Inscuteable-independent apicobasally oriented asymmetric divisions in the *Drosophila* embryonic CNS

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Inscuteable is the founding member of a protein complex localised to the apical cortex of *Drosophila* neural progenitors that controls their asymmetric division. Aspects of asymmetric divisions of all identified apicobasally oriented neural progenitors characterised to date, in both the central and peripheral nervous systems, require *inscuteable*. Here we examine the generality of this requirement. We show that many identified neuroblast lineages, in fact, do not require *inscuteable* for normal morphological development. To elucidate the requirements for apicobasal asymmetric divisions in a context where *inscuteable* is not essential, we focused on the MP2 > dMP2 + vMP2 division. We show that for MP2 divisions, asymmetric localisation and segregation of Numb and the specification of distinct dMP2 and vMP2 identities require *bazooka* but not *inscuteable*. We conclude that *inscuteable* is not required for all apicobasally oriented asymmetric divisions and that, in some cellular contexts, *bazooka* can mediate apicobasal asymmetric divisions without *inscuteable*.

INTRODUCTION

Neural progenitors of the *Drosophila* embryonic central nervous system (CNS) divide asymmetrically with their mitotic spindles aligned along the apical/basal (A/B) axis and cell-fate determinants localised to the basal cell cortex. An apically localised protein complex that includes Inscuteable (Insc) (Kraut and Campos-Ortega, 1996; Kraut *et al.*, 1996) and Bazooka (Baz) (Schober *et al.*, 1999; Wodarz *et al.*, 1999) mediates these divisions. However, the proposed role of apical complex molecules like Insc in specifying CNS cell fate is based on

studies involving a small number of identified early born neurons from a few well-characterised lineages (e.g. Buescher *et al.*, 1998; Yu *et al.*, 2000). Here we use cell transplantations to examine the requirement for *insc* during the development of complete neuroblast lineages; we show that the morphology of several characterised ventral neuroblast lineages (Bossing *et al.*, 1996; Schmid *et al.*, 1999) does not require *insc*. We focused on the MP2 > dMP2 + vMP2 asymmetric division, in which the resolution of distinct daughter fates requires *Notch* and *numb* (Spana *et al.*, 1995; Spana and Doe, 1996). We show that for the A/B-oriented MP2 divisions, asymmetric localisation and segregation of Numb and the specification of distinct dMP2 and vMP2 identities occur normally in the absence of *insc*. Although Insc is dispensable, there is a requirement for Baz for the MP2 asymmetric division; when *baz* is limiting, Numb is cortical in dividing MP2, leading to the duplication of dMP2 at the expense of vMP2. We conclude that *insc* is not required for all A/B-oriented asymmetric divisions; in some cellular contexts (Lu *et al.*, 2001), *baz* can mediate both planar (Bellaiche *et al.*, 2001) and A/B asymmetric divisions (MP2) in the absence of *insc*.

RESULTS AND DISCUSSION

Cell transplantations and immunohistochemistry were performed to uncover the role of *insc* in the development of complete neuroblast (NB) lineages. Cells removed from the ventral neuroectoderm of *insc*²² (an amorphic allele of *insc*) mutant embryos were isotopically and isochronically implanted into wild-type (WT) hosts (Figure 1; Table I). The resulting NB lineages were

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Fig. 1. The resolution of distinct fates for the progeny of the MP2 asymmetric division does not require *insc* function. Representative horseradish peroxidase (HRP)-labelled MP2 clones obtained by transplanting WT and mutant donor cells into WT hosts are shown. (A–C) HRP lineages in a lateral view. (A'–C') Summary of morphologies of typical clones from the corresponding experiments in a horizontal orientation. Anterior is to the left. (A) The WT MP2 lineage consists of two cells. The dMP2 neuron (black arrowhead) projects its axon posteriorly, while its sibling neuron vMP2 (white arrowhead) sends projections in the anterior direction. (B) MP2 clone obtained from heterogenetic transplantations of homozygous *N^{55ell}* (an amorphic allele of *N*) donor cells into WT hosts. The vMP2 neuron is transformed into the dMP2 neuron, as seen by the duplication of the dMP2 fate and the absence of the vMP2 projection. (C) MP2 clone obtained from heterogenetic transplantations of *insc²²* donor cells into WT hosts. The clone is indistinguishable from its WT counterpart.

