**Drosophila** neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis

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Over the past decade, many of the key components of the genetic machinery that regulate the asymmetric division of *Drosophila melanogaster* neural progenitors, neuroblasts, have been identified and their functions elucidated. Studies over the past two years have shown that many of these identified components act to regulate the self-renewal versus differentiation decision and appear to function as tumor suppressors during larval nervous system development. In this paper, we highlight the growing number of molecules that are normally considered to be key regulators of cell cycle events/progression that have recently been shown to impinge on the neuroblast asymmetric division machinery to control asymmetric protein localization and/or the decision to self-renew or differentiate.

The machinery that drives neuroblast asymmetry and the differential fate of the daughters

One of the best *Drosophila melanogaster* models for studying asymmetric division are the neural progenitors, or neuroblasts, which go on to generate the majority of the cells of the central nervous system. Neuroblasts undergo asymmetric divisions, generating two daughter cells of distinct size and fate. The larger daughter retains neuroblast identity and can continue to divide asymmetrically and self-renew, whereas the smaller daughter, namely the ganglion mother cell (GMC), is committed to the differentiation pathway and divides terminally to produce two neurons or glial cells. Through repeated self-renewing asymmetric divisions, neuroblasts, like other stem or progenitor cells, can generate a large number of differentiated progeny during their lifetime.

Many key components of the genetic machinery that facilitate the neuroblast asymmetric division have been identified and characterized (Egger et al., 2008; for review see Yu et al., 2006). There are in essence three key features associated with the neuroblast asymmetric division: (1) cell fate determinants, which act as differentiation factors, are asymmetrically localized as cortical crescents during mitosis; (2) the mitotic spindle is oriented orthogonal to the cortical protein crescents to ensure their exclusive segregation to the GMC daughter; and (3) the mitotic spindle is itself asymmetrical, resulting in the production of a larger neuroblast daughter and a smaller GMC daughter. All three features of this asymmetric division appear to be regulated by a set of proteins localized to the apical cell cortex starting during the late G2 phase of the cell cycle. These key components and their roles in mediating the neuroblast asymmetric division are summarized in Fig. 1 A. The cell fate determinants are localized to the basal cell cortex of embryonic neuroblasts, and the mitotic spindle is aligned along the apicobasal axis. A subset of these embryonic neuroblasts become quiescent, and proliferation is reinstated during larval development. The basic machinery involved in the asymmetric division of these larval neuroblasts appears to be conserved with embryonic neuroblasts; however, larval neuroblasts of the central brain divide without a fixed orientation.

Failure in asymmetric division, overproliferation, and tumor formation

The *Drosophila* larval brain neuroblast has recently emerged as a novel model for the study of stem cell self-renewal and tumorigenesis. Several types of studies have led to the view that defective asymmetric division may lead to the generation of tumors. First, brain tissue mutant for several of the components that control neuroblast asymmetric division (e.g., Miranda, Prospero, Numb, lethal giant larvae [Lgl], Brat, and Partner of Inscurtable [Pins]) will undergo massive overgrowth upon transplantation into the abdomen of wild-type hosts, killing the host within weeks (Caussinus and Gonzalez, 2005; Beaucher et al., 2007). These implanted cells exhibit many of the hallmarks of malignant neoplastic growth. They appear to be immortal and can be serially transplanted into successive hosts over years. They exhibit genome instability as indicated by high frequencies of cytologically
abnormal karyotypes as well as defects in centrosome morphology and number. These transplanted cells can also exhibit metastatic behavior, migrating away from the site of the primary tumor, passing through several cell layers, and establishing secondary colonies. Because the tumors derived from tissues mutant for different components of the neuroblast asymmetry machinery are essentially indistinguishable, it seems likely that they arise from a common mechanism: the disruption of neuroblast asymmetry and the production of excess self-renewing cells.

