Int. J. Dev. Biol. 42: 379-383 (1998)

Asymmetry and cell fate in the *Drosophila* embryonic CNS

SAL FUERSTENBERG, JULIE BROADUS and CHRIS Q. DOE

Department of Cell and Structural Biology, University of Illinois, Urbana, USA

ABSTRACT *Drosophila* CNS precursors, neuroblasts, repeatedly divide to produce a large neuroblast and a smaller GMC. This division is asymmetric with regard to sibling cell size, mitotic potential and gene expression. Recent work has identified a number of molecules that show a polarized distribution during neuroblast mitosis: prospero RNA and Inscuteable, Miranda, Prospero, Staufen, and Numb proteins. The process of asymmetric localization of proteins and RNAs is cell cycle dependent, microfilament dependent and coordinated with the positioning of the mitotic spindle, which results in the unequal distribution of cell fate determinants to a specific daughter cell at cytokinesis.

KEY WORDS: *prospero, numb, inscuteable, neuroblast, asymmetric division*

The precursor cells of the *Drosophila* central nervous system (CNS) are derived from the ventral neurogenic ectoderm of the developing embryo. Single cells within this region leave the epidermal layer and come to lie between the ectoderm and mesoderm, forming the neural primordium. These progenitors of the CNS, called neuroblasts, repeatedly divide unequally to regenerate a larger apical neuroblast and "bud off" a smaller cell, called a ganglion mother cell (GMC) from their basal side. The neuroblast remains associated with the ventral neuroectoderm, while the smaller GMC is pushed dorsally into the interior of the embryo. Each GMC divides once to produce a pair of postmitotic neurons or glia (for a comprehensive description see Campos-Ortega, 1993; Goodman and Doe, 1993).

Neuroblast cell division is inherently asymmetric, generating sibling cells which differ in size, in capacity for self renewal, and in gene expression. This asymmetry may be directed by extracellular (extrinsic) factors which affect a single sibling cell after mitosis; or the asymmetry may be intrinsic, through the unequal segregation of intracellular determinants which direct disparate fates in each of the daughters (Horvitz and Herskowitz, 1992). Genetic, biochemical and cell biological analyses have revealed several potential fate determinants which are partitioned during neuroblast division (Fig. 1); the recent data are reviewed here.

Proteins and RNA are asymmetrically localized into the GMC

The best characterized asymmetric "determinant" in neuroblasts is the Prospero protein (Fig. 1a). Prospero is a divergent homeodomain transcription factor (Doe *et al.,* 1991; Vaessin *et al.,* 1991; Matsuzaki *et al.,* 1992) which is synthesized in the neuroblast but required in GMCs to activate GMC-specific gene expression (e.g., *even-skipped* and *fushi tarazu*; Doe *et al.,* 1991), and to repress neuroblast-specific gene expression (e.g., *asense* and *deadpan*; Vaessin *et al.,* 1991). Prospero is colocalized with F-actin at the neuroblast cortex in a highly cell cycle-dependent fashion (Hirata *et al.,* 1995; Knoblich *et al.,* 1995; Spana and Doe, 1995). At late interphase, Prospero resides at the apical neuroblast cortex in a diffuse crescent (Spana and Doe, 1995). As the neuroblast enters mitosis, Prospero is transported to the basal side of the neuroblast where it forms a tight crescent centered over the basal centrosome (Hirata *et al.,* 1995; Knoblich, *et al.,* 1995; Spana and Doe, 1995). As the GMC buds from the neuroblast during anaphase, Prospero is tightly associated with the basal cell cortex, and is ultimately segregated into the GMC where it is translocated into the nucleus. Prospero localization is cell cycle regulated: in G2-arrested neuroblasts (in *string* mutant embryos), Prospero remains apical in the neuroblast, suggesting that basal localization is dependent upon entry into mitosis (Spana and Doe, 1995); similarly, colcemid-treated neuroblasts arrested in metaphase retain Prospero protein at the basal cortex indefinitely, suggesting that exit from mitosis is necessary for cortical release and nuclear translocation (Broadus and Doe, 1997).