analysed and benchmarked against corresponding WT lineages with respect to the composition of their neurons and/or glia, cell positions and axonal morphologies. We obtained a total of 112 mutant ventral nervous system clones from donors homozygous for *insc²²*. The 112 mutant lineages represented 15 out of the 17 described neuroblast lineages from the ventral neuroectoderm (Bossing *et al.*, 1996). We limit our discussion to nine out of the 15 ventral *insc* mutant lineages, each of which was represented by at least three independent mutant clones (Table I).

Three *insc* mutant lineages, NB1-1, NB4-2 and NB7-1, showed deviations from the WT morphology (Table I; data not shown). In all three mutant lineages, *insc* appears to be required specifically for the cell-fate resolution of the earliest born neurons, later born components of these three lineages appear not to require or can partially bypass *insc* for WT morphological development. A detailed description of the roles of *insc*, *Notch*, *Delta* and *kuzbanian* in neuroblast lineage development will be presented elsewhere. Here we are concerned with NB lineages that do not require *insc*. All of the *insc* clones associated with six of the NB lineages (NB1-2, NB3-2, NB4-1, NB5-2, NB7-2 and MP2) were morphologically WT in terms of cell numbers, positions and axonal projections (Table I). We focused on the simple MP2 lineage in an attempt to understand the nature of at least some of these *insc*-independent asymmetric divisions. The MP2 precursor forms adjacent to the ventral midline in each

segment and undergoes a single A/B-oriented asymmetric division to produce two daughter cells, vMP2 and dMP2 (Spana *et al.*, 1995). The vMP2 neuron projects a single axon anteriorly, while dMP2 sends out a single axon into the ipsilateral connective where it bifurcates into an anterior and posterior branch (Figure 1A and A'). Mutations in *N* cause a transformation of vMP2 into dMP2 (Spana and Doe, 1996). The same phenotype was seen in transplanted *N^{-/-}* MP2s (Figure 1B and B'). Loss-of-function mutations in the cell-fate determinant Numb cause the reverse transformation dMP2>vMP2, as Numb apparently antagonises *N* signalling directly by protein–protein interactions (Spana and Doe, 1996). Thus, one might expect mutations in *N* and *insc* to be similar with respect to terminal phenotypes of sibling neurons, if *insc* were to be responsible for the asymmetric localisation and segregation of Numb.

We analysed 11 MP2 clones, which were derived from *insc* mutant neuroectodermal cells, and all the clones were WT with respect to cell positioning and axonal projections (Figure 1C and C'). This finding was further substantiated using molecular markers. Odd-skipped (*Odd*) is expressed in the MP2 precursor and the newly born dMP2 and vMP2 neurons. Expression is extinguished in vMP2 at stage 11, but maintained in dMP2. *Odd* is also expressed in the midline lineage, MP1. The axonal marker 22C10 also labels both MP2 progeny, but not MP1. The enhancer trap line AJ96 expresses β -Gal strongly throughout the

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Table 1. Clonal types from the ventral neuroectoderm (0–20% VD) obtained by transplanting *insc* mutant neuroectodermal cells

Clone	No.	Phenotype (with respect to WT and <i>Notch</i> mutants)
NB1-1	9	Partially transformed
NB4-2	6	Partially transformed
NB7-1	15	Partially transformed
MP2	11	Wild type
NB1-2	8	Wild type
NB3-2	8	Wild type
NB4-1	3	Wild type
NB5-2	13	Wild type
NB7-2	7	Wild type

Shown above are a subset of the ventral NB clones obtained by transplanting *insc* mutant neuroectodermal cells. NB clones derived from the dorsal neuroectoderm (20–50% VD) and midline clones as well as neuroblast lineages derived from the ventral ectoderm for which less than three independent clones were obtained were not included in the analysis. The phenotypes indicated have been benchmarked against corresponding WT lineages and, in the case of NB1-1, NB4-2 and NB7-1 clones, also against *N* mutant lineages. Wild type refers to clones that are indistinguishable from their WT counterparts. Partially transformed refers to clones that are clearly not WT but in which the mutant morphology is not as severe as those of their corresponding *N* mutant lineages. VD, ventro-dorsal diameter.

