Asymmetric localization and function of cell-fate determinants: a fly’s view
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One mechanism to generate daughter cells with distinct fates is the asymmetric inheritance of regulatory proteins, leading to differential gene regulation in the daughter cells. This mode of cell division is termed ‘asymmetric cell division.’ The nervous system of the fly employs asymmetric cell division, both in the central nervous system, to generate neural precursors, neurons and glial cells; and in the peripheral nervous system, to create sensory organs that are composed of multiple cell types. These cell lineages are excellent models to examine the gene expression program that leads to fate acquisition, the cell-fate determinants that control these programs and how these determinants, in turn, are distributed through cell polarity machinery.

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Introduction
Diverse organisms, from bacteria to humans, have evolved means by which to create multiple cell types through a mitotic cell division. One method of generating sister cells with distinct identities is the asymmetric segregation of cell fate determinants, resulting in an ‘asymmetric cell division’ [1]. The nervous system of the fruit fly has been an invaluable model for understanding the mechanisms underlying asymmetric cell division. This review focuses on the mechanisms by which cell fates are acquired within the neuroblast lineage of the central nervous system (CNS), as well as the sensory organ precursor lineage of the peripheral nervous system (PNS) in Drosophila melanogaster. In addition, the possible function of vertebrate homologs of cell fate determinants in flies is discussed.

Within the nervous system of D. melanogaster, the neuroblast (NB) and sensory organ precursors (SOP) undergo asymmetric cell division. Embryonic neuroblasts (NBs) delaminate basally from the surface epithelium and then undergo an asymmetric cell division, giving rise to a smaller, basal ganglion mother cell (GMC) and a larger, apical NB. NBs continue to divide in this manner, while each GMC divides once, asymmetrically, to form two different daughter neurons (or two glial cells, in some lineages). Thus, both NB and its daughter, the GMC, undergo an asymmetric cell division (Figure 1a). The adult bristle sensory organs are also produced by asymmetric cell divisions (Figure 1b). The sensory organ precursor (SOP), or pI cell, divides within the plane of the epithelium, yielding a posterior pIa cell and an anterior pIb cell. The pIa cell divides to form the posterior socket cell and the anterior hair cell, whereas, the pIb cell produces the neuron, the sheath cell and a glial cell that is eliminated via apoptosis (Figure 1b) [2–4]. The ability to create distinct daughter cells is crucial, both for maintaining the stem-cell-like identity of the NB in the NB lineage and for generating a complete sensory organ, composed of multiple cell types that are derived from the SOP.

The generation of daughter cells with different cell fates involves three levels of control (Figure 1c): (i) regulated programs of gene expression, controlling cell fate; (ii) unequally-segregated fate determinants that, in turn, regulate programs of gene expression in only one of the two daughter cells; (iii) cell polarity machinery that coordinates asymmetric localization of fate determinants, with respect to the cell division plane. This review discusses current knowledge about each of these three processes in both the NB and SOP asymmetric cell divisions in Drosophila. We also review current knowledge of possible asymmetric cell divisions within the developing CNS in vertebrates. The large amount of data concerning the homologous cell polarity machinery in Caenorhabditis elegans is not discussed, as it is beyond the scope of this review (for a review, see [5–7]).

Specifying distinct daughter cell fates
Several mechanisms can enable one daughter cell to activate a program of gene expression that is required...
for a particular cell fate, while the other daughter cell does not. The NB division accomplishes this through asymmetric inheritance of the transcription factor Prospero (Pros) (Figure 1a). Division of the SOP, however, uses a different mechanism that enables the activation of the transcription factor Suppressor of Hairless (Su(H)) in one daughter cell and the translational repression of the transcription factor Tramtrack69 in the other daughter cell.

Asymmetric transcription factor segregation
Pros is a homeodomain transcription factor that accumulates in the nuclei of GMC, but not NB. In pros mutant embryos, GMCs fail to express normal GMC marker proteins (such as Even-skipped) and their progeny neurons display axonal defects [8–12], indicating that Pros regulates the GMC-specific program of gene expression. Differential expression of target genes is controlled through the asymmetric segregation of Pros to daughter cells during NB division [10–12]; however, the direct transcriptional targets of Pros that are required for GMC cell fate are not well characterized.