Recently, it has been shown that *prospero* RNA is also localized in the neuroblast in a cell cycle-dependent manner, and segregated into the GMC following mitosis (Li *et al.,* 1997; Broadus *et al.,* 1998). Like Prospero protein, *prospero* RNA is apical at interphase, forming an intense crescent which extends from the nucleus to the cell cortex (Broadus *et al.,* 1998). This may represent the vectoral transport of newly transcribed RNA to a cortical anchoring site (Francis-Lang *et al.,* 1996). During metaphase, *prospero* RNA moves to the basal neuroblast cortex, and as the neuroblast divides, *prospero* RNA is partitioned solely into the GMC where it is released into the cytoplasm. Not all RNAs show asymmetric

***Address for reprints:** Department of Cell and Structural Biology, HHMI, University of Illinois, Urbana, IL 61821, USA. FAX: 217-244-3982. e-mail: c-doe@uiuc.edu

0214-6282/98/$10.00

Printed in Spain

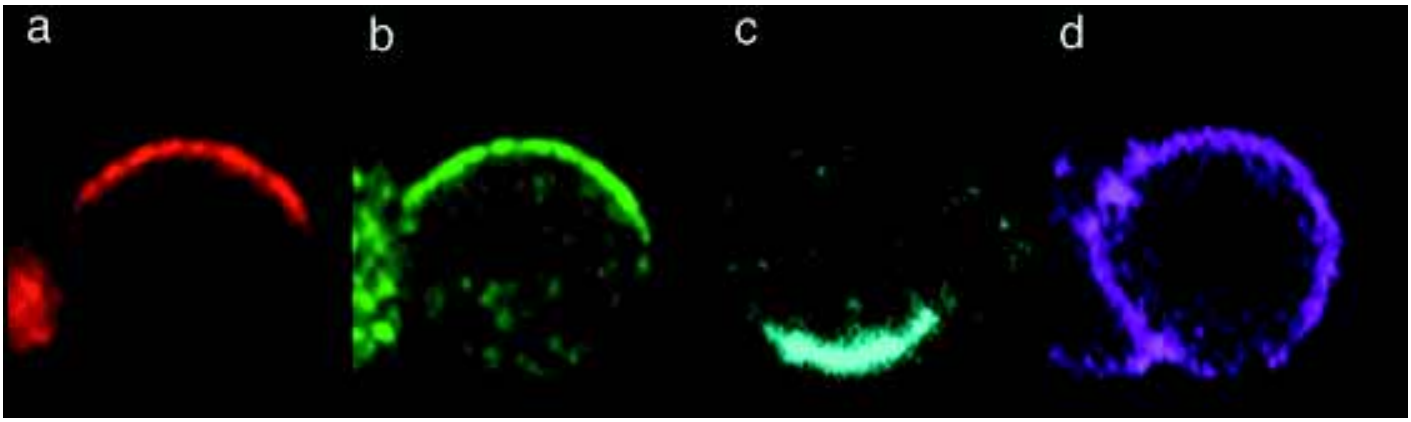

Fig. 1. Localization of Prospero, Staufen, Inscuteable and microfilaments during mitosis *in vitro*. **(a)** *Prospero (red) forms a basal crescent;* **(b)** *Staufen (green) forms a basal crescent;* **(c)** *Inscuteable (blue) protein forms an apical crescent.* **(d)** *Phalloidin staining shows that actin filaments (magenta) are distributed uniformly at the cell cortex.*

distribution in neuroblasts; *seven-up* RNA is uniformly distributed in neuroblasts at all stages of the cell cycle (Broadus *et al.,* 1998).

