The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*

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**SUMMARY**

Both intrinsic and extrinsic factors are known to regulate sibling cell fate. Here we describe a novel mechanism for the asymmetric localization of a transcription factor to one daughter cell at mitosis. The *Drosophila* CNS develops from asymmetrically dividing neuroblasts, which give rise to a large neuroblast and a smaller ganglion mother cell (GMC). The *prospero* gene encodes a transcription factor necessary for proper GMC gene expression. We show that the prospero protein is synthesized in the neuroblast where it is localized to the F-actin cell cortex. At mitosis, prospero is asymmetrically localized to the budding GMC and excluded from the neuroblast. After cytokinesis, prospero is translocated from the GMC cortex into the nucleus. Asymmetric cortical localization of prospero in neuroblasts requires entry into mitosis; it does not depend on *numb* function. prospero is also observed in cortical crescents in dividing precursors of the peripheral nervous system and adult midgut. The asymmetric cortical localization of prospero at mitosis is a mechanism for rapidly establishing distinct sibling cell fates in the CNS and possibly other tissues.

Key words: prospero, *Drosophila*, CNS, numb, string, neuroblast, GMC

**INTRODUCTION**

How one precursor cell can divide and give rise to two cells with different identities is a central question in developmental biology. Two mechanisms can be imagined for generating asymmetric cell fates. At one extreme, extrinsic cues could specify the fate of two initially ‘naïve’ daughter cells; at the other extreme, an intrinsic or inherited factor could be asymmetrically localized to one daughter cell, leading to different cell fates. Recently, a great deal has been learned about how extrinsic cues specify cell fate in both invertebrate and vertebrate systems (see Zipursky and Rubin, 1993; Artavanis-Tsakonas et al., 1995; Johnson and Tabin, 1995); however, few intrinsic factors have been characterized for both expression and function (see Rhyu et al., 1994; Guo and Kemphues, 1995).

Among the best characterized localized determinants are the RNAs and proteins that generate the anterior and posterior fates of the *Drosophila* embryo (Driever, 1993; St Johnson, 1993). For example, staufen and oskar proteins are asymmetrically localized to the posterior end of the developing *Drosophila* oocyte where they are required for establishment of the pole plasm. However, these determinants ultimately control the fate of nuclei within the syncytial early embryo, rather than being partitioned into one daughter cell at mitosis. Similarly, par-1 protein is localized to the posterior half of the *C. elegans* embryo where it becomes incorporated into the P1 cell after division (Guo and Kemphues, 1995). The par-1 gene is required for the asymmetry generated in the first embryonic division (Kemphues et al., 1988). In *Drosophila*, the numb protein is asymmetrically localized to one daughter cell during mitosis of external sensory organ (ES) precursors (Rhyu et al., 1994) and the CNS MP2 precursor (Spana et al., 1995). In both lineages, numb is necessary and sufficient to specify the cell fate of the cell in which it is observed (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995; this manuscript).

The *Drosophila* CNS develops from stem cell-like precursor cells, called neuroblasts (see Goodman and Doe, 1993). Neuroblasts repeatedly divide asymmetrically to regenerate a large neuroblast and ‘bud off’ a smaller ganglion mother cell (GMC). The division is oriented such that the GMC usually buds off from the basal (inner) surface of the neuroblast. Each GMC divides once to produce a pair of neurons or glia. Neuroblast and GMC daughter cells are different in size, gene expression profile, and cell lineage. Asymmetric neuroblast divisions can occur normally in vitro (Luer and Technau, 1992; Huff et al., 1989), suggesting that intrinsic factors control many aspects of the neuroblast/GMC cell fates. We are interested in identifying factors that regulate sibling cell fates following the asymmetric neuroblast cell division.