Supporting this view, a second series of recent studies have shown that all of the basal cell fate determinants (Prospero, Brat, and Numb as well as their adaptor molecules Miranda and Partner of Numb; Bello et al., 2006; Betschinger et al., 2006; Choksi et al., 2006; Lee et al., 2006a, c; Wang et al., 2006), which facilitate their asymmetric localization, can act as tumor suppressors (Fig. 1). Larval neuroblasts homozygous for mutations in any of these genes produce supernumerary self-renewing daughters at the expense of differentiated progeny. These observations suggest
that the loss of or a failure to correctly asymmetrically localize these determinants in larval neuroblasts can result in the failure to correctly specify the fate of their daughters, which can, in turn, lead to overproliferation and tumorigenesis. Consistently, several earlier studies showed that mutations in three genes, discs large (dlg), (lgl), and scribble (scrib), which induced the formation of malignant neoplastic tumors of the nervous system, also caused defects in the asymmetric localization of the cell fate determinants in neuroblasts (Ohshiro et al., 2000; Peng et al., 2000; Betschinger et al., 2006; Lee et al., 2006b). Lgl functions to restrict atypical PKC (aPKC) to the apical daughter (self-renewing cell), and it is also the target of aPKC phosphorylation (Fig. 1; Lee et al., 2006b). Together, these studies suggest a causal link between defects in neuroblast asymmetric division and over-proliferation/tumorigenesis in the larval brain. These findings have recently been reviewed and will not be discussed in detail here (for reviews see Yu et al., 2006; Gonzalez, 2007).

**Cell cycle genes can regulate asymmetric division and act as tumor suppressors**

Recent published and unpublished studies have reinforced an earlier view that cell cycle regulators can impinge on the asymmetric division machinery. Mutations in several genes encoding key regulators of cell cycle events can affect asymmetric protein localization, specification of distinct daughter cell fates, and/or the decision to self-renew or differentiate. In addition, the activation of cell cycle proteins, including CDK1, aurora A, and Polo, at prometaphase and metaphase coincides with the timing of asymmetric protein localization during neuroblast divisions, leading to a delicate temporal control of asymmetric division.

**cdc2/CDK1 levels can determine whether a neural progenitor division is symmetric or asymmetric**

The first indication that cell cycle regulators might also control aspects of the asymmetric division of neural progenitors came from a study on Cdc2/CDK1 (Tio et al., 2001). A dominant-negative allele of cdc2, cdc2E51Q, was isolated in a genetic screen designed to identify mutations that converted asymmetric GMC divisions that produced two daughter neurons with distinct identities into symmetric divisions generating two neurons of identical fate. Cdc2 in complex with the A- or B-type cyclins provides the kinase activity (CDK1) that is necessary to drive cells from G2 to mitosis, and cells lacking CDK1 activity arrest in G2 phase. Analysis using cdc2E51/Q as well as a temperature-sensitive allele of cdc2 under conditions in which the activity of cdc2 was attenuated, but not sufficiently so to prevent cells from entering mitosis, resulted in the failure to asymmetrically localize both the apical and basal components of the neuroblast asymmetric machinery, causing asymmetric divisions to be converted to symmetric divisions. Therefore, it appeared that there exists an intermediate level of cdc2 activity that enabled neural progenitors (and muscle progenitors) to divide but did not allow the division to be asymmetric because of a failure in asymmetric protein localization.

A direct demonstration that cdc2 activity was required during mitosis for asymmetric protein localization was facilitated by the knowledge that asymmetric protein localization does not require intact microtubules. In neuroblasts arrested at prometaphase using a microtubule-depolarizing drug in which all (both maternal and zygotic) of the cdc2 is temperature sensitive, normal apical and basal protein crescents are formed at the permissive temperature. However, after a shift to the non-permissive temperature, asymmetric protein localization cannot be maintained. If it is CDK1 activity that is responsible for the maintenance of asymmetric protein localization, attenuating cyclin levels might also be expected to cause defects in asymmetric protein localization. Cyclin A is degraded at prometaphase, whereas cyclin B and B3 are degraded during anaphase. In neuroblast double mutants for the late degrading cyclin B and B3, mislocalization of both apical and basal components can be seen at metaphase coinciding temporally with cyclin A degradation. These observations support the view that high levels of CDK1 activity are required during mitosis to maintain asymmetric protein localization and that it is possible to convert an asymmetric division into a symmetric division by altering the levels of CDK1 activity.