MP2 lineage (Menne and Klambt, 1994). In stage 13–14 *insc*²², AJ96 embryos, we found an essentially WT staining pattern of one Odd⁺ and β-Gal[−] cell (MP1) and one Odd and β-Gal double-positive cell (dMP2) in all the analysed hemisegments (*n* = 500; Figure 2D–F). Using mAb22C10 (data not shown), we observed the WT pattern of anterior dMP2 projections and posterior vMP2 projections in all analysed *insc*²² hemisegments (*n* = 110). We confirmed these findings with another allele, *insc*^{P49}. In addition, we removed possible maternal (and zygotic) component(s) of *insc* using the FLP-FRT/ovo^D technique (Chou and Perrimon, 1996). All hemisegments (*n* = 260) analysed showed a WT phenotype (data not shown), ruling out the involvement of an *insc* maternal component. These data show that the resolution of distinct dMP2 and vMP2 fates does not require *insc*.

As the MP2 asymmetric divisions are unaffected by *insc* loss of function, we asked whether *Insc* is expressed in the MP2 progenitor in WT embryos. We stained homozygous AJ96 embryos with anti-*Insc* and anti-β-Gal, and detected apically localised crescents of *Insc* at mitosis (*n* = 20; Figure 3A). The mitotic MP2 precursor was identified by diffused cytoplasmic AJ96 β-Gal staining and DNA stain. In addition, we checked the expression patterns of several proteins known to be asymmetrically localised during NB mitosis. *Baz* and *Pins* are expressed as apical crescents in the dividing MP2 in a manner similar to *Insc* (Figure 3B and C). *Numb* (Uemura et al., 1989; Rhyu et al., 1994) and *Partner of Numb* (*Pon*) (Lu et al., 1998) are asymmetrically localised to the basal cortex of the MP2 precursor (data not shown; Figure 3D). *Prospero* (*Pros*) protein is nuclear in the MP2 precursor and equally distributed to both the progeny after division (Skeath and Doe, 1996). This is in contrast to typical NBs, in which *Pros* localisation is basal; only in the ganglion mother cell (GMC) is *Pros* released from the cortex to assume a

Fig. 2. *baz* is required for dMP2 and vMP2 cell-fate determination. Confocal images of WT and mutant embryos (stage 14) in AJ96 background double labelled with anti-β-Gal (green) and anti-Odd (red), and superimposed images. In WT (A–C), *insc* (D–F) and *pins* (G–I) embryos, both vMP2 (arrowhead) and dMP2 (arrow) are β-Gal positive (green); anti-Odd also labels dMP2 (red) and MP1 (red arrow). In *baz* RNAi (J–L) embryos, the anti-β-Gal pattern remains unchanged but both progeny cells of MP2 are Odd positive (red), suggesting a vMP2>dMP2 cell-fate transformation. There are additional Odd-positive cells, but these are AJ96 negative and are probably supernumerary MP1s. One segment of embryos is shown in all panels. Anterior is towards the top.

nuclear localisation (Hirata et al., 1995; Knoblich et al., 1995). Lastly, we checked the localisation of *Miranda*, which is required for the basal localisation of *Pros* in NBs (Ikeshima-Kataoka et al., 1997; Shen et al., 1997; Schuldt et al., 1998). We could not detect *Miranda* immunoreactivity at any stage in MP2 (data not shown).

We next examined the localisation of *Pon/Numb* in dividing MP2s of *insc* mutant embryos. We found that *Pon* and *Numb* remain asymmetrically localised to the basal cortex of dividing *insc* mutant MP2s (*n* = 19; Figure 3H; data not shown). Moreover, the spindle is oriented largely within 45° of the A/B axis, as in WT (33/37). These findings, together with the transplantation results, indicate that although *Insc* is expressed and asymmetrically localised in MP2, it is not required either for *Pon/Numb* asymmetric localisation or for the resolution of distinct dMP2/vMP2 fates.

