Asymmetric cell division

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With the recent identification of intrinsic cell-fate determinants for asymmetric cell division in several systems, biologists have begun to gain insight into the cellular mechanisms by which these determinants are preferentially segregated into one of the two daughter cells during mitosis so that the daughter cells acquire different fates.

Asymmetric cell division, in which a cell divides into two cells of different developmental potentials, is a fundamental means of generating cell diversity. In principle, asymmetric cell divisions may involve extrinsic or intrinsic factors. With extrinsic factors, daughter cells are initially equivalent but adopt different fates as the result of the interactions of the daughter cells with each other or with their environment. With intrinsic factors, unequal amounts of cell-fate determinants are partitioned into the two daughter cells. The mechanisms underlying asymmetric cell division have been studied in organisms ranging from bacteria, yeast, worms (Caenorhabditis elegans) and flies (Drosophila) to mammals.

Identification of the intrinsic cell-fate determinants

The unicellular budding yeast divides with a characteristic polarity and asymmetry of cell fate. A smaller ‘daughter’ cell buds off from the larger ‘mother’ cell. The mother cell can switch mating type but the daughter cannot. This asymmetry exists because the mother, but not the daughter, expresses the HO endonuclease which catalyses the genetic recombination event that leads to mating-type switching.

The Ash1 protein is an intrinsic determinant for this asymmetric division. Ash1 is normally found only in the daughter cell of the SOP as a result of interphase. Ash1 is required for a cell-fate determinant, Numb, to be asymmetrically localized.

Asymmetric cell division of at least some CNS lineages has been observed in Drosophila. In principle, there are several distinct (but not mutually exclusive) means by which a protein such as Numb could be asymmetrically localized in a dividing precursor cell so as to be preferentially segregated into one of the two progeny cells. For example, the asymmetric localization of this protein could be the result of asymmetric localization of the mRNA that encodes the protein. The protein could also be localized asymmetrically by a certain transport system. It is also possible that the protein is selectively degraded in a portion of the cell, leaving the remaining protein to form a crescent. In order to gain insight about the underlying mechanisms, genetic and biochemical studies have established a rudimentary genetic pathway for asymmetric localization of Numb and other factors (Fig. 2). Of the genes identified so far, inscutable is at the top of the hierarchy. It coordinates three aspects of asymmetric cell divisions in the nervous system as follows.

**Figure 1** Asymmetric localization of determinants. a, The budding yeast divides asymmetrically to produce a mother cell and a smaller daughter cell. Asymmetric localization of Ash1 mRNA causes the daughter cell to inherit the Ash1 protein, a negative transcription regulator that turns off HO expression. b, In the fruit fly, a neural precursor cell divides asymmetrically to produce two different daughter cells. Inscutable first forms a crescent at one side of the neural precursor cell during interphase. Inscutable is required for a cell-fate determinant, Numb, to form a crescent at the opposite side during metaphase. After division, one of the daughter cells inherits Numb and assumes a fate distinct from its sibling.
Asymmetric localization of Numb, Prospero and Miranda.

Prospero and Miranda, like Numb, show cell-cycle-dependent asymmetric localization in dividing neural precursors in the CNS and the PNS. Prospero is a homeobox-containing transcriptional regulator that is required for proper neuronal differentiation, neuronal cell-fate specification and axon growth and guidance. Whereas Prospero is normally a nuclear protein, it translocates to the cell membrane after nuclear envelope breakdown during mitosis. By metaphase, the Prospero protein forms a crescent and is colocalized with Numb. After cell division, both Numb and Prospero are segregated into the same daughter cell with Numb staying at the membrane and Prospero entering the nucleus. Although Numb and Prospero are colocalized during part of the mitotic cycle, their localizations are independent of each other; the Prospero crescent forms normally in numb mutants and the Numb crescent is normal in prospero mutants. Because the Numb protein appears to accumulate as the crescent forms, it seems more likely that protein translocation rather than selective degradation is the primary cause of Numb asymmetric localization. However, one cannot exclude the possibility that selective degradation plays a role.

Asymmetric localization of Prospero requires Miranda, a new membrane-associated protein for which the coiled-coil structure has been predicted. In dividing neural precursors, Miranda also forms a crescent and colocalizes with Numb and Prospero during metaphase. Miranda can bind Prospero and appears to be an adaptor protein required to bring Prospero to the cell membrane to form a crescent. Miranda loss-of-function mutants, Prospero is distributed uniformly in the cytoplasm and fails to reach the cell membrane.