**Aurora A and Polo kinases act as tumor suppressors by preventing excess self-renewal**

Two other highly evolutionally conserved kinases, aurora A and Polo, have recently been shown to impinge on the neuroblast asymmetric division machinery and exhibit tumor suppressor properties in the larval brain (Lee et al., 2006a; Wang et al., 2006, 2007). Both kinases were initially identified as centrosomal proteins that have roles in mediating a multitude of mitotic processes. Loss of function mutations in either gene had previously been described as causing defects in centrosome maturation, delay/arrest at metaphase, or defects during cytokinesis (Llamazaeres et al., 1991; Glover et al., 1995; Carmena et al., 1998). Surprisingly, however, it was shown recently that mutations in aurora A or polo cause massive overgrowth in the brain but not other tissues (Lee et al., 2006a; Wang et al., 2006, 2007).

Live imaging (for aurora A mutants) and clonal analyses indicate that mutant brain neuroblasts can produce two self-renewing daughters, leading to an excess of neuroblast-like cells at the expense of differentiated neurons. Asymmetric localization of Numb and Pon (but not Prospero, Miranda, and Brat) is adversely affected in the aurora A and polo mutant neuroblasts. Presumably as a result of the partial loss of function, cell division can occur, although asymmetric protein localization is disrupted. Although this defect is one of several (see the next two paragraphs) caused by aurora A and polo mutants, it alone is sufficient to cause the observed overproliferation because clones in the larval central brain derived from single neuroblasts mutant for numb or pon exhibit excess proliferation at the expense of differentiation. Moreover, this overproliferation observed in aurora A and polo mutants can be largely but not completely reversed by overexpressing wild-type Numb. Interestingly, clones derived from single neuroblasts expressing a constitutively activated form of Notch in the central brain also exhibit an over-proliferation phenotype similar to that seen in aurora A and numb loss of function. However, Notch is not required for neuroblast proliferation in the ventral nerve cord, suggesting that its role...
in neuroblast proliferation differs in different tissues (Almeida
and Bray, 2005). Attenuating Notch in either aurora A or polo
homozygous mutant background can suppress the overprolifera-
tion phenotype, albeit partially. These findings suggest that a
genetic hierarchy comprising aurora A/polo, numb, and the neuro-
blast act to ensure that Notch is preferentially activated only in
the daughter cell, which adopts progenitor identity where it acts
to promote self-renewal.

Little is known about the biochemical substrates through
which Aurora A might act to suppress excess proliferation. How-
ever, Pon has been shown to be a functionally important down-
stream target of Polo kinase for the regulation of neuroblast
asymmetric division (Wang et al., 2007). Numb asymmetric
localization is facilitated by Pon, which is itself asymmetrically
localized. The C-terminal localization domain (Pon-LD), which is
necessary and sufficient to mediate Pon asymmetric localization,
contains a serine residue (S611) that matches the consensus
phosphorylation site for Polo. Both in vitro and in vivo ex-
periments suggested that Polo can directly phosphorylate Pon.
The significance of this phosphorylation is demonstrated by the
fact that Pon S611 phosphorylation is essential for Pon asym-
metric localization. Thus, Polo can regulate the asymmetric
division of neuroblasts by phosphorylating and, thereby, facili-
tating the asymmetric localization of Pon. Consistently, Polo is
also required for the asymmetric localization of Numb during
neuroblast divisions.

These findings illustrate the importance of Numb/Pon as
downstream components of aurora A and polo in mediating the
asymmetric fates of the neuroblast daughters. However, it is
important to emphasize that pololaurora A loss of function, in
addition to impinging on Pon/Numb asymmetric localization,
also affects several distinct pathways/components that can also
contribute to the self-renewal versus differentiation decision.
Neuroblasts mutant for pololaurora A also fail to asymmetrically
localize aPKC, which has properties consistent with that of a
proliferation factor. In addition, the tight coupling seen in
wild-type neuroblasts, in which the mitotic spindle is always
oriented orthogonal to the cortical protein crescents, is disrupted
in pololaurora A mutants. It is known that neuroblasts mutant
for components of the centrosome, like centrosomin and mush-
room body defect, which disrupt mitotic spindle orientation,
can also exhibit overproliferation, although this effect is weak
(Bowman et al., 2006; Izumi et al., 2006; Lee et al., 2006a;
Siller et al., 2006). During mammalian neurogenesis, spindle
orientation has also been shown to be an important determinant
for the choice of asymmetric division versus symmetric division.
Loss of function of several centrosomal components (i.e., abnor-
mal spindlelike microcephaly associated) results in predominant
asymmetric division and premature differentiation of neural pro-
genitors and the formation of a smaller brain (the related disease
is termed microcephaly in human patients; Bond et al., 2002).
In another study, knockdown of mouse inscuteable expression
changed the division plane of neural progenitors and resulted in
more frequent symmetric divisions that lead to enhanced pro-
liferation (Zigman et al., 2005). Thus, the phenotype induced by
pololaurora A mutants is not merely caused by disruption of the
Numb–Notch pathway but the sum of the effects exerted
on multiple pathways. In view of the pleiotrophic nature of these
kinases, it is not surprising that although expressing a phospho-
mimetic form of Pon in polo mutant neuroblasts can restore
asymmetric Numb localization, the overproliferation, spindle ori-
entation, and aPKC asymmetric localization defects remain.