Two obvious candidate molecules that might be responsible for mediating the MP2 asymmetric division are *Baz* and *Partner of Inscuteable* (*Pins*) (Parmentier et al., 2000; Schaefer et al., 2000; Yu et al., 2000). *pins* appears not to be a major player (Figure 2G–I) since, in embryos lacking both maternal and zygotic *pins*, only 5.7% of the hemisegments show dMP2 duplication (*n* = 140), as demonstrated by three odd-positive cells (data not shown). Assessing the role of *baz* was problematic since loss of zygotic function had no effect, and removing both the maternal and zygotic *baz* resulted in embryos with severe morphological defects that prevent scoring of dMP2 and vMP2

Fig. 3. Localisation of proteins asymmetrically localised in NBs in dividing MP2. Superimposed confocal images showing Insc (red), Baz (red), Pins (red) and Pon (red) in dividing MP2 (green); DNA is blue. In WT embryos, Insc (A), Baz (B) and Pins (C) are localised to the apical cortex of MP2, while Pon forms a basal crescent (D). In *insc* embryos, although the signal intensity is weaker than WT, Baz (F) and Pins (G) apical localisation is not affected. Localisation of both Insc and Pins in metaphase MP2 of *baz* (RNAi) embryos remains apical (I and K). Asymmetric localisation of Pon in MP2 is defective and becomes cortical in *baz* (RNAi) embryos (L). In *pins* embryos, Insc is cytoplasmic (M), as seen in other neuroblasts; Baz apical crescent is rather weak, sometimes undetectable (N). In *insc* and *pins* mutants, the basal localisation of Pon is not affected (H and P).

fates in older embryos. We circumvented these problems by performing RNAi (Kennerdell and Carthew, 1998) with *baz* double-stranded RNA on AJ96 embryos (see Figure 2 legend), which yielded ~25% embryos with reduced Baz protein but without the severe morphological defects that prevented scoring of vMP2 and dMP2 identities. In such embryos, vMP2>dMP2 transformations are observed in the great majority of hemisegments (57/60), as demonstrated by the presence of two cells double positive for β -Gal and Odd (Figure 2J–L). Moreover, localisation of Pon (Figure 3L) and Numb (data not shown) becomes cortical in dividing MP2 (38/40). However, there does not appear to be a dramatic defect on the orientation of the cell division, with almost all of the MP2 divisions oriented within 45° of the A/B axis (32/34). These defects associated with *baz* RNAi

cannot merely be due to secondary effects associated with a disruption of the epithelium since, in *crumbs* loss of function, Baz (12/12) and Pon (10/10) remain correctly localised to the MP2 apical and basal cortex, respectively. These results indicate that Baz is required for the asymmetric localisation of Pon and Numb in the MP2 asymmetric division.

Although apical complex members, like Baz, Insc and Pins, are expressed and apically localised in both MP2 and NBs, their behaviour appears to differ somewhat in the two cellular contexts. For example, when *baz* function is attenuated, Insc is in the cytosol of NBs whilst Insc remains localised as an apical crescent in most dividing MP2 cells (24/32; Figure 3I); similar results are seen in dividing MP2s of embryos derived from *baz*^{Xi106} germline clones, which lack both maternal and zygotic

function (data not shown). Moreover, it is interesting to note that in the absence of *insc* function, Baz (22/22) and Pins (20/20) can be localised to the apical cortex of metaphase MP2s (Figure 3F and G), although the intensity of staining is always reduced compared with WT MP2s, and in some cases the weak apical crescent of Baz can be difficult to detect. These observations suggest that even a small amount of apically localised Baz is sufficient to mediate basal localisation of Pon and direct the MP2 asymmetric division. Strikingly, Baz (Figure 3L), but not Insc (Figure 3H) and Pins (Figure 3P), seems to play a dominant role in mediating Pon/Numb basal localisation in MP2. When *baz* function is attenuated, Pon/Numb become cortically localised (Figure 3L; data not shown) even though Insc and Pins can remain apically localised (Figure 3I and K). These observations indicate that the precise requirements for asymmetric protein localisation differ between MP2s and NBs.

