Confocal images of wild type embryos double-labeled for eve (green) and gsb-d (A,B) or gsb-p (C,D) (red); co-expression appears yellow. (A) At stage 10, GMC 1-1a is eve*, gsb- (arrow) and lies just posterior to the eve*, gsb+ GMC 7-1 (arrowhead). (B) At early stage 11, both GMCs have divided to produce a pair of neurons. GMC 1-1a divides to produce eve*, gsb- neurons 1-1a1 and 1-1a2 (thick arrows). GMC 7-1a divides to produce eve*, gsb+ neurons 7-1a1 and 7-1a2 (arrowheads). A second eve*, gsb+ GMC is observed in close apposition to neurons 7-1a1 and 7-1a2. The eve* GMC 4-2a forms anterior to the gsb stripe (thin arrow). (C) Two focal planes of a late stage 11 embryo. The second eve*, gsb+ GMC has divided to produce an additional pair of eve*, gsb+ neurons. (C') In the ventral focal plane, one eve*, gsb+ neuron is observed (arrowhead). Neurons 1-1a1 and 1-1a2 have migrated anteriorly (thick arrows), forming a 6-cell cluster with the eve*, gsb+ neurons. Neurons 1-1a1 and 1-1a2 now reside in the gsb+ domain, yet they do not express gsb. GMC 4-2a has divided to produce RP2 and the smaller RP2sib, both of which are eve*, gsb- (thin arrow). (C') In a slightly dorsal focal plane, the remaining three eve*, gsb+ neurons are visible (arrowheads). (D,D') Two focal planes of a stage 16 embryo showing the mature embryonic eve pattern. All eve* cells assume characteristic positions in the dorsal-ventral axis. (D) Dorsal surface of the CNS. RP2 (thin arrow); neurons 1-1a1 and 1-1a2 are identified as aCC/pCC (thick arrows); candidate fpCC is located just ventral to aCC/pCC. RP2, aCC/pCC, and candidate fpCC are eve*, gsb+. The RP2sib has lost eve expression by this stage. (D') One pair of eve*, gsb+ CQ neurons lie medially and in an intermediate dorsal-ventral plane (arrowheads). Three additional eve*, gsb+ CQ neurons are positioned at the ventral surface of the CNS and have migrated laterally from their medial position (arrowheads). The level of gsb protein decreases in these cells after stage 13. The eve* lateral (EL) cluster, which consists of 8-12 eve*, gsb- neurons, is also ventrally positioned. A single hemisegment is shown at each stage. Anterior, top; ventral midline, left.
which one produced the aCC/pCC neurons. Individual neuroectodermal cells were labeled and the resulting NB was identified in living embryos based on the following criteria: time of segregation, position relative to other NBs, position relative to morphological markers, and position of the daughter cells. The majority of labeled NBs scored as NB 1-1 generated the aCC/pCC clone (59%; \( n = 29 \)); this is consistent with the cell lineage derived from the analysis of molecular markers (see above). A minority of labeled NBs scored as NB 1-1 produced a variety of different clones characteristic of several NBs adjacent to NB 1-1 (NBs 1-2, 2-2, and MP2; T. Bossing, C.Q. Doe and G. Technau, unpublished results). The observed variability is likely due to mis-identification of the NB (a NB was scored as NB 1-1 when it was really an adjacent NB), because it is difficult to identify NBs with absolute certainty in living embryos.

Similar results were obtained with other NBs, showing that the labeled NB can be accurately identified in most, but not all, cases. For example, when the labeled NB was scored as a NB located adjacent to NB 1-1 (NB 1-2, 2-2, or MP2), it usually generated a single type of clone representing that particular NB, but in a minority of cases other clones were observed, including aCC/pCC clones (for NBs scored as 1-2 (\( n = 30 \)), 83% ‘NB 1-2 clones’ and 17% other clones; for NBs scored as 2-2 (\( n = 47 \)), 72% ‘NB 2-2 clones’ and 28% other clones; for NBs scored as MP2 (\( n = 46 \)), 78% ‘MP2 clones’ and 22% other clones). The observation of multiple clones for a single identified NB is almost certainly due to mis-identification of the labeled NB, rather than variability of NB lineages. This is certainly true for the MP2, which always divides nearly symmetrically to produce the dMP2 and vMP2 neurons (Skeath et al., 1995; Spana et
Fig. 6. A DiI-labeled NB at the 1-1 position produces the aCC/pCC neurons. An embryo containing a DiI labeled NB at the 1-1 position was allowed to develop to the beginning of stage 12 before fixing, photoconverting the DiI signal (brown), and staining for eve (blue). (A) Ventral focal plane showing the DiI-labeled NB; (B) dorsal focal plane showing the two most dorsal cells of the clone are larger, have migrated slightly anterior from the other cells of the clone, and contain eve immunoreactivity. The most anterior cell has the darkest eve signal. The anterior migration of the eve+ cells and their position at the posterior of the eve+ cluster is characteristic of the aCC/pCC neurons.