numb is asymmetrically localized to the GMC during neuroblast cell division, but there are no detectable alterations of GMC fate in *numb* mutants (Uemura et al., 1989; Rhyu et al., 1994; Spana et al., 1995). In contrast, embryos mutant for the prospero (pros) gene have dramatic alterations in GMC gene expression. For example, activation of *fushi tarazu* (ftz) and *even-skipped* (eve) expression in a subset of GMCs requires pros function (Doe et al., 1991); in addition, repression of
deadpan and asense in GMCs also requires pros function (Vaessin et al., 1991). The pros gene is transcribed in all neuroblasts and GMCs (Doe et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992); however, pros protein has only been observed in GMC nuclei, not in neuroblasts (Vaessin et al., 1991; Matsuzaki et al., 1992). These data suggest pros plays a key role in regulating GMC development.

To characterize further the role of pros in establishing GMC cell fate, we generated monoclonal antibodies against the pros protein. Surprisingly, we find pros protein in neuroblasts, but it is localized to the cortex and excluded from the nucleus. Detailed analysis of pros expression during the neuroblast cell cycle reveals that pros is asymmetrically localized to the budding GMC at mitosis, and subsequently translocated into the GMC nucleus. We find that asymmetric pros localization follows centrosome migration to the basal pole of the neuroblast during mitosis; that this localization is blocked in G2-arrested neuroblasts; and that pros and numb localization into the GMC are independent of each other. Asymmetric localization of pros protein to the cortex is also detected in precursors of the peripheral nervous system and adult midgut.

MATERIALS AND METHODS

*Drosophila* strains

For wild-type expression of pros, the line *y w* was used. Mutant lines examined were *w*; *pros*¹⁣⁴/TM3-flacZ and *pros*¹⁷/TM3 (Doe et al., 1991); *numb*¹⁴ pr cn Bc/CyO-flacZ (Uemura et al., 1989); and *stg*⁷⁸ e/TM3-flacZ (Jurgens et al., 1984; Cui and Doe, 1995).

Antibody production

Degenerate PCR primers were used to amplify the region between nucleotides 3888 and 4262 of the pros cDNA. This fragment was then cloned into the pGEX-2T vector in the BamHI and SmaI sites (Smith and Johnson, 1988). The fusion protein produced contained GST fused in frame to amino acids 1196 to 1320 of the pros protein. Nucleotide and amino acid numbering according to Matsuzaki et al. (1992). The fusion protein was purified to homogeneity and injected into mice until the diluted raw sera showed immunohistochemical staining on *Drosophila* embryos. Following the fusion, approximately 850 hybridoma lines were screened on embryos, resulting in four confirmed positives. Two monoclonal lines, MR1A and MR2A, showed the strongest signals and were the antibodies used for subsequent experiments. Both give identical staining patterns at similar intensities.

Antibody staining and confocal microscopy

Stainings were performed essentially as described in Doe (1992). Mouse anti-pros mAbs MR1A and MR2A were used at 1:4 dilution. Rabbit anti-numb (Rhyu et al., 1994) was used at 1:1000 dilution after preabsorbing against wild-type embryos. Mouse anti-β-galactosidase (Promega) was used at 1:1000. Rabbit anti-γ-tubulin (Y. Zheng, B. Oakley and B. Alberts, unpublished) was used at a concentration of 1:1000. Secondary antibodies coupled to DTAF, LRSC or Cy5 (Jackson Immunoresearch) were used at 1:400. Histochemical detection was done with an Elite Kit (Vector Labs). DNA was visualized using sonicated para-phenylenediamine (Lundell and Hirsh, 1994). The pros/phalloidin double label was on embryos fixed in heptane-formaldehyde, but devitellinized by hand in PBS, since methanol treatment destroys phalloidin staining. BODIPY-phalloidin (Molecular Probes, Inc.) was used at a 1:50 dilution of the recommended stock during the secondary antibody incubation. Embryos were mounted in 70% glycerol with 4% N-propyl gallate or sonicated para-phenylenediamine and viewed on a Biorad MRC1000 confocal microscope.