The asymmetric distribution of *prospero* RNA suggests that RNA binding proteins may also be differentially partitioned during neuroblast mitoses. The Staufen RNA binding protein is necessary for the specific distribution of the maternal RNAs *bicoid* and *oskar* RNA in the oocyte, and is a candidate for playing a role *prospero* RNA localization, due to its presence in the embryonic CNS (St. Johnston *et al.,* 1991). At interphase, Staufen protein forms in a gradient, highest at the apical cortex and lowest near the nucleus (Broadus *et al.,* 1998), similar to *prospero* RNA. During neuroblast mitosis, Staufen colocalizes with Prospero protein and *prospero* RNA in a tight basal crescent (Fig. 1b); all three molecules are inherited by the GMC, where Staufen/*prospero* RNA become cytoplasmic and Prospero protein enters the nucleus (Broadus *et al.,* 1998). Thus, Staufen protein displays cell cycle-specific localization precisely corresponding to that of *prospero* RNA, and can bind *prospero* RNA directly *in vitro* (Li *et al.,* 1997). In embryos lacking Staufen function (either zygotic null or maternal and zygotic null embryos), the majority of neuroblasts fail to localize *prospero* RNA apically at interphase or basally at mitosis (Li *et al.,* 1997; Broadus *et al.,* 1998). However, Prospero protein is localized normally. These data show that *prospero* RNA and protein localization are independently regulated, and that *prospero* RNA localization is not required for Prospero protein localization.

Why segregate *prospero* RNA into the GMC? It has been thought that *prospero* is transcribed in GMCs, since they contain a large amount of *prospero* mRNA (Vaessin *et al.,* 1991; Matsuzaki *et al.,* 1992; Doe *et al.,* 1991). However, the use of intron-specific probes to reveal sites of active transcription (Kopczynski and Muskavitch, 1992) shows that *prospero* is transcribed solely in the neuroblast and not in the GMC (Broadus *et al.,* 1998). This highlights an interesting situation: both *prospero* RNA and protein are made in the neuroblasts where they are not required (rendered inactive by translational control of the RNA and by cortical localization of the protein), and asymmetrically localized to the GMC which absolutely requires *prospero* function but does not transcribe the gene (they are activated by translational derepression of the RNA and by cortical release/nuclear translocation of the protein). The functions of asymmetrically localized *prospero* RNA and Prospero protein have not yet been tested independently of each other: it may be necessary to keep both out of the neuroblast, or localization of both may be required to provide sufficiently high concentrations in the GMC. Loss of RNA localization (in *staufen* mutants) does not obviously alter GMC identity, but its appears that RNA and protein localization provide redundant functions (Broadus *et al.,* 1998; see Fig. 2).

Numb was the first protein to be detected as asymmetrically distributed in neuroblasts, where it is segregated into the GMC at neuroblast division (Rhyu *et al.,* 1994). In the neuroblast, Numb is associated with the plasma membrane and shows the same cell cycle dependent basal localization as seen for Prospero (Knoblich *et al.,* 1995; Spana *et al.,* 1995). This colocalization is mutually independent, since Numb localizes normally in pr*ospero* mutants, and Prospero segregates normally in *numb* mutant embryos (Knoblich *et al.,* 1995; Spana and Doe, 1995). There is no reported function of *numb* in the GMC. It does, however, play an important role in the specification of sibling cell fates in the peripheral nervous system (Uemura *et al.,* 1989; Rhyu *et al.,* 1994; Knoblich *et al.,* 1995), the MP2 lineage (Spana *et al.,* 1995) and in many sibling neurons of the CNS (J. Skeath and Doe, 1998). In all of these cases, *numb* appears to act by inhibiting Notch signaling (reviewed in Doe and Spana, 1995). Numb is a basic protein containing several consensus proline-containing SH3 binding sites (Src homology 3) and a PTB domain (phosphotyrosine binding). These motifs potentially link Numb to tyrosine kinase pathways and also identify it as a signaling adapter molecule belonging to the dshc, drk, and dck family (Uemura *et al.,* 1989; Pawson, 1995; Li *et al.,* 1997). It is not clear which, if any, of these motifs is required for antagonizing Notch signaling.

