Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate

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The generation of cellular diversity is essential in embryogenesis, especially in the central nervous system. During neurogenesis, cell interactions or asymmetric protein localization during mitosis can generate daughter cells with different fates1–4. Here we describe the asymmetric localization of a messenger RNA and an mRNA-binding protein that creates molecular and developmental differences between Drosophila neural precursors (neuroblasts) and their daughter cells, ganglion mother cells (GMCs). The prospero (pros) mRNA and the RNA-binding protein Staufen (Stau) are asymmetrically localized in mitotic neuroblasts and are specifically partitioned into the GMC, as is Pros protein5–7. Stau is required for localization of pros RNA but not of Pros protein. Loss of localization of Stau or of pros RNA alters GMC development, but only in embryos with reduced levels of Pros protein, suggesting that pros RNA and Pros protein act redundantly to specify GMC fate. We also find that GMCs do not transcribe the pros gene, showing that inheritance of pros RNA and/or Pros protein from the neuroblast is essential for GMC specification.

The Drosophila central nervous system (CNS) develops from stem-cell-like precursors called neuroblasts. Smaller daughter cells (GMCs) ‘bud off’ from the basal side of the neuroblasts; most GMCs then produce two postmitotic neurons. An important regulator of GMC development is the homeodomain protein Pros. Pros localizes to the apical cytoplasm of neuroblasts at late interphase6; at mitosis, it is translocated to the basal cortex where it forms a membrane-associated crescent, and it is subsequently inherited by the GMC in which Pros translocates into the nucleus5,7. Asymmetric cortical localization of Pros is believed to keep Pros protein out of the neuroblast nucleus, and to rapidly generate nuclear Pros protein in the newborn GMC, where Pros establishes GMC-specific gene expression6,8,9.

Here we describe the cell-cycle-specific asymmetric localization of pros mRNA in neuroblasts, which results in its selective partitioning into the daughter GMC. During interphase, most pros RNA is localized to the apical side of the neuroblast, where it is found either in the cytoplasm or associated with the cortex (Fig. 1a, Table 1). During mitosis, pros RNA is asymmetrically localized to the opposite side of the neuroblast in a basal cortical crescent (Fig. 1a, Table 1). At cytokinesis, pros RNA is specifically segregated into the GMC. Localization of pros RNA does not reflect a general
movement of poly(A\(^+\)) RNA, as a different transcript (seven-up) is not localized at any phase of the neuroblast cell cycle (results not shown). Asymmetric cortical crescents of pros RNA can also be observed in mitotic sensory organ precursors and adult midgut precursors (results not shown).

The asymmetric localization of pros RNA suggests that one or more RNA-binding proteins may also be differentially segregated during neuroblast division. A good candidate for such a protein is the RNA-binding protein Stau, which is asymmetrically localized in the oocyte, is required for localization of bicoid and oskar RNA, and is detected in the embryonic CNS\(^{10,11}\). We find that Stau shows cell-cycle-specific asymmetric localization in neuroblasts in a pattern that precisely matches the pattern of localization of pros RNA and Pros protein (Fig. 2). In late interphase, Stau is localized to the apical cortex (Fig. 2a). From metaphase through to telophase, Stau is asymmetrically localized as a basal crescent, and segregates into the daughter GMC (Fig. 2b–e). A recent report\(^{12}\) did not detect basal localization of Stau in neuroblasts, but we observed basal localization of Stau both in vivo and in vitro\(^{13}\), with two different antibodies, and this is consistent with a role for Stau in the basal localization of pros RNA (see below). Although Stau and Pros proteins are precisely co-localized in mitotic neuroblasts (Fig. 2f)\(^{13}\), Stau protein localization is normal in pros null embryos (Fig. 2g), and Pros protein localization is normal in stau null embryos (Fig. 2h). Thus, Stau and Pros proteins are independently localized to the same region, probably by binding to a common anchoring protein.

To determine whether the localization of Stau and pros RNA in neuroblasts is microtubule-dependent, as is the localization of Stau and bicoid RNA in precellular embryos\(^{14}\), we depolymerized microtubules with colcemid, arresting neuroblasts in metaphase. Basal crescents of pros RNA (Fig. 1b) and Stau protein\(^{12}\) were easily observed, and the percentage of pros RNA crescents increased from 7% to 63% (\(n = 80\)) during colcemid treatment, indicating that new pros RNA crescents can form in the absence of microtubules. In contrast, actin microfilaments are absolutely required for anchoring Stau to the basal cortex\(^{13}\). Localization of Stau and RNA in neuroblasts could occur by diffusion and microfilament-based anchoring.