Embryo staging was done according to Campos-Ortega and Hartenstein (1985); neuroblast staging was done according to Doe (1992).

RESULTS

prospero expression in the CNS

Histochemical detection of pros protein (Fig. 1A) reveals strong nuclear expression in the GMCs, as seen previously (Vaessin et al., 1991; Matsuzaki et al., 1992). Immunofluor-
prospero asymmetric cortical localization is cell cycle dependent. Wild-type embryos were stained for pros protein (green; A-E) and DNA (blue; F, G, I and J) or pros protein and F-actin (red; H). The same neuroblast is shown for each stage (e.g. A and F, B and G, etc.). All panels are lateral views with anterior to the left and dorsal up. Apical is down; basal is up. During late interphase (A,F) pros is found asymmetrically localized to the apical side of the neuroblast. The DNA is not fully condensed yet. During metaphase (B,G), pros is asymmetrically localized to the basal side of the neuroblast. The DNA is found condensed at the metaphase plate. During anaphase (C,H), pros is found co-localized with F-actin only at the cortex of the budding GMC. After cytokinesis, pros is found only at the GMC cortex (D,I). We believe this stage represents an early interphase, because cytokinesis has completed, but the DNA has not fully decondensed. After the DNA decondenses, pros is translocated into the GMC nucleus (E,J). The length of the neuroblast cell cycle is approximately 40 minutes (Hartenstein et al., 1987).

prospero is localized to the apical cortex and nucleus in stg neuroblasts. Wild-type (A-C) and stg7B embryos were triple labeled for pros (green), γ-tubulin (red), and DNA (blue). Each panel is a lateral view with anterior to the left and basal up. Each panel is of the same magnification. (A) At interphase, pros is asymmetrically localized to the apical side of the neuroblast with one γ-tubulin positive centrosome (large arrowhead). (B) At late G2, a small centrosome (small arrowhead) migrates from the apical to the basal side of the neuroblast, while the large centrosome (large arrowhead) remains at the apical side. pros is still localized to the apical side. (C) At metaphase, the smaller centrosome (small arrowhead) is found at the basal side and the larger centrosome (large arrowhead) on the apical side of the neuroblast. pros is asymmetrically localized to the apical side. (D) In young stg embryos (about stage 9-10), neuroblasts contain multiple γ-tubulin-positive spots which resemble centrosomes of reduced size (large arrowhead). This neuroblast has two larger spots and one small spot on the apical side and one small spot on the basal side. pros expression in this cell is found lightly in the nucleus, and enriched on the apical side of the cortex. (E) In older stg embryos (about stage 11), neuroblasts show more intense pros staining at the apical cortex and in the nucleus. The γ-tubulin spots are more numerous and even smaller. The DNA signal in D and E has been decreased to allow better visualization of pros nuclear staining.
prospero is cortically localized in neuroblasts and asymmetrically localized to GMCs

Wild-type embryos were stained for pros protein and mounted in a DNA stain to determine the stage of the neuroblast cell cycle (Figs 1C, 2). Neuroblasts at embryonic stages 9-10 were examined for pros expression during the neuroblast cell cycle.

Pros protein first starts to accumulate at the apical cortex in neuroblasts during interphase (Fig. 2A,F). It is possible that this localization starts to occur during either the S or G2 stages of the cell cycle and remains on the apical side of the neuroblast until mitosis. During mitosis pros protein is localized to the basal cortex of the neuroblast (Fig. 2B,G). We have not been able to identify an intermediate between the apical and basal localization between late G2 and metaphase, perhaps because it is of very short duration. During anaphase, pros is asymmetrically localized to the cortex of the budding GMC (Fig. 2C,H). We have termed the localization of the pros protein found in the neuroblast “cortical” because it co-localizes with the F-actin cortex, as revealed by double labeling for pros and phalloidin, which labels F-actin (Fig. 2H).