Miranda regulates membrane anchoring and release of Prospero protein

How are Prospero, Numb and Staufen tethered at the neuroblast cortex? The subcellular localization of these proteins suggests that they interact directly or indirectly with an already localized component of the peripheral cytoskeleton. Miranda is a pioneer protein which was cloned from a two-hybrid screen using a portion of the Prospero protein containing the asymmetric localization domain (ALD; Ikeshima-Kataoka *et al.,* 1997; Shen *et al.,* 1997).

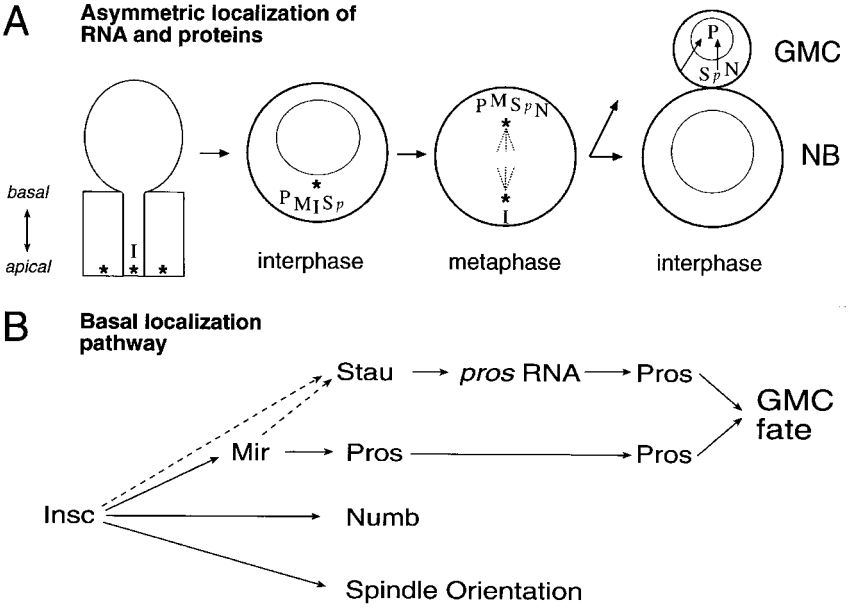

Fig. 2. Regulation of protein/RNA localization and spindle orientation. (A) *In late interphase neuroblasts, Inscuteable, Prospero and Staufen and prospero RNA reside at the apical cortex. During metaphase, Inscuteable remains apical; and coordinates spindle orientation with the basal localization of Miranda and Prospero proteins, Numb protein, and perhaps Staufen and prospero RNA. After cytokinesis, Inscuteable is retained in the neuroblast; while basal factors are segregated into the GMC. In the GMC, Prospero becomes nuclear, Staufen and prospero RNA cytoplasmic, and Numb is retained at the GMC cortex.* **(B)** *Inscuteable independently regulates the orientation of the mitotic spindle; the basal localization of Numb; and the basal localization of the Miranda/Prospero complex. Inscuteable is also required to localize Staufen protein, perhaps via Miranda (dashed arrows). Staufen protein directs the localization of prospero RNA.*

The ALD is necessary and sufficient for Prospero localization (Hirata *et al.,* 1995; F. Matsuzaki, personal communication), and is also necessary for the Prospero-Miranda interaction in yeast two hybrid assays (Ikeshima-Kataoka *et al.,* 1997). *In vitro*, Miranda binds to Prospero and Numb proteins (Shen *et al.,* 1997).