The tumor suppressor function of Aurora A and Polo in
Drosophila larval brains is in contrast to the previously re-
ported and widely accepted view that they act as oncogenes in
mammalian cells (Zhou et al., 1998). Both mammalian Aurora A
and Pololike kinase 1 can phosphorylate tumor suppressor p53,
leading to its destabilization and degradation, and, thus, appear
to act as negative regulators of p53 (Ando et al., 2004; Katayama
et al., 2004). Conversely, the overexpression of Aurora A or Polo
can induce oncogenic transformation, presumably through down-
regulating p53 functions. Overexpression of Aurora A or Polo-
like kinase 1 can also lead to the generation of multiple centrosomes
through defects in cell division and consequent tetraploidiza-
tion, thereby leading to tumor progression (Meraldi et al., 2002).
Recently, it was shown that lymphomas in p53-deficient mice
exhibit the frequent deletion of the Aurora A gene and/or re-
duced protein expression, whereas normal tissue from the same
mutant mice had increased Aurora A protein levels (Mao et al.,
2007). These apparent discrepancies between flies and mamma-
lian cells are currently unresolved, and elucidating the function,
if any, of Aurora A and Polo during mammalian neurogenesis
will be of great interest.

**Cyclin E can act downstream of homeotic genes to convert a symmetric division into an asymmetric division**

Cyclin E, a G1/S cyclin, is a molecule with a key role in regulating
the G1- to S-phase transition. It also plays a necessary and suffi-
cient role in making the thoracic neuroblast 6-4 (NB6-4t) divide
asymmetrically, whereas its abdominal counterpart (NB6-4a) does
not (Berger et al., 2005). NB6-4t localizes Prospero asymmetrically
and divides to produce a Prospero+ glioblast daughter and a
Prospero- neuroblast daughter (which produces only neurons).
In contrast, NB6-4a divides symmetrically to produce two Prospero+
daughters of glial fate. This thoracic versus abdominal difference
appears to be imposed by the differential expression of cyclin E
in NB6-4t but not NB6-4a. In cyclin E mutants, both NB6-4t and
NB6-4a fail to localize Prospero, and both divide symmetrically
to produce daughters of glial fate. Conversely, the ectopic expression
of cyclin E in NB6-4a is sufficient to cause it to divide asymmetrically
like NB6-4t. Cyclin E expression is negatively regulated by
genes of the bithorax complex; thus, in NB6-4a, in the abdominal
neuromeres where AbdA and AbdB are expressed, cyclin E
expression is repressed. The role of cyclin E in mediating asymmet-
ic division and specifying cell fate appears to be independent of its
role in cell proliferation. Neither loss nor gain of function of
Decapo, the Drosophila homologue of the P21/Cip/Kip family
of cyclin E–Cdk complex inhibitors, or dE2F, which is activated
by cyclin E and required for the initiation of S phase, had any effect
on cell fate in the NB6-4a or NB6-4t lineages, although cell num-
bers were affected. Thus, cyclin E can apparently act independently
of its role in proliferation and downstream of homeotic function to
autonomously specify the NB6-4t asymmetric division.
Cyclin E has also been attributed to have the ability to confer self-renewing asymmetric division potential to GMCs (Bhat and Apsel, 2004) and the establishment of cortical polarity in *Caenorhabditis elegans* (Cowan and Hyman, 2006). Cyclin E expression has been reported to be down-regulated by the fate-determining factor Tramtrack in the asymmetric divisions of the *Drosophila* sensory bristle lineage (Audibert et al., 2005). Up-regulation of cyclin E has been observed in both imaginal and brain tumors (Moberg et al., 2001; Betschinger et al., 2006; Wang et al., 2006). Interestingly, elevated levels of cyclin E have also been observed in a subset of human tumors, including those of the breast and ovary (Keyomarsi and Herlich, 1997).