Asymmetric transcription complex assembly
Cell fate within the SOP lineage is, primarily, regulated through the asymmetric control of transcriptional complexes, bound to DNA via Su(H). Su(H) is a DNA-binding protein that associates with either co-repressor or co-activator complexes. The processed form of the transmembrane receptor Notch (N) is a member of a potent co-activator complex for Su(H). Following the SOP division, N is activated in the posterior cell by
The determinants of cell fate
Having discussed the downstream events allowing binary cell fate decisions through asymmetric control of transcriptional programs, we now focus on the upstream events that generate the asymmetry. In the NB division, localization of Pros into GMCs is dependent on unequal segregation of adaptor molecules. In the SOP, cell-to-cell N signaling is directionally biased by regulatory proteins that either inhibit (Numb) or promote (Neuralized) N signaling.

Prospero and its regulation in the NB division
Asymmetric segregation of Pros is the result of two redundant mechanisms: pros mRNA localization through the Staufen RNA binding protein [20, 21], and localization of Pros protein through its anchor protein, Miranda [10–12, 22, 23]. In contrast to Pros protein localization, that of pros mRNA does not play an essential role in cell fate determination, except when Pros protein is limiting [20, 21]. In addition to localizing Pros protein, Miranda regulates pros mRNA localization by anchoring Staufen to the basal cortex of the dividing NB [24–26]. Null alleles of miranda result in symmetric segregation of Pros and pros mRNA into daughter cells and, therefore, inappropriate GMC cell-fate specification [22, 25].

Regulation of Su(H)-dependent transcription in the SOP
In the SOP lineage, activation of N switches on Su(H)-dependent transcriptional programs. How, then, does N-signaling occur in one daughter cell but not in the other? To date, two proteins are known to enable ‘directional’ N-signaling, ensuring that it occurs only in the pIIa cell and not in the pIIb cell: Numb and Neuralized.

Numb
Loss of numb function results in transformation of the pIIb cell into a second pIIa cell. During the pI division, Numb protein segregates preferentially to the anterior daughter cell, which, therefore, adopts the pIIa fate [27]. In the embryo, Numb localization is, in part, dependent on an interacting protein; ‘Partner of Numb’ (Pon) [28]. Numb inhibits N receptor signaling in the anterior daughter cell, whereas NICD activates Su(H) in the posterior daughter cell [29–31]. Numb also segregates in an asymmetric manner in the NB lineage [27, 32]; however, within most NB lineages, loss of numb function does not effect the GMC fate, but plays a role in specifying neuron identity at the GMC division [27, 33–35]. An exception to this is the MP2 NB lineage, which undergoes a single, terminal division that is regulated by Numb and N signaling [30, 32].
Recent work has begun to elucidate the way that Numb impinges on N signaling. Berdnik et al. [36,37] have found that the z-adaptin, a protein involved in endocytosis, associates with Numb and is asymmetrically segregated to the pIIb cell in a numb-dependent manner (Figure 2). Given the ability of Numb to associate with the NICD [31,38,39], Numb may downregulate the N receptor through z-adaptin-dependent endocytosis (Figure 2) [37]. This model predicts that Notch would be endocytosed specifically in the pIIb cell; but this has not yet been demonstrated. Recently, a second model has been proposed for Numb function. Sanpodo (Spdo) is a transmembrane protein that is specifically expressed in asymmetrically dividing cells and co-immunoprecipitates with Numb and Notch. Genetic analysis indicates that spdo is required for N signaling, generating two neurons during GMC cell divisions. spdo acts downstream of Delta and antagonistically to numb. Strikingly, the subcellular localization of Spdo appears to be regulated by Numb. Spdo is found at the cell surface in the absence of Numb (i.e. in the cells that do not inherit Numb or in numb-mutant cells), whereas, in the presence of Numb (i.e. in the cells that inherit Numb), Spdo accumulates in uncharacterized intracellular structures that might correspond to endocytic compartments [40–42]. This would, therefore, suggest that one function of Numb is to inhibit Spdo activity by mediating its endocytosis, and that Spdo cell surface membrane localization is essential for N signaling. Whether Numb regulates the localization of Numb signaling components other than Spdo is, at present, unclear, as is the mechanism by which Spdo acts to aid N signaling.