The structural analysis of Miranda reveals a central core comprised of a region of coiled coil structure similar to the myosin rod, which is thought to provide an interface for protein-protein interactions (Lupas, 1996; Ikeshima-Kataoka *et al., 1997*; Shen *et al.,* 1997). In addition, there are two leucine zipper motifs, seven consensus phosphorylation sites for Protein Kinase C (Ikeshima-Kataoka *et al.,* 1997) and four divergent ubiquitin-dependent destruction signals resembling those found in mitotic cyclins (Shen *et al.,* 1997), suggesting that Miranda activity is highly regulated. During interphase, Miranda is uniformly cortical or at low levels in the cytoplasm; at metaphase, it forms a basal crescent at the cortex which precisely coincides with that of Prospero, and both are segregated into the daughter GMC at anaphase (Ikeshima-Kataoka *et al.,* 1997). After cytokinesis, Prospero is released from the membrane and enters the GMC nucleus, and Miranda becomes undetectable (Ikeshima-Kataoka *et al.,* 1997; Shen *et al.,* 1997).

Six mutant alleles of *miranda* have been generated in a large scale EMS screen intended to identify genes which affect neural fates in the embryonic CNS (Skeath and Doe, 1998). All six *miranda* mutations result in a failure to correctly localize Prospero protein during or after neuroblast cell division. In five alleles, Prospero is present throughout the cytoplasm of the neuroblast, is equally distributed to both progeny at neuroblast division, and enters the nucleus of both neuroblast and GMC (Ikeshima-Kataoka *et al.,* 1997). A *miranda* transgene rescues Prospero localization in these *miranda* mutant embryos (Ikeshima-Kataoka *et al.,* 1997). Similar results have been observed in embryos homozygous for a deficiency which removes *miranda* (Shen *et al.,* 1997). Importantly, neuroblasts lacking Miranda show normal localization of Numb, normal spindle orientation, and normal unequal cleavage to produce a large apical neuroblast and smaller basal GMC (Ikeshima-Kataoka *et al.,* 1997; Shen *et al.,* 1997).

The sixth *miranda* allele shows a quite different distribution of Prospero: the localization and subsequent segregation of Prospero into the GMC is normal, but Prospero is not released from the GMC cortex after cytokinesis. These results strongly suggest that Miranda tethers Prospero to the basal cell cortex, and cell cycle dependent regulation of Miranda activity allows the release of Prospero into the GMC nucleus. Sequence analysis of the *miranda* mutant alleles suggest that amino acids 445-727 interact with Prospero, and amino acids 727-830 regulate the release of Prospero from the GMC cortex. The consensus Protein Kinase C sites lie within this final one hundred amino acids, suggesting that phosphorylation may regulate the release of Prospero from the GMC cortex. None of these *miranda* alleles show aberrations in the localization of Numb protein, indicating that basal localization of Numb must be independently regulated (Ikeshima-Kataoka *et al.,* 1997).

Inscuteable independently regulates protein localization and spindle orientation

How are the processes of crescent formation and spindle orientation orchestrated? Several potential determinants must be localized and anchored –Miranda and Prospero, Numb, Staufen and *prospero* RNA– and their basal positioning must be coordinated with the apical/basal orientation of the mitotic spindle. One protein identified so far fills the criteria for such a regulator: Inscuteable, which was identified in an enhancer trap screen for genes expressed in neuroblasts and SOPs (Kraut and Campos-Ortega, 1996). *Inscuteable* encodes an apparent cytoskeletal adapter protein, containing a SH3 binding domain (proline rich), ankyrin repeats (a protein-protein interaction domain), a nuclear targeting sequence (perfect, but of unknown function, as Inscuteable appears to be cortical or cytoplasmic; Kraut and Campos-Ortega, 1996) and putative WW domain (WW domains associate with proline-rich modules; Chan *et al.,* 1996; Li *et al.,* 1997).