Immediately after cytokinesis, pros is cortically localized around the entire GMC, and is excluded from the neuroblast (Fig. 2D,I). Subsequently, pros is translocated from the GMC cortex into the GMC nucleus (Fig. 2E,J). During the division of an identified neuroblast in adjacent segments, we have observed anaphase cortical localization in one segment and nuclear GMC localization in the other segment (data not shown). Because adjacent segments develop nearly synchronously, the translocation of pros into the nucleus must occur quite rapidly after cytokinesis. It is likely that the same pros protein is moving from the cortex into the nucleus, because the process occurs too rapidly for de novo transcription and translation of new pros in the GMC. We find that pros is cortically localized in the neuroblast and asymmetrically distributed to the budding GMC in every neuroblast at each mitosis examined.

Basal localization of pros occurs during mitosis of the neuroblast. Another cellular component that migrates to the basal pole of the neuroblast at mitosis is the centrosome that establishes the basal half of the mitotic spindle (Fig. 3A-C). We stained wild-type embryos for pros, DNA and γ-tubulin (a centrosomal marker) to examine the relationship between centrosome migration and pros localization (Fig. 3). During interphase, pros and one large centrosome are localized to the apical side of the neuroblast (Fig. 3A). At late G2, a smaller centrosome can be detected migrating to the basal side of the neuroblast; at this stage pros is still localized to the apical side (Fig. 3B). During metaphase, both pros and the small centrosome are localized to the basal side of the neuroblast (Fig. 3C).

Pros is a nuclear protein in MP2 and GMCs

Pros expression in every neuroblast is identical, except for the MP2 precursor. MP2 forms as part of the S1 neuroblast array, and is morphologically identical to other neuroblasts (Doe, 1992); however, MP2 divides just once to produce a pair of post-mitotic neurons: dMP2 and vMP2 (Doe et al., 1988; Spana et al., 1995). Pros is nuclear in MP2 from the time of its formation (Fig. 1C). Interestingly, MP2 and all GMCs have nuclear pros protein and divide just once to produce a pair of neurons. In both MP2 and all GMCs, pros does not become cortically localized at mitosis, but instead fills the cell, similar to a traditional transcription factor (data not shown). Thus, there is a dramatic difference in the subcellular localization of pros in asymmetrically dividing neuroblasts (cortical) and the more equally dividing MP2 and GMCs (cytoplasmic). After MP2 or GMC cytokinesis, pros protein is transiently observed in the nuclei of the sibling neurons (data not shown). In addition to nuclear expression in MP2 and GMCs, and transiently in new-born neurons, pros is also nuclear in the longitudinal glia and a lateral cluster of cells in the nerve cord throughout embryogenesis (Campbell et al., 1994).

Mechanism of pros basal localization in neuroblasts

The asymmetric localization of pros during the neuroblast cell cycle implies that the neuroblast has a mechanism to localize pros differently at each stage of the cell cycle. We examined whether pros localization is dependent on entry into mitosis and whether pros localization at mitosis is dependent on numb, which is asymmetrically localized at mitosis.

Pros basal localization is blocked in G2-arrested neuroblasts

The cell cycle-specific localization of pros protein in the neuroblast and GMC led us to examine whether pros localization is dependent on the cell cycle. We examined embryos mutant for stg, the Drosophila homologue of the S. pombe cdc25 gene (Edgar and O’Farrell, 1989). stg encodes a phosphatase required to activate the p34cdc2 kinase, which regulates entry into mitosis. In stg mutant embryos, the cell cycle is arrested in G2 of interphase 14 (Edgar and O’Farrell, 1989). Despite this cell cycle arrest, G2-arrested neuroblasts develop in their correct positions, but do not divide to produce GMCs (Cui and Doe, 1995).

