

Review

When timing is everything: role of cell cycle regulation in asymmetric division

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Abstract

Asymmetric division is a fundamental mechanism of generating cell diversity during development. One of its hallmarks is asymmetric localization during mitosis of proteins that specify daughter cell fate. Studies in *Drosophila* show that subcellular localization of many proteins required for asymmetric division of neuronal progenitors correlates with progression through mitosis. Yet, how cell cycle and asymmetric division machineries cooperate remains unclear. Recent data show that (1) key cell cycle regulators are required for asymmetric localization of cell fate determinants and for cell fate determination and (2) molecules that mediate asymmetric division can also act to modulate proliferation potential of progenitor cells.

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Keywords: *Drosophila*; Cell fate determination; Neuroblast; Sensory organ precursor; Cyclin-dependent kinase

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1. Introduction

Ability to divide is a fundamental property of a cell. In single cell organisms, it serves the purpose of self-renewal and propagation, while in metazoans it is the basis of development as well as regeneration to maintain tissue homeostasis.

However, the problem that any multicellular organism faces is how to achieve in an orchestrated manner (both in time and within the confinements of spatial boundaries) an astonishing variety of cell types that make up tissues and organs. One common mechanism that was probably widely exploited and reused throughout evolution in all phyla is asymmetric, or unequal, cell division. Asymmetry of divisions is the fundamental mechanism of generating cell diversity, and hence, achieving structural and functional diversity. During an asymmetric division, a mother cell divides unequally to generate

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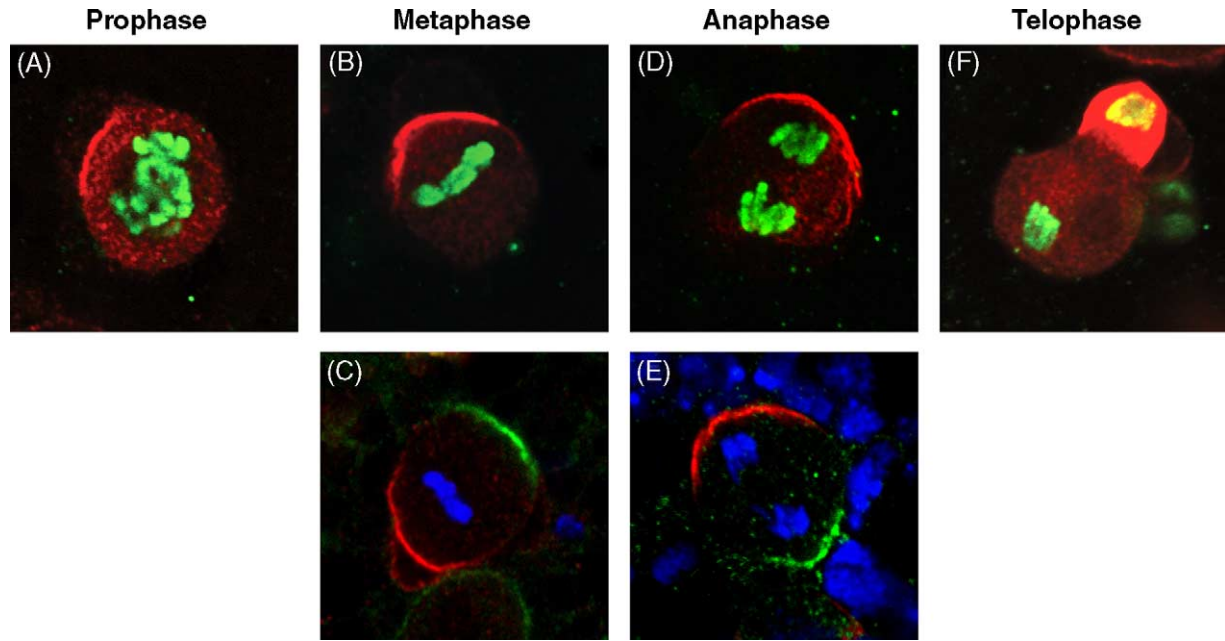


Fig. 1. Cell cycle-dependent localization of proteins that specify daughter cell fate. Asymmetric localization of Miranda (A–E, red), Partner of Inscuteable (C, green) and atypical PKC δ (E, green) in brain neuroblasts of wild-type wandering third instar *Drosophila* larvae. Brains were counterstained with anti-phosphohistone H3 antibody (A, B, D and F, green; C, blue) to mark mitotic chromosomes or with TO-PRO-3 (E, blue) to visualize DNA. During mitosis, Miranda (as well as proteins that specify cell fate) forms an asymmetric crescent (basal in embryonic neuroblasts) starting in prophase (A). Miranda crescent persists throughout mitosis (B and D). In telophase (F), Miranda is segregated exclusively to a smaller ganglion mother cell. Several other proteins (for example, (C) Partner of Inscuteable and (E) atypical PKC δ) form crescents at the opposite pole of the cell (apical in embryonic neuroblasts). Formation of the functional apical complex is required for normal basal localization of Miranda, Prospero, Numb and other proteins and for mitotic spindle rotation.

two daughters of different developmental potential (or cell fate). From a general standpoint, asymmetric cell division involves several events: (1) establishing axis of polarity and coordinating it with the body axes prior to division, (2) assembling mitotic apparatus and reorienting it along the axis of polarity, (3) distributing cell fate determinants along the polarity axis in a polarized, asymmetric manner and segregating them unequally between two daughter cells and (4) specifying distinct cell fate identities of daughter cells as a consequence of unequal distribution of determinants as well as cell–cell interactions.

Asymmetric divisions have been described from bacteria and yeast to *C. elegans* and *Drosophila* to vertebrates [1–3]. For example, during neurogenesis in the developing *Drosophila* embryo neuronal progenitors (neuroblasts (NBs) in the central nervous system and sensory organ precursors (SOPs) in the peripheral nervous system) undergo asymmetric divisions to generate sibling daughter cells with distinct cell fates. In the central nervous system (CNS), neuroblasts divide asymmetrically to give rise to another neuroblast and to a ganglion mother cell (GMC). The GMC undergoes one more round of asymmetric division to produce two terminally differentiated cells, neurons or glial cells (reviewed in [4,5]). Unequal or asymmetric distribution of cell fate determinants during divisions of neuroblasts and ganglion mother cells in the CNS and sensory organ precursors in the peripheral nervous system (PNS) results in the formation of two daughters of different identities. Localization of cell

fate determinants during cell division to form apical or basal crescents of proteins shows cell cycle dependence and correlates with progression through mitosis (Fig. 1). For example, a cell fate determinant protein Numb [6] is asymmetrically distributed to the progeny of asymmetrically dividing neuroblasts in the CNS and pI sensory organ precursors in the PNS in developing *Drosophila* embryos [7–9]. Starting in late prophase, Numb forms an asymmetrically localized crescent oriented basally in neuroblasts and anterior/laterally in SOPs. Since asymmetric localization of Numb (and other cell fate determinants) is coupled to mitosis, it is of little surprise that progression through the cell cycle is required to form Numb cortical crescent and, by definition, for asymmetric division of neuroblasts to form two unequal (in size and fate) daughter cells. Although subcellular localization of cell fate determinants shows dependence on the cell cycle, involvement of components of cell cycle machinery in asymmetric divisions has not been appreciated until recently.