Neuralized
Regulation of N-signaling within the SOP is also controlled by Neuralized (Neur), which is essential for specification of the pIa cell [41,42,43–47]. Neur is a ring-finger containing E3 ubiquitin ligase that targets DI for endocytosis [44–47]. Similar to Numb, Neur is asymmetrically segregated to the pIIb cell during the pI division, suggesting that Neur acts in the pIIb cell to promote N-signaling in the pIa cell (Figure 2). Clonal analysis has, indeed, demonstrated a non-autonomous role for neur in promoting DI endocytosis and N-signaling [43–46], although a cell-autonomous role in DI endocytosis has also been proposed [41,42]. By what mechanism DI endocytosis promotes N signaling is not yet understood. Additionally, a direct cortical anchor of Neur has yet to be identified. It will be of interest to determine whether Neur is similarly asymmetrically localized in the NB lineage and regulates cell fate therein.

Through their asymmetric inheritance, Pros, Numb and Neur, thus enable acquisition of distinct daughter fates. We now examine how the localization of these regulators and their anchors is coordinated, by cell polarity machinery, with the cell division axis.

Segregating cell fate determinants
Despite the different downstream events regulated by Numb, Neur, and Pros, conserved mechanisms exist that co-regulate their localization and link it to spindle orientation, allowing asymmetry to be generated upon cell division. The emerging theme is that the coordinated effort of several protein complexes and the actin cytoskeleton leads to the establishment of cortical domains that are crucial for segregation of cell fate determinants, spindle alignment and daughter cell size.

The apical complex of the NB
Within the NB, two conserved protein subcomplexes act together with the actin cytoskeleton to divide the cell cortex into apical and a basal domains. One of the subcomplexes is composed of the GoLoCo motif-containing protein, ‘Partner of Insuteable’ (Pins) and its associated G-protein z subunit (Gzzi) [48–51]. A second subcomplex consists of the atypical protein kinase C (DaPKC) and two PDZ (PSD95/Discs large/ZO1 domain)-containing proteins, DmPar6 and Bazooka (Baz) [52–55] (see [7] for review). An adaptor protein, Insuteable (Insc), forms the ‘apical complex’ by linking these two subcomplexes. The apical localization, and in many cases, the stability of each member of this complex is interdependent on the other ‘apical complex’ members in the NB. The G-protein z and y complex (Gzβγ), which competes with Pins for Gzzi binding, is also essential for apical complex localization and stability [51,56]. The loss of any component of the apical complex results in loss of metaphase localization of the Pon-Numb and Miranda-Pros crescents to the basal cortex, although crescent formation can occur later in telophase, through an unknown mechanism of ‘telophase rescue’. The apical complex also coordinates the alignment of the mitotic spindle with cell fate determinants and regulates spindle asymmetry, thereby controlling daughter cell size [11,49,50,51,52–54,56–58].