The proper localization of Numb, Prospero and Miranda requires Inscurtable. However, this requirement varies to some extent in different parts of the nervous system. In the neuroblasts of inscurtable mutants, Numb, Prospero and Miranda are either delocalized or form crescents that are no longer localized to the basal cortex but exhibit random orientation. In contrast, the inscurtable mutation has much weaker effects in the PNS, suggesting that additional factors are involved.

Asymmetric RNA localization. The mRNAs that encode some of the asymmetrically localized proteins are also asymmetrically localized in the fly neuroblasts. prospero mRNA is asymmetrically localized to the apical cell cortex in the interphase neuroblast and relocates to the basal cell cortex during mitosis. This RNA localization requires the Staufen protein. Staufen encodes an RNA-binding protein that is part of the machinery required for the localization of the anterior determinant nanos during fly oogenesis.

In contrast to the prospero mRNA localization being dispensable for Prospero asymmetric localization, the asymmetric Ash1 mRNA localization is a prerequisite for the asymmetric location of Ash1 to the daughter nucleus. In Ash1 mutants, the Ash1 mRNA localization is at least in part mediated through its 3'-UTR (untranslated region).

Mitotic spindle orientation. During fly neurogenesis, the neuroblasts delaminate from a monolayer of ectodermal cells. The ectodermal cells at the surface of the Drosophila embryo divide with the axes of their mitotic spindle parallel to the plane of the ectodermal monolayer. In contrast, a neuroblast divides along the apical–basal axis, so that the axes of its mitotic spindles are perpendicular to the plane of ectodermal layer. Thus, the mitotic spindle of the neuroblast has to reorientate by 90 degrees from the plane of the ectodermal layer. The rotation requires Inscurtable. In inscurtable loss-of-function mutants, the orientation of the mitotic spindle in dividing neuroblasts becomes random. Conversely, when inscurtable is misexpressed in the ectodermal cells (which normally do not express inscurtable), those cells reorientate their mitotic spindles and divide along the apical–basal axis.

The orientation of the mitotic spindle correlates with the location of the Numb, Prospero and Miranda crescents during mitosis of neuroblasts. The three proteins are colocalized in a crescent overlying the basal spindle pole. This ensures that the basal daughter will inherit most of these proteins (Fig. 1b). Both spindle orientation and asymmetric protein localization are under the control of Inscurtable. However, they appear to be controlled independently: when spindle orientation is disrupted by microtubule depolymerizing agents, these proteins still form a basal crescent.

How might Inscurtable function? Inscurtable is asymmetrically localized to the apical pole of dividing neural precursors before they enter prophase. As the cell cycle progresses, Miranda, Prospero and Staufen become transiently localized to the apical pole, raising the possibility that Inscurtable may interact with these proteins directly or indirectly and, through an unknown mechanism, localize these proteins to the basal pole. By metaphase, Miranda, Staufen, Prospero and Numb form a basal crescent that is well separated from the apical crescent of Inscurtable.

Inscurtable is a new protein with a central portion containing several repeats that bear moderate resemblance to ankyrin repeats. This central portion is responsible for Inscurtable asymmetric localization as well as its ability to reorientate the mitotic spindle and to localize Numb and Prospero (J. A. Knoblich et al., manuscript in preparation). Inscurtable localizes Numb, Prospero and prospero mRNA through intermediaries (Fig. 2). Miranda, a multidomain adaptor that is capable of binding Inscurtable, Prospero and Staufen, is such an intermediary that is required for the asymmetric localization of Prospero, Staufen and prospero mRNA.

Localization may depend on cytoskeletal elements

For asymmetric localization of proteins in fly neural precursors, the proteins may have to be first localized to the membrane either directly or through an adaptor. In the absence of membrane localization, as in the case of Prospero in miranda mutants or the Numb or Inscurtable protein with its membrane-localization...
domain deleted (ref. 27 and J. A. Knoblich et al., manuscript in preparation), the proteins stay in the cytoplasm and fail to be asymmetrically localized. The membrane association of these proteins before their asymmetric localization may be an example of ‘reduction of dimensionality’ \(2^5\); the time it takes for a molecule to reach a particular location can be greatly reduced by restricting its diffusion to two-dimensional instead of three-dimensional space.