In this review, we will discuss recent results that highlight the relationship between cell cycle regulation and asymmetric division (Table 1; Fig. 4). Due to space limitations, we limit our discussion to *Drosophila melanogaster*. However, aspects of asymmetric cell divisions in other model organisms have been extensively reviewed elsewhere (see [10,11] and other citations in this Section). To present the problem, we will use five model systems of asymmetric division in *Drosophila*: (1) neuroblasts in the embryonic

Table 1
Proteins at the interface between cell cycle regulation and asymmetric division

Protein name	Function	Model system	Effects of loss-of-function mutation		Other phenotypes
			Lineage transformation	Protein expression	
Cdk1/Cdc2	G2/M kinase	Dividing neuroblasts in the embryonic CNS. NB4-2 lineage: NB4-2 → GMC4-2a → RP2 + RP2sib	GMC4-2a → RP2 + RP2. RP2 duplication	INSC, BAZ, PON, MIRA are abnormally localized	Abnormal orientation of mitotic spindle
Cyclin B, Cyclin B3	G2/M cyclins	Dividing neuroblasts in the embryonic CNS		INSC is abnormally localized. Only in Cyclin B, Cyclin B3 double mutant, not in single mutants	
Aurora-A	Serine/threonine kinase, spindle formation, chromosome segregation	pI lineage in the PNS: pI → pIIa (Numb ⁻) + pIIb (Numb ⁺)	pI → pIIa + pIIa. Duplication of external cells (two shaft and two socket cells)	Numb is segregated symmetrically to two pIIa cells during pI division	Abnormal spindle morphology, defects in centrosome maturation
Wee, Myt1	Inhibitory kinases, regulation of Cdc2 activity and mitotic entry	pI bristle (microchaete) lineage of the adult fly: pI → pIIa (Numb ⁻) + pIIb (Numb ⁺). Four divisions generate four cells that form a bristle mechanosensory organ	pI → pIIb. Two divisions. Sensory organs consist of only two cells, lack shaft and socket cells. Severe bristle loss	INSC, PROS (pIIb markers) are ectopically expressed in pI	
String/Cdc25	Tyrosine phosphatase, regulation of Cdc2 activity and mitotic entry				
Tribbles	Serine/threonine kinase, proteolytic degradation of String/Cdc25				
Cyclin A Regulator of cyclin A1 (Rca1) String/Cdc25	G2/M cyclin Inhibitor of anaphase-promoting complex/cyclosome	GMC lineages in the embryonic CNS: GMC4-2a → RP2 (Numb ⁺) + RP2sib (Numb ⁻). GMC1-1a → aCC (Numb ⁺) + pCC (Numb ⁻)	GMC4-2a → RP2. GMC1-1a → aCC	In the absence of division, Numb remains in GMC4-2a and GMC1-1a	When Numb is <i>also</i> lost—opposite lineage transformation: GMC4-2a → RP2sib. GMC1-1a → pCC
Cyclin E	G1/S cyclin	GMC4-2a → RP2 + RP2sib	GMC4-2a → RP2		Upregulation of Cyclin E in GMC4-2a induces self-renewing asymmetric divisions of GMC4-2a. Increase in the number of RP2 or RP2sib
		NB6-4 lineage in the embryonic CNS: NB6-4t → 3 glial cells (PROS ⁺) + 4-6 neurons (PROS ⁻). NB6-4a → two glial cells (PROS ⁺)	NB6-4t-to-NB6-4a transformation. Conversion of asymmetric divisions to symmetric. Same phenotype—upon ectopic expression of Abd-A or Ubx	PROS is expressed in both daughter cells of NB6-4t	NB6-4a-to-NB6-4t transformation: upon ectopic expression of Cyclin E or upon loss of Abd-A or Abd-B
Prospero	Homeodomain transcription factor, cell fate determinant	Dividing GMCs in the embryonic CNS			Ectopic transcription of <i>string/cdc25</i> , <i>Cyclin A</i> , <i>Cyclin E</i> . Continued mitotic activity of GMCs
		Proliferation of the LG in the embryonic CNS: longitudinal glioblast → 10-12 LG	Reduction in LG number: longitudinal glioblast → 8 LG	PROS is expressed in a subset of LG arrested in G1 and maintains their mitotic potential	
Bazooka	PDZ domain protein, involved in cell polarity and asymmetric localization of cell fate determinants	pI lineage in the PNS: generates 5 cells. pIIa (divides once) → shaft cell + socket cell	pIIa undergoes two rounds of division. pI lineage generates 7 cells	PON and Numb do not localize asymmetrically	

→ → Denotes cell fate transformation. *Abbreviations*: BAZ, Bazooka; CNS, central nervous system; GMC, ganglion mother cell; INSC, Inscuteable; LG, longitudinal glia; MIRA, Miranda; NB, neuroblast; PNS, peripheral nervous system; PON, Partner of Numb; PROS, Prospero. See text for details and for references.

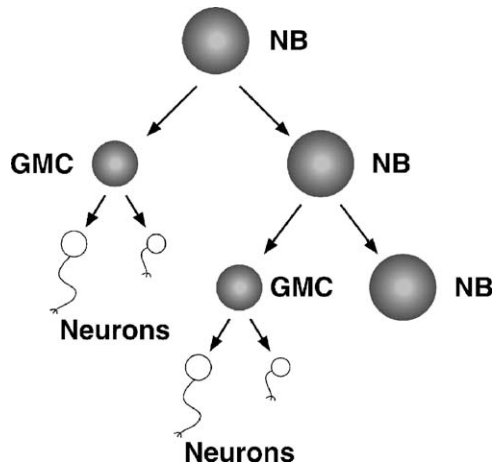


Fig. 2. A typical neuroblast lineage in a *Drosophila* embryo. Neuroblasts (NBs) have stem cell-like properties and divide asymmetrically to self-renew and to produce a series of ganglion mother cells (GMCs). GMCs do not self-renew, but instead undergo one terminal asymmetric division to produce two distinct neurons. Some neuroblasts generate both neurons and glial cells and are called neuroglioblasts. A unique neuroblast identity is defined by the time and position at which they are formed in the embryonic neuroectoderm and by combinatorial pattern of expression of transcription factors and, in turn, determines its lineage (number and timing of successive divisions and the identity of its progeny).

CNS (Fig. 2), (2) GMCs in the embryonic CNS, (3) SOPs in the embryonic PNS, (4) pI bristle (microchaete) lineage in the adult PNS (Fig. 3) and (5) cardiac progenitors in the embryonic mesoderm.

2. Cdc2 and coordination of cell cycle and asymmetric division in neuroblasts

Like in other eukaryotes, the decision to enter mitosis during embryonic cell cycles in *Drosophila* is governed largely by Cdc2/Cdk1 phosphorylation state and by levels of mitotic cyclins (reviewed in [12–14]). Cdc25/String protein tyrosine phosphatase [15–17] is required to activate Cdc2 [17–19] and to promote mitotic entry. The *string* (*stg*) mutant embryos have a markedly reduced number of cells during late stages of embryogenesis compared to wild-type embryos. Mutants undergo the first 13 nuclear divisions with normal timing and morphology but arrest in the interphase of the 14th mitosis. Three final embryonic mitotic cycles 14–16 do not occur [15]. Similarly, in *string* mutant embryos neuroblasts are arrested at the G2/M transition of cycle 14 and do not enter mitosis [8]. In addition, asymmetric distribution of Numb is disrupted and it remains evenly distributed at the neuroblast cortex. However, this suggests merely that formation of the Numb crescent is dependent on entry into mitosis. When cell cycle is arrested, cell fate determinants do not localize asymmetrically (in a cell cycle-dependent manner), neuroblasts fail to divide and retain their original identity (see also [20,21]).