Lgl as a target of DaPKC
Elegant studies by Betschinger and co-workers [59–61], recently, have shed light on the mechanism used by the apical complex to direct localization of cell-fate regulators to the basal cortex (Figure 3). This mechanism involves lethal(2) giant larvae (lgl), a gene required for asymmetric segregation of cell-fate determinants [60,61]. Lgl interacts with DaPKC and DmPar6, and is a direct phosphorylation target of DaPKC [59–61]. In NBs, Lgl localizes at the cortex and its cortical localization depends on Discs-large (Dllg), a protein that is also required for the basal localization of cell-fate determinants [60,61]. In cells expressing either a version of DaPKC that is no longer restricted to the apical cortex, or a non-phosphorylatable Lgl, Miranda is no longer restricted to the basal cortex but is either cytoplasmic (former) or found around the entire cell cortex (latter). These results have led to a model, whereby, phosphorylation of Lgl by DaPKC at the apical cortex inactivates...
Regulation of Lgl and basal component localization in the NB. Recent work has suggested a model for the way that apically localized components lead to basal cell-fate regulator localization. aPKC phosphorylates and inactivates Lgl at the apical surface (where aPKC is localized with other apical complex components), thus, resulting in asymmetric Lgl activity at the basal cortex [59**]. Lgl, in turn, negatively regulates the localization of Myosin II, resulting in MyoII localization to the apical cortex [62**]. Cortical MyoII (presumably myosin filaments) is involved in exocytic trafficking [64]. The function of the actin cytoskeleton in localizing cell-fate determinants is not well understood. First, actin-dependent myosin motors might transport cell-fate determinant to the basal cortex. For instance, the myosin VI Jaguar has been shown to co-immunoprecipitate with Miranda and to be required for Miranda crescent formation, raising the possibility that myosin VI-dependent transport localizes Miranda to the basal cortex [68]. Consistent with this hypothesis, treatment of cells with the myosin motor inhibitor butanedione-2-monoxime (BDM) abolishes crescent formation of Pon, Miranda and Neur [28,43**,65–67]. The function of the actin cytoskeleton in localizing cell-fate determinants is not well understood. First, actin-dependent myosin motors might transport cell-fate determinant to the basal cortex.

**Role of the actin cytoskeleton**

Disruption of cytoskeletal structures, through drug treatments, has defined the requirements for cortical localization of the cell-fate determinants. The localization of Numb, Pon, Neur, Pros and Miranda, during mitosis into basal (NB) or anterior crescents (SOP), is dependent on the actin cytoskeleton, but independent of polymerized microtubules [25,28,43**,65–67]. The function of the actin cytoskeleton in localizing cell-fate determinants is not well understood. First, actin-dependent myosin motors might transport cell-fate determinant to the basal cortex. For instance, the myosin VI Jaguar has been shown to co-immunoprecipitate with Miranda and to be required for Miranda crescent formation, raising the possibility that myosin VI-dependent transport localizes Miranda to the basal cortex [68]. Consistent with this hypothesis, treatment of cells with the myosin motor inhibitor butanedione-2-monoxime (BDM) abolishes crescent formation of Pon, Miranda and Neur [28,43**,65–67]. The function of the actin cytoskeleton in localizing cell-fate determinants is not well understood. First, actin-dependent myosin motors might transport cell-fate determinant to the basal cortex.

**Regulation of cortical domains within the SOP**

In opposition to the NB, the SOP cell divides planar to the epithelium and establishes separate cortical domains of Pins–Gzi (at the anterior cortex) and Baz–DaPKC–DmPar6 (at the posterior cortex) [51,71]. Localization of these two complexes in separate domains is dependent on the lack of insc expression in the SOP [71]. Pon–Numb co-localizes with Pins–Gzi at the anterior cortex [27,51,71]. Interestingly, Dlg binds to Pins and is co-localized to the anterior cortex [71]. Dlg–Pins–Gzi and Baz, as in the NB, are required for proper localization of Numb [51,71]; likewise, segregation of Neur to the pIIb is also dependent on Dlg and Pins [43**]. The role played by Lgl in the SOP for asymmetric segregation of Numb and cell fate is somewhat controversial. Ohshiro and co-workers [60] have reported that, as in the NB, lglts3 mutant SOPs possess delocalized Numb crescents, as well as bristle phenotypes, consistent with a pIIa-to-pIIb cell fate transformation that might be associated with Numb mis-
segregation. Justice et al. [72] have recently reported a contrasting result. In mitotic clones of a complete loss of function \(lg\) allele, Numb crescent formation at the anterior cortex is unperturbed. Additionally, pIIb-to-pIIa cell fate transformations were observed and genetic studies suggest that Lgl acts upstream of NICD in the pIIa vs pIIb fate decision and, in contrast to the NB, downstream or in parallel to numb. The mechanism of Lgl action is an important issue to resolve, to understand how the cell-fate determinants are localized within the SOP.