Inscuteable protein is localized to the apical cortex of the neuroblast, and is first detected in the apical “endfoot” which remains associated with the epithelial surface in delaminating

neuroblasts; this becomes a discrete apical crescent in fully delaminated interphase cells. The apical crescent of Inscuteable persists at least through metaphase. At later stages of the cell cycle it appears to be delocalized or degraded *in vivo* (Kraut *et al.,* 1996), although *in vitro* cultured neuroblasts show apical Inscuteable localization throughout the entire cell cycle, with selective segregation into the neuroblast after mitosis (Fig. 1c; Broadus and Doe, 1997); the persistent detection of Inscuteable *in vitro* may be due to the greater sensitivity of antibody detection. In *inscuteable* mutant embryos, Prospero and Numb are colocalized during mitosis, but are homogeneously cortical or in crescents whose positions are randomized with respect to surrounding tissue. Moreover, the orientation of the spindle is also random with respect to the apical/basal axis, and coordination of the Prospero/Numb crescent with respect to spindle orientation is lost as well. In *prospero* or *numb* mutants, Inscuteable is positioned normally (Kraut *et al.,* 1996). Inscuteable appears to independently regulate both spindle orientation and the basal localization of Prospero and Numb during mitosis.

Does *inscuteable* also regulate the positioning of *prospero* RNA and Staufen protein? In a two-hybrid screen designed to find proteins that interact with Inscuteable, one class of positive clones encoded the C-terminal domain of Staufen. This interaction requires the C-terminal 108 amino acids of Inscuteable, and is inhibited by the addition of 86 additional N-terminal amino acids of Inscuteable. An *inscuteable* construct representing the C-terminal 289 amino acids restores binding to the Staufen peptide (Li *et al.,* 1997). This suggests that an inter- or intramolecular interaction may regulate the binding of Inscuteable to Staufen. The effect on Staufen protein in *inscuteable* mutants is subtle: there is a decrease in the number of neuroblasts with apical localization of Staufen at interphase (Li *et al.,* 1997); basal localization was not assayed in this paper. Surprisingly, although apical localization of Staufen depends upon *inscuteable*, and apical localization of *prospero* RNA is not *inscuteable*-dependent, Li *et al.* (1997) find that the basal localization of *prospero* RNA requires *inscuteable*. We think it is likely that Inscuteable is required for basal localization of Staufen (similar to its requirement for basal localization of Prospero and Numb), which is why basal localization of *prospero* RNA is affected in *inscuteable* mutants.

Regulation of asymmetric protein localization

An initial asymmetry is already established in the neuroblast when it is born, inherited from the polarized epithelium from which it delaminates. Inscuteable protein is apically localized in delaminating neuroblasts that still have an attachment to the ectoderm (Kraut *et al.,* 1996). Once Inscuteable localization is established, all known molecular and cellular asymmetries assayed to date are positioned with respect to Inscuteable: the apical localization of Staufen; the basal localization of Numb, Miranda and Prospero, *prospero* RNA; and the positioning of the mitotic spindle.

How is Inscuteable localization regulated? *In vitro* culture of isolated neuroblasts provides some clues (Broadus and Doe, 1997). Neuroblasts isolated *in vitro* divide to produce a smaller GMC and regenerate a larger neuroblast; in the absence of any continuous extrinsic signal, the apparent asymmetry of division is maintained. In these mitotic neuroblasts, Inscuteable is apical with regard to the positioning of the GMC bud, and Prospero and Staufen are positioned opposite to that of Inscuteable during mitosis and are segregated into the GMC, identical to the *in vivo* situation. These results cannot distinguish whether apical localization of Inscuteable in metaphase neuroblasts is random or in response to an apical cue that is maintained *in vitro*; in either case, all subsequent aspects of asymmetric protein localization and neuroblast division occur normally *in vitro*.