The strongest evidence to date that there is a direct connection between cell cycle and asymmetric division comes from functional analysis of Cdc2 mitotic kinase in *Drosophila* [22,23] (Table 1). Initial genetic analysis of *cdc2* (*DmCdk1*) [17,18] demonstrated its role in G2/M transition [19,24]. Loss of zygotic function of Cdc2 causes lethality at the larval–pupal interphase due to proliferation defects in imaginal disks and larval brains. However, inactivation of both maternal and zygotic Cdc2 function (using a temperature-sensitive allele of *cdc2*) resulted in mitotic arrest during embryonic divisions, most notably in late divisions in the peripheral and central nervous systems. A mutant screen for mutations affecting asymmetric localization of cell fate determinant Prospero (PROS) [8,25–28] in dividing neuroblasts led to identification of a novel allele of *cdc2*, *cdc2^{E51Q}* [23]. Embryos homozygous for *cdc2^{E51Q}* mutation show late embryonic lethality and duplication of the RP2 neuron, reminiscent of mutant phenotype of *inscuteable* (*insc*) [29,30] and *partner of inscuteable* (*pins*) [31]. RP2 is generated through an asymmetric division of GMC4-2a, the first GMC produced from the NB4-2 neuroblast (GMC4-2a → RP2 + RP2sib). Duplication of the RP2 neuron is caused by an RP2sib → RP2 transformation, indicating that the normally asymmetric division of GMC4-2a was converted to a symmetric division (GMC4-2a → RP2 + RP2). These defects in asymmetric divisions correlate with abnormal localization of several apical and basal determinants—Inscuteable (INSC), Bazooka (BAZ), Partner of Numb (PON), Miranda (MIRA)—in both neuroblasts and GMCs and with abnormal orientation of mitotic spindle. It appears that initial apical localization of apical determinants (BAZ, INSC and PON) during interphase does not require Cdc2 activity. However, Cdc2 is required to maintain proper localization of apical complex proteins during mitosis as premature attenuation of Cdc2 activity in metaphase neuroblasts results in mislocalization of both apical and basal proteins. Therefore, Cdc2 activity is required to maintain polarity of neuroblast asymmetric divisions during mitosis.

A question remains as to what is the exact biochemical nature of *cdc2^{E51Q}* mutation. This E51Q missense mutation has a much stronger phenotype than a complete loss of *cdc2* function mutation [19,24]. Overexpression of Cdc2^{E51Q} protein in transgenic animals [23] caused the same phenotype as *cdc2^{E51Q}* loss-of-function mutation, notably conversion from asymmetric to symmetric division of neuronal and muscle progenitors. This and other lines of evidence suggest that *cdc2^{E51Q}* acts as a maternally inherited wild-type Cdc2 protein. Despite earlier thorough functional analysis of *cdc2* in *Drosophila* using loss-of-function mutations [19,24] and expression of peptide aptamers in transgenic flies [32], it is a unique genetic nature of this allele of *cdc2* discovered fortuitously that led to unraveling of an unexpected role of Cdc2 mitotic kinase in asymmetric division.

How does Cdc2 coordinate tight temporal correlation of asymmetric localization of protein complexes that determine

asymmetric division and the cell cycle? Is there a cross-talk between two molecular pathways? Do the machineries of cell cycle and asymmetric division link directly? These are some of the questions that are yet to be addressed.

Since Cdc2 activity is required for asymmetric divisions in neuronal and muscle precursor cells, one would expect that changes in levels of mitotic cyclins or changes in a phosphorylation state of Cdc2 may also affect asymmetric divisions. There are three mitotic cyclins in *Drosophila*—Cyclin A, Cyclin B and Cyclin B3 [33–35]. Destruction of all three cyclins is required for late anaphase, cytokinesis and exit from mitosis. To address role of mitotic cyclins in asymmetric division, Tio et al. followed temporal distribution of apically localized INSC protein in mitotic neuroblasts in different cyclin mutants and in their double mutant combinations [23]. INSC had normal apical localization in *Cyclin A*, *Cyclin B* or *Cyclin A*, *Cyclin B3* double mutants. However, in *Cyclin B*, *Cyclin B3* double mutants INSC crescents were mislocalized in the majority of dividing neuroblasts [23]. Therefore, although Cyclin A appears to be dispensable, both B- and B3-type cyclins are required to maintain apical localization of INSC (and likely, other components of asymmetry machinery) during mitosis.

In summary, attenuating function of Cdc2/Cdk1, B- and B3-type cyclins and String/Cdc25 results in delocalization of normally asymmetrically localized determinants (INSC, MIRA, Numb and PON) in dividing neuroblasts. These determinants have cell-autonomous effects, and when they are distributed equally to two daughter cells, the polarity of cell divisions changes—symmetric divisions become asymmetric. In addition, active Cdc2/Cyclin complexes are not required to initiate apical complex formation in interphase neuroblasts, but are necessary to maintain the asymmetric localization of apical components during mitosis.

3. Aurora-A mitotic kinase, G2/M transition and asymmetric division

The only other mitotic kinase implicated, in addition to Cdc2/Cdk1, in asymmetric divisions in the fruit fly is Aurora-A. *Drosophila* Aurora-A [36] is the founding member of the Aurora family of conserved serine/threonine protein kinases (reviewed in [37]). In contrast to Cdc2, Aurora kinases are required in a subset of mitotic events, but not for cell cycle progression or mitotic entry. Aurora-A is an important regulator of spindle formation, and hence, is essential for chromosome segregation. Mutations in *aurora-A* (*aurA*) do not arrest the cell cycle, but interfere with centrosome maturation and separation, formation and maintenance of the bipolar spindle, and chromosome segregation. A genetic screen for mutations that affect asymmetric localization of Numb revealed a new role for Aurora-A in asymmetric division of sensory organ precursor cells that is unrelated to its known functions in mitosis [38] (Table 1). The rationale of the screen was that mutations that cause defects in asymmetric localization of

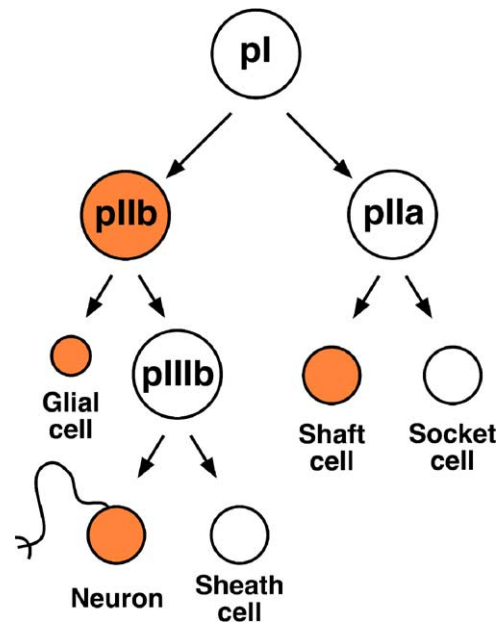


Fig. 3. The sensory organ precursor lineage. The same lineage was described in the embryonic peripheral nervous system and in the bristle (microchaete) lineage of the adult fly. Cells within the lineage that inherit Numb cell fate determinant are shown in orange. See text for details.