In a different experiment, two embryos with a labeled NB scored as l-1 were fixed at early stage 12, the DiI label photoconverted, and the embryos stained for eve protein. In both embryos, eve expression is detected in one or two of the cells that have migrated anterior, away from the rest of the clone (Fig. 6). Based on their migration and eve expression, these are likely to be the aCC/pCC neurons.

3. Discussion

We have described new markers for subsets of NBs in the embryonic Drosophila CNS; these markers have been placed in the map by analyzing their expression relative to each other and previously characterized NB markers (Doe, 1992). The new markers have allowed us to trace the lineage of the first two eve+ GMCs with certainty. NB 1-1 gives rise to the mir-lacZ+, eve+ GMC 1-1a. NB 7-1 makes the en-lacZ+, gsb-d, eve+ GMC 7-1a. The GMCs 1-1a and 7-1a divide to make neurons that become closely associated, but GMC 1-1a progeny (the aCC/pCC neurons) remain identifiable due to their lack of gsb expression. The combination of multiple molecular markers allows us to be certain that the progeny of GMC 1-1a differentiates into the well characterized aCC/pCC neurons, while the progeny of GMC 7-1a differentiates into two of the CQ neurons. In addition, DiI lineage tracing has been used to confirm that a NB at the 1-1 position does produce eve+ neurons. This information should be valuable for interpreting the phenotype of mutations affecting the aCC/pCC and other eve+ neurons. In addition, the lineages described here are likely to lead to specific experiments, such as examining the function of genes expressed early in the NB 1-1 and NB 7-1 lineages for a role in the development of the aCC/pCC or CQ neurons.

The new molecular markers used in this study have revealed that the NB generating the aCC/pCC lineage differs from the NB previously suggested as the origin of this lineage (Doe, 1992). Accordingly, we have changed the name of this NB, to maintain the name of the aCC/pCC precursor as NB 1-1. We chose to change the NB name to '1-1' to reflect the significant homologies between the NB 1-1 in Drosophila and Schistocerca: (1) DiI lineage studies in Drosophila and direct observation lineage analysis in Schistocerca suggest that NB 1-1 generates the aCC/pCC lineage (du Lac et al., 1986; this paper); (2) both NBs generate an eve+ GMC 1-1a and progeny aCC/pCC neurons (N.H. Patel, pers. commun.; this paper); (3) both NBs are the most medial, anterior NBs in every segment (Doe and Goodman, 1985; this paper); (4)
both NBs are among the earliest forming NBs (Doe and Goodman, 1985; this paper); and (5) both NBs are en-, but are located adjacent and medial to the only en+ NB in the anterior portion of the segment (Condon et al., 1994; this paper). Thus, cell lineage, NB position, gene expression, and time of NB formation are all in close agreement for NB 1-1 in both Drosophila and Schistocerca. In addition, we changed the name of NB 1-2 so that in both Drosophila and Schistocerca the single en+ NB in the anterior of each segment is called NB 1-2; the names of NBs 2-2 and 2-3 were also changed to match their positional homologues in Schistocerca. In the future, gene expression and cell lineage studies in both insects will be required to determine the complete relationship between positionally homologous NBs.

4. Experimental procedures

Standard methods were used to rear flies and collect embryos (Roberts, 1986). Developmental stages and morphological landmarks are described in Campos-Ortega and Hartenstein (1985) and Doe (1992).

4.1. Cell-specific CNS markers and sources

The following NB markers, sources, and staining methods are described in Doe (1992): engrailed; wingless; fushi tarazu-lacZ; achaete; seven-up-lacZ (H162 enhancer trap line); ming-lacZ (1530 enhancer trap line); unplugged-lacZ (1912 enhancer trap line); Chiang, Young, and Beachy (pers. commun.); huckebein-lacZ (5953 enhancer trap line).

Additional NB markers described in this paper are: mirror-lacZ, an enhancer trap insertion in the mirror gene (H. McNeill and M. Simon, unpublished results), detected using an antibody against β-gal; engrailed-lacZ, an enhancer trap insertion into the engrailed gene called ryXho25 (Hama et al., 1990), detected using an antibody against β-gal; prospero protein, detected with mouse monoclonal MR1a or MR2a used at 1:1 (Spana and Doe, 1995); odd-skipped, detected with a rabbit antiserum used at 1:5000 (E. Ward and D. Coulter, St. Louis University); eagle, an RNA expression pattern determined by S. Higashijima and H. Okano for communicating results on eagle prior to publication; T. Tabata for the en-lacZ stock; and S. Benson for establishing the "hyper-NB map." This work was supported by an NIH Systems and Integrative Biology Predoctoral training grant (J.B.), a Damon Runyon Postdoctoral fellowship (J.B.S.), an NIH Cell and Molecular Biology Predoctoral training grant (E.P.S.), the Deutsche Forschungsgemeinschaft (G.T.), and the Howard Hughes Medical Institute (C.Q.D.). C.Q.D. is an Assistant Investigator with the Howard Hughes Medical Institute.

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