**Unequal segregation of Numb and asymmetric cell division in Vertebrates**

The fundamental importance of asymmetric cell division in the development of the nervous system in invertebrates raises the question of whether unequal segregation of fate determinants also contributes to the generation of various cell types in the vertebrate nervous system. The molecular conservation of the fate determinants Numb and Neur is consistent with this suggestion [38,73–76]. By contrast, no Miranda homologs have been identified in sequenced vertebrate genomes, and there is no evidence for unequal distribution of Pros homologs at mitosis. The identification of Neur as a cell-fate determinant in the fly is too recent to have permitted an analysis of the role of Neur homologs in asymmetric divisions in vertebrates; but various studies have examined the possible role of Numb in asymmetric cell division.

In the developing cortex of mammals, neuroepithelial cells are polarized along their apical–basal axis and have been shown to divide parallel (horizontally), perpendicular (vertically) or with an oblique angle, relative to the lumen of the tube. Results from live imaging analyses suggested that horizontal divisions of progenitor cells were proliferative (symmetrical), thus, augmenting the pool of neural progenitor cells, whereas, vertical divisions were differentiative (asymmetrical), generating one apical cell that remained in the proliferative zone and one basal cell that behaved as a newly born neuron [77]. A recent double knockout analysis of numb and numb-like mutant mouse embryos has indicated that Numb and Numb-like play an essential (yet partly redundant) role in maintaining neural progenitor cell populations [78]. Mouse Numb has been shown to localize at the apical cortex of dividing neuroepithelial cells, although Numb has also been suggested to preferentially accumulate into the neuronal daughter (i.e. the presumed ‘basal’ daughter [79]), bind mouse Notch and inhibit Notch signaling [38,80]. Therefore, a model is suggested where orientation of the division regulates the equal versus unequal partitioning of Numb, hence the fate asymmetry in the choice between neural progenitor and neuron. Consistent with this model, vertical divisions have been observed in fixed tissue studies of chick and rat retina [81,82], and chick cortex [39]; furthermore, unequal partitioning of Numb is correlated with a fate difference, following \(ex \ v i c o\) divisions of isolated progenitor cells from mouse cortex [79]. Finally, a recent time-lapse analysis of rat retina explants has suggested that vertical divisions tend to produce cells with distinct identities, while horizontal divisions exhibit the opposite tendency [83].

Despite these exciting observations, direct \(i n \ v i c o\) evidence that unequal segregation of Numb in vertical division regulates a fate difference between the two daughter cells is still lacking. And more significantly, this simple model has been challenged by the following observations. First, the number of vertical divisions throughout neurogenesis appears to be too low to account for the large number of emerging neurons [77,81]. Second, no correlation was observed between the orientation of the division, Numb segregation and the expression of an early neural marker in the chick retina [82]. Third, a recent time-lapse analysis of divisions in living zebrafish embryos has indicated that most neurons are born from divisions that produce two neurons and that no correlation exists between the apical–basal orientation of the division and the fate of the daughter cells [84]. To what extent these contradictory conclusions reflect differences in model organisms, regional identity within the nervous system, experimental approaches and/or marker used, remains to be examined. Additionally, interpretation of some of the published data is complicated by two confounding points. First, as initially pointed out by Huttner and Brand in 1997 [85], unequal segregation of apical Numb might also result from oblique cleavage in which the apical domain is unequally bisected. Thus, divisions that are described as horizontal could be asymmetric, in terms of Numb partitioning. Second, the axis of polarity used for unequal partitioning of Numb (or other fate determinants) is not necessarily the apical–basal axis. For instance, the polarization machinery uses the anterior–posterior polarity of the SOP to localize Numb and Neur at the anterior pole. Interestingly, a recent \(i n \ v i c o\) imaging analysis of division in the zebrafish retina has suggested that the orientation of progenitor cell divisions is regulated by planar central–peripheral polarity cues [86]. This study nicely illustrates the importance of green fluorescent protein (GFP) imaging in the study of asymmetric cell division. Clearly, combining live imaging in whole organisms and tissue explants with GFP probes, revealing both orientation of division and, most importantly, unequal partitioning of fate determinants, should help to establish the role of Numb unequal partitioning in generating cell-fate diversity.