Numb will lead to cell fate transformation in the SOP lineage in the *Drosophila* PNS and cause loss or duplication of bristles within mitotic clones generated on the fly head. During PNS development, SOP cells undergo a series of asymmetric cell divisions (Fig. 3) to generate four different cell types that form an external sensory (ES) organ [39,40]. First, pI SOP cell divides asymmetrically along the anterior–posterior axis within the epithelium to form a small anterior pIb cell that is Numb-positive (Numb⁺) and a large posterior pIIa that is Numb⁻. Then, pIb delaminates and divides to generate a Numb⁺ glial cell that migrates away and does not become part of the organ, and a Numb⁻ pIIIb. pIIa divides within the epithelium to form two large cells that form the external structures of the ES organ—a Numb⁺ anterior shaft (hair) cell and a Numb⁻ posterior socket cell. Finally, pIIIb divides to produce a small Numb⁺ internal neuron and the Numb⁻ sheath cell. Each of these four cell types can be easily identified based on cell size and expression of cell-specific markers (Numb and transcription factor Suppressor of Hairless Su(H)).

One of the mutations identified in the screen was a novel allele of *aurora-A* (*aurA*³⁷) that impairs asymmetric localization of Numb in pI divisions. When Numb distributes uniformly throughout the cortex and segregates to both daughter cells, *aurA*³⁷ mutant SOP cells, instead of dividing asymmetrically, divide equally into two Numb⁺ pIIa cells. Mutant *aurA*³⁷ ES organs consist of four large cells—two hairs and two sockets—indicating that inner cells were transformed into additional outer cells. In contrast to Numb, asymmetric localization of proteins that establish anterior–posterior axis prior to pI division—Bazooka (at the posterior cell cortex) and the inhibitory G protein α subunit, G α i (at the anterior

cell cortex)—is not affected. Hence, although the pI precursor established normal anterior–posterior polarity in interphase, Numb segregated symmetrically into pI progeny.

Furthermore, the authors demonstrate that defects in Numb localization are not indirect consequences of abnormal spindle morphology and defects in centrosome maturation, which are also observed in *aurA*³⁷ mutant cells [38]. In fact, neither functional centrosomes nor the mitotic spindle are required for Numb localization. In summary, during asymmetric division of pI precursors in the PNS Aurora-A is required for asymmetric protein localization and this function is independent of its other roles in mitosis. It is not required for setting up polarity during interphase (mediated by Bazooka and G α i) but, instead, functions later to interpret this polarity information to initiate asymmetric localization of Numb at the onset of pI mitosis and to establish asymmetry of cell division.

The molecular mechanism through which Aurora-A controls asymmetric division remains unknown. Remarkably, during asymmetric division of neuroblasts in the CNS, Cdc2/Cdk1 mitotic kinase is also required for asymmetric localization of Numb and other cell fate determinants (see Section 2). The peak activities of both Cdc2 and Aurora-A parallel the onset of mitosis [41,42]. Whether the two kinases interact and if they do, then how, is not clear. It appears that in dividing mammalian cells in culture Aurora-A activation depends on the activation of Cdc2/Cyclin B complex at the G2/M transition [42] placing Cdc2 upstream of Aurora-A. Similarly, Cdc2 kinase activity is necessary and sufficient for Aurora-A activation in *Xenopus* oocytes [43], although other authors claim that Cdc2 inhibitors do not block Aurora-A activation [44]. Activation of Aurora-A at the G2/M transition correlates with its phosphorylation [42]. In *Xenopus* M phase-arrested egg extracts, Aurora-A protein is found to be phosphorylated on at least three residues [43,44] that regulate differentially its kinase activity. However, Cdc2 does not directly phosphorylate and activate Aurora-A suggesting that other kinases or phosphatases under the control of Cdc2 may regulate Aurora-A activity during meiosis. Together, these results suggest that Aurora-A is phosphorylated and activated downstream of Cdc2/Cyclin B kinase during mitosis and meiosis in different species. Identity of kinases and their opposing phosphatases that control phosphorylation state of Aurora-A during cell division remains unknown. They can be Cdc2-dependent kinases that directly phosphorylate and activate Aurora-A, or Cdc2-dependent phosphatases that inhibit Aurora-A and prevent its auto-phosphorylation and auto-activation, or Cdc2-dependent adaptors or co-factors of Aurora-A. Furthermore, there may be additional physiologically relevant phosphorylation sites in Aurora-A that may have other unrelated functions (structural, targeting for degradation, etc.).

In addition to several known substrates of Aurora-A (reviewed in [45]), Numb or a component of Numb localization machinery could be regulated through Aurora-A-mediated phosphorylation. Numb contains several putative Aurora-A

phosphorylation sites, although it appears that *Drosophila* Aurora-A does not phosphorylate Numb in vitro [38]. Several proteins are required for Numb localization in *Drosophila* neuroblasts: ankyrin-like domain protein Inscuteable as well as other apical complex proteins, two tumor-suppressor proteins—WD-40 repeat protein Lethal (2) giant larvae (LGL) and PDZ domain protein Discs large (DLG) and two proteins that interact with phosphotyrosine-binding (PTB) domain of Numb—transmembrane Numb-interacting protein (NIP) and Partner of Numb [30,46–50]. One of them or an unknown, yet to be identified component of the Numb localization machinery, may be phosphorylated by Aurora-A at the onset of mitosis when Numb forms an asymmetrically localized crescent. Numb localization also requires activation of Cdc2/Cyclin B complex (see Section 2).

An intriguing possibility linking the two kinases together was proposed in a recent study [51]. It appears that at least in HeLa cells, Aurora-A and CDC25B interact in vivo and in vitro. Aurora-A phosphorylates CDC25B on serine-353. Both activated Aurora-A and phosphorylated CDC25B localize at the centrosome in early prophase. Furthermore, levels of phosphorylated CDC25B correlate with expression of Aurora-A and specific targeting of S353 (using antibodies against CDC25B phosphopeptide) was sufficient to delay entry into mitosis. Therefore, in HeLa cells, a major function of Aurora-A at prophase appears to be S353 phosphorylation of CDC25B to regulate the function of CDK1/cyclin B complexes at the centrosome. To date, such observation suggesting a direct biochemical link between Aurora-A and Cdc25 to regulate the activity of Cdk1/cyclin B complex and to promote entry into mitosis is limited to cell culture. It remains to be seen if the same mechanism operates in other model systems and if activation of Numb localization machinery requires both Cdk1 and Aurora-A-dependent phosphorylation. At present, it is not clear if Aurora-A is required exclusively for asymmetric localization of cell fate determinants in SOPs in the PNS [38], or if Cdc2/Cdk1 has a more universal role in asymmetric divisions, in addition to its reported function in asymmetric division of neuroblasts in the CNS and muscle progenitors in the mesoderm [22,23]. To complicate matters further, phosphorylation by Aurora-A does not change in vitro catalytic activity of CDC25B [51]. Hence, even if the two work in tandem to promote mitotic entry in asymmetrically dividing cells, CDC25B phosphorylation, instead of being an activating modification, may trigger, for example, conformational changes required for interaction with other regulatory proteins or for docking of a substrate or a cofactor.