In newly formed neuroblasts in both wild-type and stg embryos, pros is localized to the apical cortex of the neuroblast; however, in stg embryos pros is also observed in the nucleus (Fig. 3D,E). The cortical and nuclear localization in the neuroblasts becomes more pronounced as the embryo ages (Fig. 3E). We never observe basal pros localization in the G2-arrested neuroblasts. In addition to being blocked in G2, neuroblasts also have centrosome defects. Multiple spots of γ-tubulin staining are present in each neuroblast; these ‘centrosomes’ are typically much smaller than normal. Most frequently the largest centrosomes are localized apically, where pros accumulates, but smaller centrosomes can be observed both apically and basally (Fig. 3D,E). Thus, the...
cortical localization of pros at mitosis

Pros and numb are independently localized to the basal cortex of neuroblasts

In addition to the pros protein, the numb protein is also asymmetrically localized to the basal cortex of the dividing neuroblast (Rhyu et al., 1994). We observe uniform numb distribution on the cortex of the interphase neuroblast, followed by asymmetric localization to the basal cortex during neuroblast mitosis (Fig. 4B). numb is then localized uniformly on the GMC cortex (data not shown). We find that pros and numb are approximately co-localized at the basal cortex of the neuroblast at mitosis (Fig. 4A–C). In both neuroblasts and sensory precursors, we observe that numb has a more restricted basal localization than that of pros (Figs 4A–C, 5A–C). This might reflect different mechanisms of localization, different efficiency of utilizing the same mechanism, or different sensitivity of each antibody.

To determine whether numb is required for pros localization in the neuroblast, we stained numb mutants for pros. We find no change in the localization of pros protein in numb embryos: asymmetric localization to the budding GMC is observed, as well as nuclear protein in the GMC (Fig. 4D). pros embryos were then stained for numb protein; no change in the localization of numb during neuroblast mitosis was observed (Fig. 4E).

Thus, pros and numb are independently localized to the basal neuroblast cortex.

Prospero is asymmetrically localized in precursors of the PNS and adult midgut

In the external sensory (ES) bristle organ lineage, the sense organ precursor (SOP) produces two cells, called A and B. The A cell produces the bristle and socket cells, while the B cell produces a neuron and a glial cell. pros RNA is observed in the SOP and the B daughter cell; however, pros protein has been detected only in the nucleus of the B cell (Vaessin et al., 1991). This pattern is similar to pros expression in the neuroblast/GMC division. We looked for pros cortical localization in the SOP using the distinctive expression of the numb protein to identify mitotic SOPs. numb is found in a cortical crescent in the mitotic SOP (Rhyu et al., 1994). Just as in the neuroblast, pros is found to colocalize with numb in a cortical crescent (Fig. 5A–C). Presumably this asymmetric cortical expression in the mitotic SOP precedes the localization of pros to the B cell, where it is found in the nucleus. This indicates that numb is asymmetrically localized with pros to the B cell where it would function to specify the B cell fate.

The embryonic expression of pros outside of the CNS and PNS is limited to the Garland gland and a subset of cells in the gut (Doe et al., 1991). We examined the expression of pros in the posterior midgut primordia by double labeling for pros and DNA. We find that the large interstitial cell precursors (ICPs) of the posterior midgut primordia asymmetrically localize pros to the cortex during mitosis, similar to neuroblasts and SOPs (Fig. 5D–F). These ICPs bud off smaller progeny that have pros nuclear localization and are probably the adult midgut precursors (AMPs; Tepass and Hartenstein, 1995).

Discussion

Prospero protein is localized to the nucleus in most cells in which it is expressed: GMCs, immature neurons, longitudinal glia, sense organ glia, adult midgut precursor cells, cone cells and R7 neuron in the eye disc, and a number of unidentified imaginal cells (Vaessin et al., 1991; Oliver et al., 1993; Campbell et al., 1994; Bronner et al., 1994; data not shown). It has a domain that is distantly related to the homeodomain (Chu-LaGraff et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992), and it is capable of sequence-specific DNA binding (H. Vaessin, personal communication). Yet in at least three tissues – the CNS, PNS and midgut – pros protein is localized to the cell cortex of asymmetrically dividing precursor cells, where it is selectively localized into one daughter cell and then translocated into the nucleus. The asymmetric cortical localization of pros is a novel mechanism for selectively segregating a transcription factor to just one daughter cell at mitosis.