**Perspectives**

During the past ten years, genetic studies of asymmetric cell division in *Drosophila* have dramatically increased our understanding of the molecular basis of asymmetric cell division. This review has discussed several mechanisms by which distinct daughter cell fates arise in the nervous system of *Drosophila*. Although the role of vertebrate...
homologs of fate determinants in flies is either debated (Numb) or unknown (Neur), Drosophila will certainly continue to contribute to our understanding. Nevertheless, despite the importance of Drosophila as a model system, we must bear in mind that acquisition of distinct cell fates during asymmetric cell division might be acquired through mechanisms other than those that have been identified, so far, in the developing Drosophila nervous system. For instance, microtubule-based directional transport [87] and/or asymmetric mRNA/protein degradation [88] have been shown to contribute to unequal partitioning of fate determinants in molluscs and worms. Future studies on asymmetric cell divisions in various systems, including vertebrates, should further our understanding of how stem cells are maintained, how distinct cell types arise and differentiate, and how these processes can become deregulated, leading to pathologies such as cancers.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


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58. Fuse N, Hisata K, Katzen AL, Matsuoka F: Heterotrimeric G proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions. Curr Biol 2003, 13:947-954. Together, these four elegant studies establish that the activity of the Gaii and Gji13F genes is essential for the basal localization of cell-fate determinants in dividing NBs and SOPs. The G0-bound form of Gai binds to Pins, and Gai is shown to localize to the apical cortex of NBs. Moreover, loss-of-function alleles of Gai give similar phenotypes to loss of pins function. Furthermore, the subcomplexes Gai–Pins and Baz–Par6–aPKC each act redundantly in NBs to regulate the geometry of the mitotic spindle, hence the daughter cell-size difference. Consistent with this, daughter cells have equal size in the Gii13F mutant embryos and Gji13F is required for apical localization of the Gai–Pins and Baz–Par6–aPKC subcomplexes.

59. Betschinger J, Mechtler K, Knoblich JA: The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. Nature 2003, 242:326-330. In this study, Lgl is found to co-immunoprecipitate in a complex with Par6 and aPKC, but not with Baz. Lgl is shown to be phosphorylated by aPKC in vitro and in vivo. Mutations of the phosphorylation sites abolish the aPKC-dependent dephosphorylation of Lgl from the cortical S2 cells. Importantly, overexpression of non-phosphorylatable Lgl in NB, but not wild-type Lgl, causes Miranda to be distributed around the entire cell cortex. Similarly, expression of a version of aPKC that cannot anchor to the apical cortex of Miranda causes Miranda to be cytoplasmic. The authors propose a model in which apically localized aPKC phosphorylates Lgl and inactivates it locally, resulting in localization of Miranda to the basal cortex, where Lgl remains unphosphorylated and active.


Here, the role that myosin II plays in the basal localization of Pros and Miranda in the NB is characterized. In embryos with strongly reduced maternal and zygotic MRLC function, Miranda no longer localized at the cortex. Additionally, the authors demonstrate that Rho kinase phosphorylation of MyoII is necessary for its function and that Lgl inhibits MyoII function. Because MyoII localized to the apical pole of the NB, the authors propose a model of asymmetric localization, based on cortical exclusion rather than transport. In this model, MyoII would block basal component localization through its localization to the apical pole, which is controlled by Lgl.


Clonal analysis, using a null allele of lgl, indicates that Lgl is not required for Numb asymmetric localization in the SOP division, but is required to promote the pIIb cell fate. Lgl acts upstream of activated N and functions either downstream or parallel to Numb to block N signaling.


Live imaging, using two-photon microscopy of developing zebrafish retina reveals that divisions are planar and that the orientation of divisions changes over time in both zebrafish and rat, from the central-peripheral axis in early neurogenesis to circumferential axis later in neurogenesis. This study raises the possibility that planar polarity cues might regulate asymmetric divisions during CNS development.