4. Cell fate determination in the absence of cell cycle progression

Studies in other model systems suggest that cell fate determination need not be linked tightly to cell cycle controls and may occur even upon cell cycle arrest. Such cell fate determination independent of cell cycle progression was described

in two other model systems in *Drosophila*—in the bristle (microchaete) lineage of the adult fly [52] and in dividing ganglion mother cells in the embryonic CNS [53–55].

In the notum, the bristle lineage starts with a single progenitor cell pI that undergoes two rounds of asymmetric cell divisions (Fig. 3) to produce four differentiated cells that form a bristle mechanosensory organ of the adult fly [56,57]. During its first division, the pI precursor divides asymmetrically to produce two secondary precursor cells pIIa and pIIb (pI → pIIa + pIIb). Asymmetric division of pIIa leads to the formation of two differentiated cells, the shaft (hair) and the socket cells (pIIa → shaft cell + socket cell) that form externally visible structures of the organ. Asymmetric division of pIIb gives rise to a tertiary precursor cell pIIIb and a glial cell that undergoes apoptosis (pIIb → pIIIb + glial cell) [56,57]. Finally, pIIIb divides unequally to form the internal neuron and sheath cell (pIIIb → neuron + sheath cell). The resulting four cells—neuron, sheath, shaft and socket—form the bristle mechanosensory organ. Asymmetric localization of Numb protein that is inherited by only one of the two daughter cells during each of these four divisions is required to establish correct cell fates within the pI lineage [56]. To understand how determination of terminal cell fate depends on progression through the cell cycle, Fichelson and Gho undertook a thorough analysis of atypical pI lineages that formed upon cell cycle delay at the G2/M transition [52].

In *Drosophila*, mitosis is driven by active Cdc2/DmCdk1 cyclin-dependent kinase [17–19]. Cdc2 phosphorylation levels are regulated by two classes of enzymes. First, Wee1 tyrosine kinases (in *Drosophila*, Wee [58,59] and Myt1 [60,61]) negatively regulate Cdc2 by addition of an inhibitory phosphate, thus, preventing mitotic entry. Second, Cdc25 protein tyrosine phosphatases (in *Drosophila*, String (STG) [15–17] and Twine (TWE) [62]) remove the inhibitory phosphate, and, hence, activate Cdc2 and promote entry into cell division cycle.

Several approaches were used to delay the cell cycle by down-regulating Cdc2/Cdk1 function or activity in pI precursor and its progeny (Table 1): (1) overexpression of Wee or Myt1 inhibitory kinases, (2) decreasing the dosage of String/Cdc25 phosphatase, (3) overexpression of Tribbles (TRBL) serine/threonine kinase that induces proteolytic degradation of String/Cdc25 [63,64] and (4) depleting Cdc2 using a temperature-sensitive mutation of *cdc2*. Whether Cdc2 levels were decreased or Cdc2 activity was attenuated (by expressing negative regulators), G2/M transition in the pI precursor was delayed. When pI finally underwent mitosis, it divided with an altered polarity. Asymmetric distribution of a cell fate determinant Partner of Numb [46] was reversed—compared to a wild-type pI division. This produced a lineage similar to the wild-type pIIb sub-lineage and gave rise to sensory organs consisting of only two cells—a neuron and a sheath cell. Since sensory organs in these flies lacked shaft and socket cells, this manifested as severe bristle loss. Taken together, these observations suggest that pI precursor underwent a cell fate transformation adopting a pIIb

identity (pI → → pIIb). Instead of undergoing its first division to produce two secondary precursor cells as in wild-type flies (pI → pIIa + pIIb), it divided twice in a pattern identical to a wild-type pIIb sub-lineage. This pI → → pIIb fate transformation occurred prior to the first division (that was delayed due to insufficient levels or activity of Cdc2) and independently of cell cycle progression. In other words, cell cycle and cell fate determination are independent events in the bristle lineage.

A similar relationship between cell cycle and cell fate determination was reported for dividing ganglion mother cells in the CNS using two GMC lineages as a model—GMC4-2a [53–55,65] and GMC1-1a [54]. Unlike neuroblasts that have stem cell-like properties and divide asymmetrically to self-renew and produce a series of GMCs (e.g., NB1 → NB2 + GMC; see [21]), GMCs undergo one terminal asymmetric division to produce two distinct neurons (Fig. 2). GMC4-2a derives from the NB4-2 neuroblast and divides to form two sibling neurons, RP2 and RP2sib. GMC1-1a derives from NB1-1 and forms aCC and pCC neurons. In each case, the two differentiated daughter neurons have unique identities and express distinct sets of molecular markers (see [54]). For example, RP2 neuron has high levels of expression of a nuclear homeodomain protein Even-skipped (EVE) compared to RP2sib where its expression is low and eventually lost. To block cell cycle progression through G2/M transition, the following loss-of-function mutations were used: *Cyclin A* (*CycA*), *Regulator of cyclin A1* (*Rca1*) [35,66,67] and *string/cdc25* (*stg*) [15–17]. *Rca1* is an essential inhibitor of the anaphase-promoting complex/cyclosome (APC). Mutations in *Rca1* prevent entry into mitosis due to premature degradation of mitotic cyclins in G2. When cell cycle is arrested in G2 (or in G1; see [65]), GMC4-2a and GMC1-1a fail to divide into sibling neurons, but yet undergo terminal differentiation and adopt exclusively the fate of one of their sibling progeny (Table 1). GMC4-2a adopts exclusively RP2 identity and GMC1-1a—aCC identity, as demonstrated by expression patterns of several molecular markers (including EVE) and by analysis of their axonal projections.

Why is this choice not random with the GMCs always adopting only one out of two possible cell fates? Like neuroblasts, GMCs have apical–basal polarity and are known to divide asymmetrically with Numb protein localized as a basal crescent to ensure its asymmetric segregation into one sibling cell [53]. For example, during GMC4-2a division Numb is segregated preferentially into a sibling cell that later adopts the RP2 identity. Hence, the sibling cell that inherits Numb differentiates into the RP2 cell. In the absence of cell cycle progression Numb protein remains in GMC4-2a. When GMC4-2a fails to divide, it adopts the fate of the Numb-positive RP2. Conversely, when Numb is not present (due to *numb* loss-of-function mutation), cell fate of GMC progeny is equalized to that of a sibling cell that normally does not express Numb (RP2sib in GMC4-2a lineage, pCC in GMC1-1a lineage). Importantly, this cell fate choice occurs independently of cell division. In summary, in the absence of cell

cycle progression through G2/M transition, GMCs can undergo a binary fate switch and differentiate in the direction of either of their sibling progeny (e.g., GMC4-2a → RP2 or GMC4-2a → RP2sib). This decision depends on the presence or absence of Numb but, also, on the activation state of the Notch transmembrane receptor pathway (reviewed in [68,69]). When Numb is present and antagonizes Notch signaling in an undivided GMC4-2a, it adopts the identity of the RP2 progeny. Conversely, if the Notch pathway is constitutively activated (or Numb is lost), GMC4-2a adopts the RP2sib fate and GMC1-1a adopts the pCC identity. Hence, acquisition of the RP2sib (in the GMC4-2a lineage) or pCC identity (in the GMC1-1a lineage) as well as pIIa identity (in the pI bristle lineage; see [52]) requires activation of the Notch pathway. Finally, these cell fate choices can occur in the absence of another sibling cell and, therefore, do not require interaction with the sibling cell but depend on the presence of intrinsic cell fate determinants. In contrast, a balance of both extrinsic and intrinsic cues is important when the neuronal progenitor cell divides to form two sibling cells that adopt different identities. Signaling by Notch ligands Delta and Serrate originating from a sibling cell are extrinsic cues necessary for cell fate determination in the pI bristle lineage in the adult PNS [70]. In neuroblast lineages both extrinsic (Delta–Notch signaling) and intrinsic (e.g., Numb; see [9] and citations in this Section) cues regulate sibling cell fate choice.