To determine whether Stau is required for localization of pros RNA in neuroblasts, we scored embryos (referred to as stau embryos) produced by homozygous stau\(^D3\) flies. These embryos lack both maternal and zygotic Stau. In mitotic neuroblasts of stau embryos, basal pros RNA localization is reduced from 71% to 27% (Table 1); apical RNA localization is reduced from 56% to 7% in

### Table 1 Loss of stau reduces asymmetric localization of pros RNA

<table>
<thead>
<tr>
<th>Neuroblast cell-cycle phase</th>
<th>Subcellular distribution of pros RNA</th>
<th>Uniform cortical/cytoplasmic location</th>
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<tbody>
<tr>
<td></td>
<td>Apical crescent</td>
<td>Basal crescent</td>
</tr>
<tr>
<td>Wild type ((n = 99))</td>
<td>0 (0%)</td>
<td>70 (71%)</td>
</tr>
<tr>
<td>Stau(^*) ((n = 91))</td>
<td>4 (4%)</td>
<td>25 (27%)</td>
</tr>
<tr>
<td>Wild type ((n = 314))</td>
<td>Interphase</td>
<td>176 (56%)</td>
</tr>
<tr>
<td>Stau(^*) ((n = 241))</td>
<td>18 (7%)</td>
<td>6 (2%)</td>
</tr>
</tbody>
</table>

\(^*\) Wild-type embryos are CyO/CyO or stau\(^D3\)/CyO embryos; stau\(^*\) embryos are stau\(^D3\)/stau\(^D3\) embryos from stau\(^D3\)/stau\(^D3\) parents.

Figure 1 Asymmetric localization of pros RNA in neuroblasts requires Stau but not microtubules. Neuroblasts in stage 9–11 embryos were stained for pros RNA (red) and DNA (green). The bottom panels show results of double-labelling. Apical side is down; basal side is up. a, Wild-type embryo. Interphase neuroblasts show apical pros RNA (arrowhead); mitotic neuroblasts have basal cortical/pros RNA (arrow). b, Colcemid-treated embryo. Neuroblasts lack microtubules yet show robust basal crescents of pros RNA (arrow). c–f, Stau mutant embryos. c, d, Interphase neuroblasts with apical (c) or delocalized (d) pros RNA (arrowheads). e, f, Mitotic neuroblasts with basal (e) or delocalized (f) pros RNA (arrows).
interphase neuroblasts (Table 1). Most stau− neuroblasts show uniform cytoplasmic or cortical pros RNA localization (Fig. 1). Similar results are seen in embryos lacking only zygotic stau expression (results not shown). It seems likely that apical Stau protein is required for apical pros RNA localization at interphase, and that basal Stau protein is required for basal pros RNA localization at metaphase. Crescents of pros RNA still form at low frequency in stau embryos (Fig. 1), however, suggesting that Stau is not the only factor that is able to localize pros RNA at the cortex. We conclude that the RNA-binding protein Stau is required for reliable apical and basal localization of pros RNA in neuroblasts.

What is the function of the asymmetric segregation of pros RNA into GMCs? Hybridization with a pros intron probe shows that pros is transcribed in 67% of neuroblasts (n = 144; Fig. 3a), but is transcribed in only 2% of GMCs (n = 851; Fig. 3b). Thus, detection of pros RNA in GMCs9,10 (Fig. 3 insets) must reflect inheritance of pros RNA from the neuroblast. As GMCs clearly require pros function9, but do not transcribe the pros gene, inheritance of pros RNA and/or Pros protein from the neuroblast is essential for GMC specification.

Loss of pros RNA localization—but not of Pros protein localization—in stau embryos does not alter GMC fate as judged by expression of the markers even-skipped (eve) or fushi tarazu (results not shown). To determine whether localized pros RNA and localized

![Figure 2](https://example.com/figure2.png)

**Figure 2** Asymmetric localization of Stau and Pros proteins in neuroblasts. a–e, Cell-cycle-specific localization of Stau protein (red) and stau DNA (green) in wild-type neuroblasts (bottom panels show results of double-labelling). Apical side is down; basal side is up. a, Interphase neuroblast with apically located Stau (large arrowhead); localization of Stau in the GMC cytoplasm is indicated by the small arrowhead. b, metaphase; c, anaphase and d, telophase neuroblasts with Stau localized to the basal cortex. e, Stau is segregated into the GMC where it is cytoplasmic (arrowhead). f, Stau (red; top) and Pros (blue; bottom, merged with Stau image) proteins are co-localized at mitosis. g, Stau is localized normally in pros− mitotic neuroblasts. h, Pros is localized normally in stau− mitotic neuroblasts.