**Anaphase-promoting complex/cyclosome function is required for the asymmetric localization of Miranda and its cargo proteins**

The anaphase-promoting complex/cyclosome (APC/C) is a protein complex with at least 11 core subunits that functions as an E3 ubiquitin ligase that normally targets proteins for degradation via the 26S proteasome (Peters, 2006). Transient associations with the activating subunits Cdc20 and Cdh1 promote mitotic transitions via several key processes, including the destruction of mitotic cyclins and inhibitors of chromosome separation as well as the regulation of DNA replication, centrosome duplication, and mitotic spindle assembly (Sigrist et al., 1995; Zur and Brandeis, 2001; Leismann and Lehner, 2003). APC/C activity has recently been shown to have cell cycle–independent roles, including the control of axon growth and patterning in the developing mammalian brain (Konishi et al., 2004), the regulation of synaptic size and transmission in both *C. elegans* and *Drosophila* (Juo and Kaplan, 2004; van Roessel et al., 2004), and establishing the anterior–posterior axis of the *C. elegans* zygote by asymmetrically distributing Par proteins and promoting association of the paternal pronucleus/centrosome with the actin-rich cortex (Rapleye et al., 2002).

Recent findings suggest that APC/C core function is specifically required for asymmetric localization of Miranda and its interacting proteins Prospero, Brat, and Staufen but for none of the other asymmetrically localized components of the *Drosophila* neuroblast asymmetry machinery (Slack et al., 2007). Mutations in any one of several APC/C core components cause Miranda and its associated proteins to mislocalize to a pericentrosomal region, the nature of which is currently undefined. Mislocalization to this compartment requires neither intact microtubules nor intact centrosomal function. Although typical APC/C mutants are arrested at metaphase with high Cdk1 activity, the delocalization of Miranda appears to be largely independent of these defects. Miranda can be ubiquitinated both in vivo and in S2 cells. The extreme C-terminal region of Miranda contains a putative APC/C motif, and removal/replacement of this region prevents Miranda ubiquitination in S2 cells. Correlating with this disruption to ubiquitination, the mutant Miranda mislocalizes to the pericentrosomal compartment in a microtubule-independent manner. Interestingly, replacement of this C-terminal region with a ubiquitin moiety can restore asymmetric localization in dividing larval neuroblasts. Thus, APC/C seems to facilitate the ubiquitination of Miranda, which appears to be required for the asymmetric cortical localization of Miranda. Given the known function of APC/C in ubiquitin-mediated degradation, it will be interesting to determine whether Miranda is a direct substrate for APC/C.

**Concluding remarks**

There is increasing evidence that cell cycle regulators can impinge on the neuroblast asymmetry machinery and control various aspects of asymmetric division, including the decision of self-renewal versus differentiation. These cell cycle regulators include protein kinases, Cdc2/Cdk1, Aurora A, and Polo as well as APC core components and cyclin E. Interestingly, the basal protein component Pon has been shown to be a phosphorylation substrate of Polo kinase, providing a direct molecular link between a cell cycle regulator and a component of the asymmetry machinery. It has been shown that Cdc2/cyclin E and APC function are important for the establishment of cell polarity in the *C. elegans* zygote, suggesting that this regulation may be evolutionarily conserved. The most intriguing observation is that some of the cell cycle regulators, including Aurora A and Polo, possess tumor suppressor activity in the *Drosophila* larval brain, at least in part through regulating Numb asymmetry. Currently, many questions remain. What are the additional downstream factors that are controlled by the Cdk1/Aurora A/Polo kinases in the regulation of asymmetric protein localization and progenitor self-renewal? What, if any, interplay is there between the Numb–Notch pathway on the one hand and Brat–Prospero on the other in regulating neuroblast self-renewal? How general a role will ubiquitination play in the process of asymmetric protein localization and asymmetric division? Future studies will provide insight into these issues.

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