The asymmetric localization of these proteins requires certain cytoskeletal elements. In embryos treated with the microfilament inhibitor cytochalasin D, Insuteable is no longer asymmetrically localized \(37\). Although Numb and Prospero are still asymmetrically localized in neuroblasts, the crescents frequently form at incorrect positions \(14, 17\). Treatment with the more potent microfilament inhibitor latrunculin A abolishes Numb and Prospero asymmetric localization \(27, 29\). It thus appears that the asymmetric localization of Insuteable differs from Numb/Prospero asymmetric localization in the requirements for intact microfilaments. The involvement of microfilaments in the asymmetric localization of determinants seems to be a general requirement. In addition to a requirement for microfilaments, the localization of Ash1 in budding yeast, P-granules in \(C. elegans\) and posterior determinants during \(Drosophila\) oogenesis also involves Myo4 (refs 7, 8), non muscle myosin II \(30\), and tropomyosin \(11, 12\), respectively, suggesting that motor-based transport systems are utilized.

Compared to microfilaments, the requirement of microtubules is more variable. Ash1 localization \(10, 11\) and the localization of Insuteable, Numb and Prospero do not require microtubules \(14, 17\). In contrast, the localization of both anterior and posterior determinants during fly oogenesis requires microtubules \(33, 34\).

What might be the mechanisms for asymmetric localization of proteins such as Numb? There are several possible and not mutually exclusive models (for a more detailed discussion, see ref. 35). (1) The transport model: Numb is bound to a motor and carried along actin or another cytoskeletal structure toward its target area. This may be analogous to the potential involvement of an actin/myosin-based transport system for the transport of Ash1 mRNA in budding yeast \(7, 8\) and the asymmetric localization of PAR-1, 2 and 3 in \(C. elegans\) \(36\). (2) The diffusion model: Numb can diffuse along the cell membrane and is then captured by a localized anchor. (3) The capping model: The Numb proteins form aggregates either with themselves or with other molecules and eventually form a cap on one side of the cell. We do not know whether one or a combination of these models accounts for Numb localization.

**How do the determinants control cell fates?**

Determinants such as Ash1 and Prospero are transcriptional regulators. Unequal segregation of those determinants between the daughter cells results in differential gene expression. Ash1 suppresses \(HO\) transcription and thereby controls mating-type switching \(44-46\). Prospero can be either a positive or a negative regulator depending on its cofactor \(36\). It may control the expression of genes involved in neuronal differentiation \(18, 19\), such as \(deadpan\), \(asene\), \(even-skipped\) and \(fushi-tarazu\).

In contrast to nuclear proteins that regulate transcription, Numb functions by modulating cell–cell interactions. Cell–cell interaction mediated by the transmembrane receptor Notch is required for the four progeny of an \(SOP\) to assume their correct fate. Without Notch activity, all four cells become neurons \(37\). During each cell division within the sensory organ lineage, Numb appears to bias the Notch-mediated cell–cell interaction by inhibiting Notch activity \(38, 39\), so that the cell–cell interaction becomes asymmetric. The daughter that inherits Numb has lower Notch activity relative to its sibling and adopts IIB fate (Fig. 3). This is an example of how an intrinsic mechanism using Numb and an extrinsic mechanism mediated by Notch can be integrated for the control of cell fate.

How might Numb inhibit Notch function? Numb is a membrane protein containing a phosphotyrosine-binding domain. Numb can bind Notch in \(in \text{ vitro}\) binding assays \(34\). However, the mechanism by which Numb inhibits Notch is unknown. Numb could inhibit Notch activity by direct interaction. Alternatively, Numb could function as an adaptor protein to recruit other proteins that regulate Notch signalling. This inhibition of Notch by Numb is used as a general binary switch rather than for the specification of a particular cell fate. Besides PNS, it is also used in specifying two alternative sibling fates in certain CNS lineages \(35, 40\) and muscle founder cell lineages in \(Drosophila\) (ref. 41 and R. Bodmer and W. Chia, personal communication). In different lineages, different downstream effectors may be used to specify the daughter cell fates. An example is the sensory organ lineage in which the asymmetric divisions of both IIA and IIB employ the Numb/Notch binary switch; however, \(Su(H)\) is used to transduce Notch signalling in IIA but not in IIB lineages \(42\).