We have documented pros protein localization throughout the neuroblast and GMC cell cycle (diagrammed in Fig. 6A). pros is first detected in the neuroblast in a crescent on the apical side of the cortex in close proximity to the position of the undivided centrosome. During metaphase, pros is localized to a crescent on the basal side of the neuroblast; at anaphase, it is localized into the budding GMC. After cytokinesis, pros is translocated from the GMC cortex to the nucleus. Thus, pros protein has three different subcellular localizations, correlated with the neuroblast cell cycle: at late interphase it is apical, at mitosis it is basal and following cytokinesis it is translocated from the cortex to the nucleus in the GMC.

Mechanisms of asymmetric prospero localization

The first detectable pros protein is localized to the apical side of neuroblasts after their delamination but before their first mitosis. This localization is one of the first signs of apical/basal polarity in neuroblasts. What generates the apical/basal neuroblast polarity that leads to asymmetric apical localization of pros? Neuroblasts delaminate from the ventral neuroectoderm, which is a classic epithelium with many aspects of apical/basal polarity (Knust, 1994). Thus, the delaminated neuroblast may retain aspects of apical/basal polarity. Two genes, crumbs and stardust, are required for normal apical/basal polarity of the neuroectoderm (Tepass and Knust, 1993), but it is not known whether they are required for apical pros localization. One other early neuroblast asymmetry is the centrosome, which is at the apical side of the neuroblast. It is not known whether the apical centrosome plays a role in apical pros localization, although apical centrosome position is correlated with pros localization (as is basal centrosome position at mitosis; see below).

The second subcellular localization of pros occurs at neuroblast mitosis, when the protein is asymmetrically localized to the basal cortex. The basal localization of pros follows the migration of the GMC centrosome to the basal side of the neuroblast. What controls the apical to basal movement of pros protein during mitosis? In stg embryos, neuroblasts are arrested in G2 of the cell cycle, they have abnormal centrosome migration and morphology, and pros basal localization does not occur. There are several possible explanations for the lack of pros basal localization in stg embryos. (1) Defects in the post-translational modification of the pros protein (e.g. due to
Fig. 4. prospero co-localizes with numb but is localized independently. Wild-type (A-C), 
numb\(^1\) (D) and \textit{pros}^{14} (E) embryos were double labeled for pros (green) and numb (red) protein. These are lateral views of neuroblasts with anterior to the left and basal up. (A) pros is asymmetrically localized to a crescent on the basal side of the neuroblast at mitosis (see Fig. 2B). This cell is at metaphase, as judged by pros expression. (B) In the same neuroblast, numb is also asymmetrically localized to a crescent on the basal side. (C) The merged image of A and B show that pros and numb co-localize on the basal side of the neuroblast at mitosis though they do not overlap completely. (D) pros is localized normally in \textit{numb}^{1} mutants. A neuroblast at anaphase shows pros localized to the cortex of the budding GMC. A GMC nucleus is located to the left of the bud. There is no numb protein expression in \textit{numb}^{1} embryos. (E) numb is localized normally in \textit{pros}^{14} mutants. A neuroblast at anaphase shows numb localized to the bud. \textit{pros}^{14} mutants do not produce detectable pros protein.