MP2 appears to be the only known A/B-oriented asymmetric division that does not require *insc*. Although MP2 delaminates from the neuroectoderm and divides in an apico-basal fashion like NBs, there are unique features that set MP2 apart from other neuroblasts. Unlike NBs that divide in a stem-cell-like mode, MP2 undergoes one differentiative division, making it more like a GMC or a pIIb division. Insc is present in both GMC/SOP lineages. A/B-oriented asymmetric GMC divisions, like those of GMC4-2a, require *insc* (Buescher et al., 1998). While the first SOP division (the anterior–posterior pl > pIIa + pIIb) does not require *insc*, recent work has shown that the spindle orientation of the strikingly GMC-like A/B division of the pIIb cell is dependent on Insc (Roegiers et al., 2001b). Finally, unlike NBs, Pros shows nuclear localisation in MP2 (Skeath and Doe, 1996). There is evidence supporting the view that Pros acts to terminate cell proliferation during *Drosophila* neurogenesis (Li and Vaessin, 2000). It is plausible that both GMCs and the MP2 precursor use nuclear Pros as a cue to reduce their mitotic potential and undergo a single differentiative division. Two recent reports have shown that planar asymmetric divisions undertaken by pl in the peripheral nervous system (Bellaiche et al., 2001; Roegiers et al., 2001a) and epithelial cells with disrupted adherens junctions (Lu et al., 2001) both require *baz* and not *insc*. We demonstrate here that *insc* is not required for all A/B-oriented asymmetric divisions. Our findings further support the view that *baz* is a more general mediator of asymmetric divisions than *insc*, and can act to promote both A/B and planar asymmetric divisions in the absence of *insc*.

METHODS

Transplantations. The transplantation procedure and subsequent handling of the recipient embryos were performed as described previously (Prokop and Technau, 1993). The genotype of the donor embryos was determined by staining each donor embryo for the presence/absence of a Blue-balancer chromosome.

RNAi experiments. RNAi experiments were carried out essentially as described (Kennerdell and Carthew, 1998). A 0.7 kb *Pst*I fragment spanning nucleotides 3618–4343 of *baz* cDNA was used as the template for double-stranded RNA synthesis. Approximately 50–100 pl of dsRNA solution (~1 mg/ml) were injected into each embryo. For the purposes of determining resolution of dMP2 and vMP2 cell fates, injected embryos were aged to stages 13–14 prior to the fixation and immunostaining; for the

purposes of determining protein localisation in dividing MP2, injected embryos were aged at 25°C for 6.5 h before fixation and immunostaining.

Immunohistochemistry and image collection. Primary rabbit antibodies used for immunostaining included anti-Insc (1:1000), anti-Pins (1:1000), anti-Baz (1:1000, a gift from F. Matsuzaki), anti-Pon (1:1000, a gift from Y.N. Jan) and rabbit anti-Odd (1:1000, a gift from Jim Skeath). Mouse anti-β-Gal (1:200) was obtained from Promega, and Cy3 and FITC-conjugated secondary antibodies were obtained from Jackson Laboratories. The images were collected with a Bio-Rad laser confocal microscope (MRC 1024) and processed with Adobe Photoshop.