Previous work using cytoskeletal inhibitors Colcemid and Cytochalasin B suggested that the asymmetric localization of Numb and Prospero was independent of both microtubules and microfilaments (Knoblich *et al.,* 1995). More recently, microfilaments have been shown to be important for localizing and anchoring Inscuteable, and although Prospero and Numb crescents form, they are frequently misoriented (Kraut *et al.,* 1995). This result suggests that microfilaments may indeed be required for the asymmetric localization of many proteins in neuroblasts. This has been confirmed by drug studies using cultured neuroblasts (Broadus and Doe, 1997). In cultured neuroblasts treated with Cytochalasin B or Latrunculin B to disrupt microfilaments, Inscuteable was either uniformly cortical or cytoplasmic and Prospero and Staufen were cytoplasmic (Broadus and Doe, 1997). In contrast, treatment with Colcemid to disrupt microtubules has no effect on protein crescents; in fact, proteins accumulate in crescents in metaphase-arrested neuroblasts to higher than normal levels. The effect of drug treatment is reversible: treatment with Colcemid (to accumulate metaphase protein crescents) followed by addition of Cytochalasin B results in cytoplasmic localization of Inscuteable, Prospero and Numb; if Cytochalasin B alone is washed out, all three proteins regain their normal asymmetric positions (Broadus and Doe, 1997). Thus, microfilaments appear to be essential for the anchoring and/or localization of Inscuteable, Prospero and Staufen to the cell cortex. Microfilaments do not provide the asymmetry, however, since they appear to be uniformly distributed around the cell cortex at all stages of the cell cycle (Fig. 1d; Knoblich *et al.,* 1995; Spana and Doe, 1995; Broadus and Doe, 1997). The simplest model is that diffusion plus microfilament-dependent anchoring controls protein localization: at mitosis, each protein could acquire an affinity for an apical or basal localized cortical component.

Collectively, these data reviewed in this paper identify several pathways that establish neuroblast polarity and sibling cell fate (Fig. 2). During late interphase, there is a pathway for localization of Inscuteable, Prospero, and Staufen/*prospero* RNA to the apical side of the neuroblast. The mechanism or cues for apical localization of Inscuteable persist through entry into mitosis, while those for apical localization of Prospero and Staufen/*prospero* RNA do not. During mitosis, there are at least two pathways for basal protein localization in neuroblasts (Miranda-dependent for Prospero and perhaps Staufen; Miranda-independent for Numb), and a third pathway which orients the mitotic spindle. All three pathways require Inscuteable and are tightly cell cycle regulated and microfilament-dependent. Asymmetric localization provides unique functions during neuroblast division: Prospero localization is necessary for GMC fate specification; Staufen/*prospero* RNA function is less well characterized, but may provide a redundant mechanism for ensuring high Prospero levels in the GMC or removing Prospero from the neuroblast; Numb function in GMCs has not been identified, but high levels in the GMC may be relevant for its subsequent

function in sibling neuron specification; finally, Inscuteable provides an essential function in coordinating basal protein localization with mitotic spindle orientation, ensuring the appropriate partitioning of determinants to neuroblast and GMC daughter cells.

References

BROADUS, J. and DOE, C.Q. (1997). Extrinsic cues, intrinsic cues, and microfilaments regulate asymmetric localization of Prospero, Staufen, and Inscuteable in *Drosophila* neuroblasts. *Curr. Biol. 7:* 827-835.

BROADUS, J., FUERSTENBERG, S. and DOE, C.Q. (1998). Staufen dependent localization of prospero mRNA contributes to neuroblast daughter cell fate. *Nature 391:* 792-795.

CAMPOS-ORTEGA, J.A. (1993). Early neurogenesis in *Drosophila melanogaster*. In *Development of Drosophila melanogaster*. (M. Bate and A. Martinez-Arias Eds.),Cold Spring Harbor Laboratory Press, New York. pp. 1091-1130.

CHAN, D.C., BEDFORD, M.T. and LEDER, P. (1996). Formin binding proteins bear WWP/WW domains that bind proline-rich peptides and functionally resemble SH3 domains. *EMBO J. 15:* 1045-1054.

DOE, C.Q., CHU-LAGRAFF, Q., WRIGHT, D.M. And SCOTT, M.P. (1991). The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. *Cell 65:* 451-464.

DOE, C.Q. and SPANA, E.P. (1995). A collection of cortical crescents: asymetric protein localization in CNS precursor cells. *Neuron 15:* 991-995.