Fig. 5. Precursor cells in the PNS and gut also localize prospero to the cortex. (A-C) pros is asymmetrically localized on the cortex of the SOP cell. Wild-type stage 11 embryos were double labeled for pros (green) and numb (red). Anterior is to the left and dorsal up. (A) pros is asymmetrically localized to the SOP cortex. (B) numb is asymmetrically localized to the cortex; this localization of numb occurs at mitosis (Rhyu et al., 1994). (C) The merged images of A and B show that pros and numb co-localize in the SOP cell, although like the neuroblasts in the CNS, the overlap is not identical. (D-F) pros is asymmetrically localized in cells of the posterior midgut primordia. Wild-type embryos were double labeled for pros (green) and DNA (red). A ventral view of a stage 11 embryo is shown with anterior to the right. (D) pros is asymmetrically localized on the cortex of the ICPs. (E) DNA staining in the same section shows the outline of the endodermal layer and that the ICPs are interior to the endodermal layer. (F) The merged images of D and E show that the ICPs are in mitosis and that the crescent is positioned toward the endodermal layer. The ICPs will bud off smaller cells, the AMPs, which localize pros to the nucleus (Oliver et al., 1993; Bronner et al., 1994).
absence of the stg phosphatase) could block pros localization. Currently there is no information about post-translational modifications of the pros protein at different stages of the cell cycle.

(2) Disruption in centrosome morphology and migration could prevent pros basal localization. In stg embryos, both centrosomal and pros staining remain at the apical side of the neuroblast. This suggests that pros localization may be triggered by centrosomal position. Although some basal centrosomal staining is observed, there is no associated basal pros localization; the basal centrosomes in stg mutants are much smaller than normal, however, and may not be functional for triggering pros localization. Mutations that alter the migration of the centrosomes in dividing larval neuroblasts (Heck et al., 1993; Sunkel et al., 1995) may lead to a clearer picture of the role of centrosomes in triggering asymmetric pros localization.

Another possible mechanism for pros basal localization is localization of pros RNA followed by anchoring of the translated protein. A precedent is the localization of oskar RNA and protein to the posterior pole of the Drosophila oocyte (St. Johnson, 1993). The pros transcript has a relatively large 3’ untranslated sequence, which is where many RNA localization motifs are found, but the distribution of pros RNA during neuroblast mitosis has not yet been examined.

It is clear that the basal localization of numb protein in neuroblasts is not required for basal localization of pros. Both proteins are localized to the basal neuroblast cortex, but there are noticeable differences in the subcellular distribution of each protein. For example, numb shows greater localization to the basal side when compared to pros in the same neuroblast (Fig. 4A-C) or sense organ precursor (Fig. 5A-C), pros is apically localized in neuroblasts; numb is not. Finally, pros is nuclear in the MP2 precursor (Fig. 1C), whereas numb is asymmetrically localized to the basal cortex of MP2 (Spana et al., 1995). Taken together, these data show that pros and numb are capable of responding differently to a cellular localization mechanism, and may utilize different mechanisms for asymmetric localization.

After localization of pros into the GMC, it is translocated from the cortex into the nucleus. We think the same pros protein is moving from the GMC cortex into the nucleus, because the process occurs too rapidly for de novo synthesis of new pros in the GMC. The pros gene is >23 kb (data not shown), and would take over 20 minutes to transcribe and translate (Thummel et al., 1990; Shermoen and O’Farrell, 1991); we observe nuclear localization rapidly after mitosis (see Results). However, it is possible that pros RNA is inherited by the GMC and could be rapidly translated and localized to the nucleus. The pros protein has a consensus nuclear localization signal, and the protein is nuclear in most cells in which it is expressed. It is likely that there is a mechanism in neuroblasts to keep pros at the cell cortex. The critical step in the translocation of pros into the GMC nucleus is probably its release from the cortex. What triggers the release of pros from the GMC cortex? pros protein might be post-translationally modified in the GMC; if the modification occurs transiently after mitosis, the effect could be limited to pros in the GMC since there is no pros in neuroblasts immediately after mitosis. Another possibility is that there are differences in the GMC and neuroblast cytoskeleton, which result in cortical localization only in the neuroblast. For example, the neuroblast centrosome is clearly larger in size than the GMC centrosome, and may lead to a different organization of the