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REFERENCES

- Bellaiche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O’Kane, C.J., Bryant, P.J. and Schweisguth, F. (2001) The Partner of Inscuteable/Discs-large complex is required to establish planar polarity during asymmetric cell division in *Drosophila*. *Cell*, **106**, 355–366.
- Bossing, T., Udolph, G., Doe, C.Q. and Technau, G.M. (1996) The embryonic central nervous system lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. *Dev. Biol.*, **179**, 41–64.
- Buescher, M., Yeo, S.L., Udolph, G., Zavortink, M., Yang, X., Tear, G. and Chia, W. (1998) Binary sibling neuronal cell fate decisions in the *Drosophila* embryonic central nervous system are nonstochastic and require inscuteable-mediated asymmetry of ganglion mother cells. *Genes Dev.*, **12**, 1858–1870.
- Chou, T.B. and Perrimon, N. (1996) The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. *Genetics*, **144**, 1673–1679.
- Hirata, J., Nakagoshi, H., Nabeshima, Y. and Matsuzaki, F. (1995) Asymmetric segregation of the homeodomain protein Prospero during *Drosophila* development. *Nature*, **377**, 627–630.
- 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**, 625–629.
- Kennerdell, J.R. and Carthew, R.W. (1998) Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell*, **95**, 1017–1026.
- Knoblich, J.A., Jan, L.Y. and Jan, Y.N. (1995) Asymmetric segregation of Numb and Prospero during cell division. *Nature*, **377**, 624–627.
- 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, L. and Vaessin, H. (2000) Pan-neural Prospero terminates cell proliferation during *Drosophila* neurogenesis. *Genes Dev.*, **14**, 147–151.
- Lu, B., Rothenberg, M., Jan, L.Y. and Jan, Y.N. (1998) Partner of Numb localizes with Numb during mitosis and directs Numb asymmetric localization in *Drosophila* neural and muscle progenitors. *Cell*, **95**, 225–235.

- Lu, B., Roegiers, F., Jan, L.Y. and Jan, Y.N. (2001) Adherens junctions inhibit asymmetric division in the *Drosophila* epithelium. *Nature*, **409**, 522–525.
- Menne, T.V. and Klambt, C. (1994) The formation of commissures in the *Drosophila* CNS depends on the midline cells and on the Notch gene. *Development*, **120**, 123–133.
- Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P. and O’Kane, C.J. (2000) Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in *Drosophila*. *J. Neurosci.*, **20**, RC84.
- Prokop, A. and Technau, G. (1993) Cell transplantation. In Hartley, E.D. (ed.), *Cellular Interactions in Development: A Practical Approach*. Oxford University Press, Oxford, UK, pp. 33–57.
- Rhyu, M.S., Jan, L.Y. and Jan, Y.N. (1994) Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell*, **76**, 477–491.
- Roegiers, F., Younger-Shepherd, S., Jan, L.Y. and Jan, Y.N. (2001a) Bazooka is required for localization of determinants and controlling proliferation in the sensory organ precursor cell lineage in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **98**, 14469–14474.
- Roegiers, F., Younger-Shepherd, S., Jan, L.Y. and Jan, Y.N. (2001b) Two types of asymmetric divisions in the *Drosophila* sensory organ precursor cell lineage. *Nat. Cell Biol.*, **3**, 58–67.
- Schaefer, M., Shevchenko, A. and Knoblich, J.A. (2000) A protein complex containing Inscuteable and the G α -binding protein Pins orients asymmetric cell divisions in *Drosophila*. *Curr. Biol.*, **10**, 353–362.
- Schmid, A., Chiba, A. and Doe, C.Q. (1999) Clonal analysis of *Drosophila* embryonic neuroblasts: neural cell types, axon projections and muscle targets. *Development*, **126**, 4653–4689.
- Schober, M., Schaefer, M. and Knoblich, J.A. (1999) Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. *Nature*, **402**, 548–551.
- Schuldt, A.J., Adams, J.H., Davidson, C.M., Micklem, D.R., Haseloff, J., St Johnston, D. and Brand, A.H. (1998) Miranda mediates asymmetric protein and RNA localization in the developing nervous system. *Genes Dev.*, **12**, 1847–1857.
- 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. (1996) The achaete–scute complex proneural genes contribute to neural precursor specification in the *Drosophila* CNS. *Curr. Biol.*, **6**, 1146–1152.
- Spana, E.P. and Doe, C.Q. (1996) Numb antagonizes Notch signaling to specify sibling neuron cell fates. *Neuron*, **17**, 21–26.
- 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.
- Uemura, T., Shepherd, 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.
- Wodarz, A., Ramrath, A., Kuchinke, U. and Knust, E. (1999) Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. *Nature*, **402**, 544–547.
- Yu, F., Morin, X., Cai, Y., Yang, X. and Chia, W. (2000) Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. *Cell*, **100**, 399–409.

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