![Table 2](https://example.com/table2.png)

**Table 2** Loss of Stau enhances a hypomorphic pros GMC phenotype

<table>
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<tr>
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<th>pros&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>pros&lt;sup&gt;+/+&lt;/sup&gt;</th>
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<tr>
<td>Stau&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>22.9 ± 4.7 (16)</td>
<td>28.2 ± 7.2 (16)</td>
<td>15.2 ± 6.5 (11)</td>
</tr>
<tr>
<td>Stau&lt;sup&gt;+/&lt;/sup&gt;</td>
<td>10.1 ± 3.7 (7)</td>
<td>8.5 ± 4.6 (8)</td>
<td>6.3 ± 3.9 (6)</td>
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Stau<sup>+/</sup> embryos were scored; the Eve<sup>+</sup> phenotypes originate in GMCs (embryonic stage 10–11), but we scored neurons (stage 15–16) because the pattern is more reproducible. Medial Eve<sup>+</sup> cells are defined as all Eve<sup>+</sup> cells except for those cells of the lateral EL cluster. Wild-type and homozygous stau<sup>+/+</sup> or stau<sup>+/</sup> embryos have ~112 medial Eve<sup>+</sup> cells; pros-null embryos have ~2 medial Eve<sup>+</sup> cells, n.a., not assayed.

![Figure 3](https://example.com/figure3.png)

**Figure 3** pros is transcribed in neuroblasts but not GMCs. A stage-10 embryo was labelled with a pros intron probe (a, b) or a pros complementary DNA probe (insets). a, Ventral view of the neuroblast layer. Most neuroblasts (arrow) show two chromosomal sites of pros transcription; not all sites of transcription are in focus. b, Ventral view of the adjacent GMC layer in the same embryo. GMCs (arrow) rarely show pros transcription; in this focal plane, none of the GMCs are transcribing pros. Insets: a pros cDNA probe shows high levels of pros RNA (arrowheads) in both neuroblasts and GMCs.

![Figure 4](https://example.com/figure4.png)

**Figure 4** Localization and function of Stau and pros RNA in neuroblasts. a, Protein and RNA localization in wild-type and stau mutant neuroblasts. P, Pros protein; S, Stau protein; t pros RNA; r, lower level of pros RNA; X, putative Pros- and Stau-binding protein; NB, neuroblast. Apical, down; basal, up. b, Basal localization pathway: Stau/pros RNA and Pros protein are independently localized, perhaps by a common protein (X), and may act redundantly to provide levels of Pros that are necessary to establish GMC-specific gene expression.
HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C


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The protein HLA-E is a non-classical major histocompatibility complex (MHC) molecule of limited sequence variability. Its expression on the cell surface is regulated by the binding of peptides derived from the signal sequence of some other MHC class I molecules1–11. Here we report the identification of ligands for HLA-E. We constructed tetramers12 in which recombinant HLA-E and β2-microglobulin were refolded with an MHC leader-sequence peptide, biotinylated, and conjugated to phycoerythrin-labelled extravidin. This HLA-E tetramer bound to natural killer (NK) cells and a small subset of T cells from peripheral blood. On transfectants, the tetramer bound to the CD94/NKG2A, CD94/NKG2B and CD94/NKG2C NK cell receptors, but did not bind to the immunoglobulin family of NK cell receptors (KIR). Surface expression of HLA-E was enough to protect target cells from lysis by CD94/NKG2A NK-cell clones. A subset of HLA class I alleles has been shown to inhibit killing by CD94/NKG2A NK-cell clones13. Only the HLA alleles that possess a leader peptide capable of upregulating HLA-E surface expression confer resistance to NK-cell-mediated lysis, implying that their action is mediated by HLA-E, the predominant ligand for the NK cell inhibitory receptor CD94/NKG2A.

The non-classical MHC class Ib molecule HLA-E has a broad tissue distribution14. Like its homologue, the mouse MHC class Ib Qa-1,15 HLA-E preferentially binds to a peptide derived from amino-acid residues 3–11 of the signal sequences of most HLA-A, -B, -C, and -G molecules, but cannot bind its own leader peptide16,17. Cell-surface expression of HLA-E depends on a functional transporter associated with antigen processing (TAP), and is upregulated by the binding of the peptides derived from MHC molecule signal sequences18. The close correlation between the surface expression of HLA-E and of certain other MHC class I molecules suggested a possible role for HLA-E in NK-cell-mediated recognition of target cells.

To test this hypothesis, we constructed tetramer complexes...