FRANCIS-LANG, H., DAVIS, I. and ISH-HOROWICZ, D. (1996). Asymmetric localization of *Drosophila* pair-rule transcripts from displaced nuclei: evidence for directional nuclear transport. *EMBO J. 15:* 640-649.

GOODMAN, C.S., and DOE, C.Q. (1993). Embryonic development of the *Drosophila* central nervous system. In *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, New York. pp. 1131-1206.

HIRATA, J., NAKAGOSHI, H., NABESHIMA, Y. And MATSUZAKI, F. (1995). Asymmetric segregation of a homeoprotein, *prospero*, during cell divisions in neural and endodermal development. *Nature 377:* 627-630.

HORVITZ, H.R. and HERSKOWITZ, I. (1992). Mechanisms of Asymmetric Cell Division: Two Bs or Not Two Bs, That is the Question. *Cell. 68:* 237-255.

IKESHIMA-KATAOKA, H., SKEATH, J.B., NABESHIMA, Y., DOE, C.Q., and MATSUZAKI, F. (1997). Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. *Nature 390:* 625-628.

KNOBLICH, J.A., JAN, L.Y. and JAN, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. *Nature 377:* 624-627.

KOPCZYNSKI, C.C. and MUSKAVITCH, M.A.T. (1992). Introns excised from the *Delta* primary transcript are localized near sites of *Delta* transcription. *J. Cell Biol. 119:* 503-512.

KRAUT, R. and CAMPOS-ORTEGA, J.A. (1996). *inscuteable*, a neural precursor gene of *Drosophila*, encodes a candidate for a cytoskeleton adaptor protein. *Dev. Biol. 174:* 65-81.

KRAUT, R., CHIA, W., JAN, L.Y., JAN, Y.N. and KNOBLICH, J.A. (1996). Role of inscuteable in orienting asymmetric cell divisions in *Drosophila*. *Nature 383:* 50-55.

LI, P., YANG, X., WASSER, M., CAI, Y. and CHIA, W. (1997). Inscuteable and Staufen Mediate Asymmetric Localization and Segregation of prospero RNA during *Drosophila* Neuroblast Cell Divisions. *Cell 90:* 437-447.

LUPAS, A. (1996). Coiled coil: new structures and new functions. *Trends Biochem. Sci. 21:* 375-382.

MATSUZAKI, F., KOISUMI, K., HAMA, C., YOSHIOKA, T. and NABESHIMA, Y. (1992). Cloning of the *Drosophila prospero* gene and its expression in ganglion mother cells. *Biochem. Biophys. Res. Commun 182:* 1326-1332.

PAWSON, T. (1995). Protein modules and signalling networks. *Nature 373:* 573-579.

RHYU, M.S., JAN, L.Y. and JAN, Y.N. (1994). Asymmetric distribution of of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell 76:* 477-491.

SHEN, C.-P., JAN, L.Y. and JAN, Y.N. (1997). Miranda Is Required for the Asymmetric Localization of Prospero during Mitosis in *Drosophila*. *Cell 90:* 449-458.

SKEATH, J.B. and DOE C.Q. (1998). Sanpodo and Notch act in oposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* 125. (In press).

SPANA, E.P. and DOE, C.Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. *Development 121:* 3187-3195.

SPANA, E.P., KOPCZYNSKI, C., GOODMAN, C.S. and DOE, C.Q. (1995). Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS. *Development 121:* 3489-3494.

ST. JOHNSTON, D., BEUCHLE, D. and NÜSSLEIN-VOLHARD, C. (1991). *staufen*, a gene required to localize maternal RNAs in the *Drosophila* egg. *Cell 66:* 51-63.

UEMURA, T., SHEPERD, S., ACKERMAN, L., JAN, L.Y. and JAN, Y.N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. *Cell 58:* 349-360.

VAESSIN, H., GRELL, E., WOLFF, E., BIER, E., JAN, L.Y. and JAN, Y.N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. *Cell 67:* 941-953.