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**Fig. 6.** prospero is asymmetrically localized to the budding GMC and is required to specify GMC cell type. (A) Schematic diagram details the localization of pros protein (green), DNA (blue) and the centrosomes (red) throughout the cell cycle of the neuroblast. Basal is up, apical is down. See text for details. (B) Schematic diagram depicts the expression of GMC genes (cyan) and neural precursor genes (magenta) in wild-type and pros embryos. The orientation is the same as in A. GMC genes like eve and ftz are not expressed in the neuroblast but activated in a subset of GMCs. The expression of these genes is lost in some lineages in pros mutants (Doe et al., 1991). Neural precursor genes like dpn and ase are expressed in neuroblasts but not in GMCs. These genes are not repressed in GMCs in pros mutants (Vaessin et al., 1991).
cortical cytoskeleton in the neuroblast. Finally, it is possible that the pros association with the cortex is intrinsically unstable, leading to pros nuclear localization after a specific time. While unlikely, we cannot rule out this mechanism.

Each of the mechanisms discussed above is consistent with the observation that pros protein is partially translocated into the nucleus in G2-arrested neuroblasts in stg embryos. The post-translational modifications occurring transiently after mitosis would affect pros in the G2-arrested neuroblasts if the timing of the modification process was not dependent on the nuclear cell cycle. Temporally accurate transcriptional activation in G2-arrested neuroblasts has been documented (Cui and Doe, 1995). If changes in the GMC cytoskeleton are required to release pros from the cortex, they may also occur in the G2-arrested neuroblast. Finally, if pros protein intrinsically loses its ability to adhere to the cell cortex, it would accumulate in the neuroblast nucleus in stg embryos.

Function of asymmetric prospero localization

As neuroblasts divide to generate smaller GMCs, each cell type rapidly establishes a unique pattern of gene expression. pros is required in GMCs to activate the expression of eve and ftz in specific lineages (Fig. 6B) (Doe et al., 1991); these genes are termed ‘GMC genes’ because they are expressed in GMCs but not neuroblasts. pros is also required to repress the ‘neural precursor’ genes dpn and ase in GMCs (Vaessin et al., 1991); these genes are expressed in neuroblasts but not GMCs. The asymmetric localization of pros protein may provide a mechanism to rapidly initiate GMC-specific gene expression. De novo transcription and translation of the pros gene takes at least 20 minutes; this time lag is eliminated by the asymmetric localization of pros protein into the budding GMC.

Cortical localization of pros might be necessary to prevent nuclear localization in neuroblasts. Misexpression of pros in the nucleus of a neuroblast might cause defects in gene expression. In stg mutants, pros protein accumulates in the neuroblast nucleus. Does pros activate GMC-specific genes in these neuroblasts? The GMC genes ftz and eve have been examined in stg mutants. eve is not expressed in stg mutants, though the expression of eve seems to require cytokinesis (Cui and Doe, 1995). ftz, on the other hand, is expressed in a subset of neuroblasts in stg mutants (Doe et al., 1988). It is not known whether the expression of ftz in these G2-arrested neuroblasts requires pros. It is also unknown whether the neural precursor genes dpn and ase are repressed in neuroblasts in stg mutants.

Is pros necessary to specify the difference between the neuroblast and GMC cell types? In pros mutants, GMCs show partial alteration of gene expression that is consistent with a transformation towards a neuroblast fate: they fail to activate several GMC-specific genes and fail to repress several neuroblast-specific genes. However, the GMCs appear to divide to produce neurons that extend axons, although motoneuron axon outgrowth is seriously delayed and the number of interneuronal projections in the CNS is dramatically reduced (Doe et al., 1991; Broadie and Bate, 1993). Thus pros is only partially necessary to establish the GMC cell fate. Whether pros expression in neuroblast nuclei is sufficient to induce GMC fate has not been determined.

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