Asymmetric divisions in the context of cell cycle progression have also been studied in mesodermal lineages in *Drosophila* [71]. During development of *Drosophila* heart, cardiac progenitors may undergo symmetric or asymmetric cell divisions to generate cardiac cell diversity. When cardiac progenitors divide asymmetrically, they produce sibling daughter cells of both myogenic and non-myogenic (pericardial) identity [72]. For example, progenitors of the Svp lineage divide asymmetrically to generate Svp myocardial cells and Svp-Odd pericardial cells and progenitors of the Eve lineage generate muscle founders DA1 and DO2 and Eve pericardial cells. Formation of cardiac cell types was examined in cell cycle mutants (*Cyclin A* and *Regulator of cyclin A1*) and in embryos in which cell cycle regulators *dacapo* (*dap*) and *retina aberrant in pattern* (*rap*, *fizzy-related*, *fzr*) were overexpressed. *dap* encodes a G1/S Cdk inhibitor related to vertebrate Cip/Kip family [73,74]. It binds to and inhibits activity of Cdk2/Cyclin E complex to maintain G1 arrest and, thus, is required for regulating exit from the cell cycle at the proper developmental time. RAP/FZR negatively regulates levels of Cyclin A, B, and B3, and is required during G1 to prevent an unscheduled accumulation of mitotic cyclins [75,76]. Loss-of-function mutations in *dap* or *rap* cause delay in normal cell cycle exit with many cells in embryonic epidermis completing an additional cycle of division.

When cell cycle was arrested, cardiac progenitors of asymmetric Eve and Svp lineages continued to differentiate and always adopted myogenic cell fate (where Numb is present and antagonizes Notch signaling; see [72]). If upon cell cycle

arrest Notch was activated (by removing Numb or by expressing constitutively active Notch), their progenitors adopted a non-myogenic pericardial cell fate (that is normally Numb-negative; see [72]). Hence, arrest of an asymmetric cell division leads the undivided progenitor to adopt the fate of a daughter cell that inherits Numb, and in the absence of Numb (or when Notch signaling is constitutively activated) the alternate, Numb-independent fate is chosen.

To date, the relationship between cell cycle progression and cell fate determination has been studied in several asymmetric cell lineages in *Drosophila* including GMC lineages in the CNS, SOP lineages in the PNS (see below), the bristle (microchaete) lineage in the adult fly and mesodermal lineages in the developing heart. It appears that in these model systems cell division per se is not essential for a progenitor cell to make a non-random cell fate choice and to adopt one of the two alternative cell fates. Rather it is determined by the activation state of the Notch signaling pathway, which in turn, depends on the presence or absence of Numb protein in a daughter cell. In the absence of cell cycle progression, the arrested progenitor always adopts a default cell fate identical to that of a wild-type sibling cell that inherits the Numb protein. This default cell fate is pIIb in the pI bristle lineage, RP2 in GMC4-2a (and aCC in GMC1-1a) lineage in the embryonic CNS, myogenic cell fate in Eve and Svp lineages in the cardiac mesoderm, and, finally [77], type II neuron in SOP lineages in the embryonic PNS. When cell division is blocked and SOPs in the embryonic PNS are arrested, they choose only one of two possible cell fate identities—type I (that give rise to external sense organs and chordotonal organs) or type II (give rise to multidendritic neurons) sensory neurons [77]. Again, as shown in other model systems of Numb-dependent asymmetric divisions, this binary choice is determined by the activation state of Notch signaling pathway. In the absence of cell cycle progression, SOP precursors differentiate preferentially into type II multidendritic neurons that inherit Numb, which in turn, keeps Notch pathway in an inactive state. Hence, for SOPs in the embryonic PNS the default neuronal fate is type II neuron. As expected, when Notch is lost, type I neurons (where Notch pathway is active) are missing while type II neurons are produced in excess [77].

5. Cell fate determination coupled to cell cycle progression

As we just discussed using four developmental model systems of asymmetric divisions in *Drosophila* as examples, it appears that there is no direct relationship between cell fate determination and cell cycle progression. Upon G2/M block, progenitor cells fail to enter mitosis and can adopt one of two alternative daughter cell fates. Despite the diversity of the four systems studied (GMC lineages in the CNS, SOP lineages in the PNS, pI bristle lineage in the adult fly and mesodermal lineages in the heart), they share one common feature. All four describe a *single* asymmetric terminal

division that produces two postmitotic cells that undergo terminal differentiation to produce specialized cells (neurons, glia and other support cells, myocardial or pericardial cells). In contrast, neuroblast divisions are different in nature. First, neuroblasts possess stem cell-like properties. They undergo a *series* of asymmetric divisions to produce pairs of mitotically active cells—another neuroblast (with a different identity) and a ganglion mother cell (Fig. 2). Second, each neuroblast has a unique identity. A neuroblast can be identified by: (1) specific position and time of delamination, (2) pattern of gene expression and (3) invariant cell lineage (and identity of the progeny produced). Neuroblasts generate distinct cell types (self-renewing neuroblasts and GMCs) in a specific order. Each GMC differs from other GMCs, even the ones from the same neuroblast lineage. GMCs from the same neuroblast lineage appear at different times in different segments of the embryo, express different sets of genes, and produce unique (neuroblast lineage-specific) neurons and glia. It appears that expression of GMC-specific genes (for example, *eve* in GMC4-2a, NB4-2 lineage) requires integration of multiple independent positive and negative transcription factor inputs that restrict gene expression to a single GMC within the neuroblast lineage [78].

Likewise, temporal cell fate specification of neuroblasts depends on their birth order and on a sequential transient expression of transcription factors [20,21]. All neuroblasts express sequentially four transcription factors—Hunchback (HB) → Krüppel (KR) → Nubbin (NUB, Pdm) → Castor (CAS). HB and KR are necessary and sufficient to specify early-born neuroblast cell fates. For example, neuroblasts in the NB7-3 lineage express sequentially HB, HB/KR (early-born), KR, KR/NUB, NUB (late-born) and divide three times to form three GMCs of unique identities. Their expression, transient in neuroblasts, is maintained in GMCs and in their differentiated progeny. However, unlike in GMC, SOP and mesodermal lineages, acquisition of successive neuroblast identities requires progression through the cell cycle. When neuroblasts were arrested at the G2/M transition (by mutating *string/cdc25*), they remained HB⁺ KR⁺ (and produced HB⁺ KR⁺ GMC) and failed to make a transition to HB⁻ KR⁺, NUB⁺, or CAS⁺ identity [21]. Similarly, in NB1-1 lineage expression of Even-skipped transcription factor is dependent on cell cycle progression [20]. In *string* mutants NB1-1 delaminates, but never divides and never adopts the EVE⁺ identity of its progeny GMC1-1a. Therefore, expression of transcription factors that specify identity of neuroblast progeny (succeeding neuroblasts, GMCs and eventually, terminally differentiated neurons) is strictly coupled to mitotic division. In the absence of cell division arrested neuroblasts retain their initial identity determined by the pattern of expression of transcription factors and do not acquire the pattern of expression (or identity) of their progeny.