\(C.\; elegans\) has an alternative way of making Notch signalling asymmetric. During the 4–8-cell stage of \(C.\; elegans\) embryogenesis, the anterior cells receive inductive signals from the posterior cells. This signal is mediated by GLP-1 (\(C.\; elegans\) Notch homologue). The asymmetry of GLP-1 signalling is at least in part achieved by selective translation in the anterior cells of \(glp-1\) mRNA, which is uniformly distributed in both anterior and posterior cells \(43\).

**Evolutionary considerations**

The molecules that mediate cell–cell interaction during asymmetric cell division appear to be well conserved, including the molecules in the Notch signalling pathway and the Wnt signalling pathway \(44-46\). By contrast, the molecules that have been found thus far to be involved in asymmetric localization of determinants in \(Drosophila\), \(C.\; elegans\) \(4-6\) or budding yeast \(7, 8\) do not bear any family resemblance, except for the involvement of Staufen in both neural development and oogenesis in \(Drosophila\). This apparent diversity of molecular mechanisms may simply reflect our current state of ignorance. Once the remaining components are identified in those systems, a common theme may emerge. Alternatively, the diversity may be genuine and reflect the different strategies evolved for achieving asymmetric localization of determinants in different situations. For example, the distance to be covered by determinants in an oocyte (up to hundreds of micrometres) is much greater than that in a neural precursor (less than 10 \(\mu\)m). Furthermore, the anterior and posterior determinants are laid down only once during oogenesis whereas determinants for asymmetric division are asymmetrically localized every time the neural precursor divides.

Homologues for a few of the genes such as \(numb\) and \(par-1\) have been found in vertebrates. The mouse Numb, when introduced into...
the fly, is asymmetrically localized and can rescue the fly numb mutant phenotype. Mouse Numb (mNumb) is also asymmetrically localized in the neural precursor cells in developing mouse cortex; however, there is an interesting difference. In mouse, mNumb crescent localization and spindle orientation are not coupled as in the fly. Whereas a fly neural precursor always divides asymmetrically and imparts Numb to one of its daughters, a mouse neural precursor may divide either asymmetrically, with one daughter inheriting most of the mNumb, or symmetrically, with both daughters inheriting mNumb. The in vivo function of mNumb remains to be determined. PAR-1 is a putative Ser/Thr kinase required for the first asymmetric cell division of a C. elegans zygote. The mammalian PAR-1 homologues, PAR-1 and MARK, are kinases that can phosphorylate microtubule-associated proteins. Further, their overexpression disrupts the microtubule cytoskeleton and alters cell shape. These results suggest that PAR-1 may have a conserved function in specifying cell polarity by regulating the microtubule cytoskeleton.

**Perspective and future directions**

Recent studies have revealed multistep machineries that localize intrinsic determinants for asymmetric cell division. Our understanding of the underlying mechanisms is still fairly rudimentary. Besides the obvious need to identify the remaining components of the machineries, questions that need to be addressed include the following. (1) Inspectable does not set up cell polarity. Instead, it probably responds to the apical–basal polarity information that is laid down early in Drosophila development (perhaps at cellular blastoderm stage). How does Inspectable transduce this apical–basal polarity information? (2) How is the apical–basal polarity used to orientate the mitotic spindle? (3) The asymmetric localization of determinants is tightly linked with the cell cycle. How does the basic cell cycle machinery regulate the asymmetric localization of determinants? Is there a cell-cycle-dependent post-translational modification and/or degradation of the machinery? (4) The asymmetry organizer appears to have two centres at opposite poles of the cell, for example an apical centre for Inspectable localization and a basal centre for the Numb/Prospero/Miranda crescent in fly neuroblasts (Fig. 1b). How do the two centres communicate with each other? (5) Are the intrinsic determinants translated by transport, diffusion or capping? (6) The Numb/Notch interaction provides one way of integrating intrinsic and extrinsic mechanisms. How are the signalling pathways such as the Wnt pathway integrated with the intrinsic determinants’ functions? (7) Have different organisms adopted different strategies, or are there common mechanisms for asymmetric cell divisions? The intensive efforts being directed at several experimental systems should soon deliver some answers.

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