Why is cell fate determination coupled to cell cycle progression in dividing neuroblasts, but not in GMC, SOP or mesodermal lineages? In addition to the unique features of neuroblast divisions already mentioned, another important

point to note is that their asymmetric divisions result in unequal distribution of cell fate determinant proteins between two daughter cells (a succeeding neuroblast and a GMC). For example, Prospero is asymmetrically localized to the cortex of the budding GMC during anaphase and upon completion of mitosis is translocated from the GMC cortex into the interphase GMC nucleus [27,28]. Nuclear PROS is required in GMCs to activate expression of GMC-specific genes [25,78]. This cell cycle-specific subcellular distribution of PROS is also cell cycle-dependent. Upon cell cycle arrest PROS fails to localize properly [25], fails to activate GMC-specific genes and to specify a proper identity of neuroblast progeny. Clearly, this effect of cell cycle arrest on cell fate determination is *cell-autonomous*. In contrast, cell cycle-independent cell fate determination described in GMC lineages in the CNS (and in SOP lineages in the PNS and in mesodermal lineages in the heart) involves *cell-extrinsic* factors, namely cell–cell interaction between two sibling cells. The directionality of this interaction depends on the activation state of the Notch signaling pathway, and in turn, on asymmetric distribution of Numb determinant into one of the two sibling cells (for details, see Section 4).

6. The role of asymmetry proteins in the regulation of cell proliferation

Key molecules that mediate asymmetric divisions of neuronal progenitor cells in the CNS and the PNS can also limit their proliferation potential. Two proteins Prospero and Bazooka appear to be required for the regulation of mitotic activity during nervous system development (Table 1), in addition to their well-established role in asymmetric division and in the specification of cell identity.

Drosophila Prospero is an evolutionary conserved homeodomain transcription factor expressed in neuronal lineages [25,26,79]. During neuroblast divisions, PROS is localized to the basal cortex and distributes asymmetrically only to one daughter cell, the GMC, that undergoes one final terminal division. Upon completion of mitosis, PROS translocates from cytoplasm into the nucleus of the GMC where it activates or represses cell type-specific genes to establish GMC cell fate [27,28]. In addition to its role in the specification of GMC cell fate, PROS also limits proliferation potential of GMCs by suppressing transcription of multiple cell cycle genes [80]. Loss of the *pros* gene results in continued mitotic activity that correlates with ectopic transcription of *string/cdc25*, *Cyclin A* and *Cyclin E* in late embryonic development. Conversely, expression of ectopic PROS leads to transcriptional repression of several cell cycle regulators (including *string/cdc25*, *Cyclin A*, *Cyclin E* and *E2f*). This down-regulation is restricted to cell cycle genes and is not a result of general shutdown of transcription by ectopic PROS. However, these experiments do not show whether all neuronal lineages undergo additional rounds of cell proliferation or how mitotic division pattern of individual GMCs

is affected. Clearly, there are lineages that show normal proliferation in *pros* mutants (NB6-4t [81], see also [65]) and hence, do not depend on PROS activity to execute their cell division program. In summary, PROS, upon its translocation from cytoplasm into the nucleus, is required in GMCs to repress transcription of multiple cell cycle regulatory genes and to prevent them from entering the next mitotic cycle. Such timely termination of cell proliferation is necessary for proper execution of developmental programs that include terminal differentiation and acquisition of a unique cell identity.

However, PROS may control cell cycle genes in different ways depending on cellular context. In contrast to its requirement to limit cell proliferation in dividing GMCs and to promote cell cycle exit, it appears to play just the opposite role in the lineage that produces longitudinal glia (LG) in the CNS [82]. Proliferation of the LG is regulated by neurons and Prospero appears to link glial proliferation and axon guidance. During growth cone guidance, PROS promotes glial proliferation in response to pioneer axons [82] by positively regulating the G1/S cyclin, Cyclin E [83,84]. In *pros* mutants, after neuronal contact there is a reduction in LG number (8 LG instead of the normal 10–12 produced by a single longitudinal glioblast) and loss of *Cyclin E* expression. Interestingly, PROS expression is maintained in a subset of the LG after the axonal bundles are formed and there are no more LG divisions. It appears that PROS is required in these glial precursors to maintain an immature, G1-arrested state. If neurons are ablated (or Cyclin E is ectopically expressed), only PROS-expressing LG cells are able to re-enter the cell cycle (but not if *pros* is mutated). In response to neuronal ablation (during development or upon spinal cord injury), LG undergo overproliferation in order to maintain a proper ratio between glial cells and neurons in the CNS. PROS maintains glial precursors in an undifferentiated state by antagonizing cyclin-dependent kinase inhibitor of the Cip/Kip family Dacapo (DAP). DAP binds to and inhibits the activity of the Cdk2/Cyclin E complexes and is required to maintain G1 arrest [73,74]. In longitudinal glia, PROS and DAP expression and function are mutually exclusive [82]. DAP promotes cell cycle exit and terminal differentiation of a subset of LG that do not express PROS. In contrast, PROS continues to be expressed in G1-arrested immature LG precursors and maintains their mitotic potential (and ability to respond to environmental cues in the CNS) by antagonizing DAP.

Depending on the developmental time point, PROS can both promote and antagonize *dap* transcription [85]. It appears that PROS translocation into the GMC nucleus is required for the timely activation of *dap* expression, arrest of cell proliferation, and terminal differentiation, as *pros* mutant embryos display a distinct delay in the appearance of *dap* transcripts [85]. However, early Prospero-positive GMCs do not express DAP, while DAP expression in the Prospero-positive neuroblast MP2 (that undergoes one terminal division similar to GMCs to produce two postmitotic neurons) is not dependent on Prospero or its translocation into the nucleus [86]. This observation argues against a universal

PROS-dependent mechanism operating in all GMCs to induce *dap* expression and to prevent further cell cycle progression. But *dap* expression is required to limit cell proliferation in some CNS lineages, since *dap* mutants have excess of Even-skipped and Eagle-positive neurons in the CNS at later stages [86]. In conclusion, nuclear Prospero regulates transcriptional activity of cell cycle genes and, depending on cellular context, can either inhibit cell proliferation and promote cell cycle exit (G0) and terminal differentiation (in GMCs) or can prevent cell cycle exit and maintain mitotic potential of G1-arrested precursors (PROS-positive LG with high level of Cyclin E). The latter is likely to be a fundamental mechanism operating in tissues that retain proliferative and regenerative capacity and respond to extrinsic cues to maintain tissue homeostasis during development and tissue repair.

A similar requirement for Cyclin E in the regulation of proliferation was reported for GMC4-2a [65]. Increasing function of CycE by either (1) overexpression or (2) maintaining high levels of two POU transcription factors Nubbin (NUB, Pdm) or Pdm2 (Mitimere) [87] or (3) attenuating function of Archipelago (AGO) required for degradation of CycE [88]—conferred self-renewing division potential to GMC precursor cells. Self-renewing GMCs retain high levels of CycE and loss of self-renewing proliferation ability coincides with the downregulation of CycE.

Drosophila PDZ domain protein Bazooka is required for apical–basal polarity in epithelial cells and for asymmetric localization of cell fate determinants, for spindle orientation, and to establish polarity of asymmetric divisions of neuroblasts [89–92]. In the PNS, BAZ is also asymmetrically localized in every dividing cell in the SOP lineage and is required for asymmetric localization of PON/Numb [93]. In *baz* mutant clones, PON–GFP fusion protein remains uniformly distributed in all four divisions of the pI lineage and never forms a crescent seen in dividing wild-type cells. More surprisingly, pIIa cells that normally divide only once to produce two external cells of the ES organ, undergo an additional round of cell division in *baz* mutant clones giving rise to a cluster of seven cells (as opposed to five in the wild-type pI lineage). Therefore, BAZ appears to limit the number of cell cycles of the pIIa to one. In addition, BAZ is required to promote differentiation instead of apoptosis, since loss of BAZ function in mitotic clones results in apoptosis of ES cell clusters in the pI lineage. To date, these roles of BAZ in executing cell cycle and differentiation programs have not yet been described in other tissues or in other model systems. It remains to be seen if these unexpected roles are unique to the SOP lineage in *Drosophila* PNS. It is also not clear why the ability of BAZ to restrict the number of divisions is limited to pIIa, although BAZ is expressed throughout the pI lineage. Analysis of *baz* mutant clones clearly showed that pI, pIIb and pIIIb divisions are not affected. What makes the pIIa cell (or its intrinsic cell cycle controls) different from other cells in the lineage, so that upon BAZ loss it can re-enter the cell cycle? Is it important from a biological or evolutionary

standpoint to have an additional mechanism to specifically limit the mitotic cycles of the pIIa cell that divides to form the shaft (hair) and the socket cells (external structures of the ES organ)? Finally, unlike with Prospero, Aurora-A or Cdc2, currently, we have very little understanding how a PDZ domain protein (Bazooka) can regulate the cell cycle machinery to promote cell cycle exit and, possibly, terminal differentiation.

7. Cell cycle control and lineage diversity

During *Drosophila* embryonic development, individual neuroblasts deriving from corresponding positions in thoracic and abdominal segments generally acquire similar fates. However, some of these serially homologous neuroblasts produce lineages that show segment specific differences [94,95]. This contributes to structural and functional diversity within the CNS. For example, in the NB6-4 lineage, thoracic NB6-4t neuroglioblast generates three glial cells and four to six neurons, while its counterpart in the abdominal segments (NB6-4a glioblast) divides only once symmetrically to generate two glial cells [96]. Notably, asymmetric division of NB6-4t results in distribution of cell fate determinant Prospero (PROS) and, possibly, Glial cells missing (GCM) transcription factor [97–99] only to the glial precursor cell [81,100] (but see also [101]). In contrast, NB6-4a divides symmetrically with both daughter cells expressing PROS and GCM.

It appears that downregulating levels of G1/S cyclin, *Cyclin E* (*CycE*) [83,84] by two homeotic genes *abdominal A* (*abd-A*) and *Abdominal B* (*Abd-B*) is required to specify the NB6-4a lineage [102]. Loss of *CycE* function causes homeotic transformation of NB6-4t to NB6-4a (but NB6-4a lineage is not affected), whereas ectopic expression of *CycE* (or loss of *abd-A* or *Abd-B* in corresponding abdominal segments) induces the opposite transformation, from NB6-4a to NB6-4t (Table 1). *CycE* acts upstream of *pros* and *gcm* to specify the neuronal sublineage. When *CycE* is mutated, *gcm* mRNA and PROS protein are strongly expressed in both daughter cells of NB6-4t and both differentiate as glial cells (like in wild-type NB6-4a lineage). Conversely, the NB6-4a-to-NB6-4t transformation leads to asymmetric distribution of PROS to one of the two progeny cells and, at later stages, increase in the number of cells (similar to wild-type NB6-4t lineage). Remarkably, *CycE* mRNA is expressed only in the NB6-4t lineage and, after the first division of NB6-4t, is detected only in the neuronal precursor, but not in the glial precursor. In other words, *CycE* is expressed in a cell lineage (NB6-4t neuroglioblast) where a progenitor cell undergoes more divisions and these divisions are either symmetric or asymmetric. In the NB6-4t lineage, *CycE* is detected in only one of the two sibling cells with distinct fates, namely in the neuronal precursor. In contrast, *CycE* is not expressed in a lineage (NB6-4a glioblast) characterized by a single symmetric division that gives rise to two cells of identical fate (two glial cells).

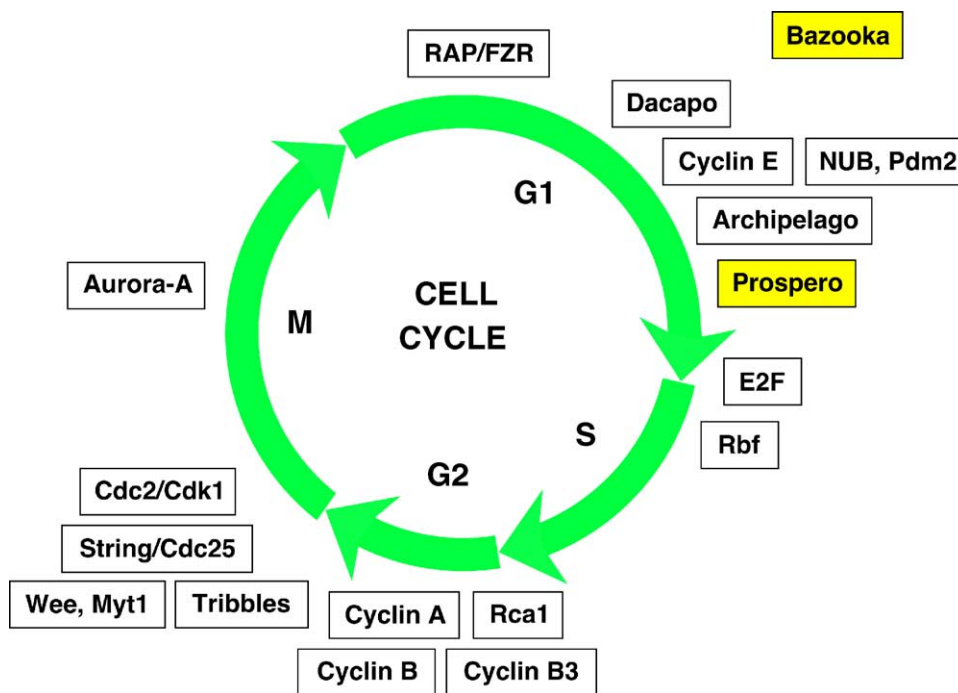


Fig. 4. Cross-talk between cell cycle and asymmetric division machineries. *Drosophila* cell cycle proteins that are also implicated in asymmetric divisions and/or cell fate determination in different model systems are shown at their respective positions in the cell cycle. Two asymmetry proteins (Prospero and Bazooka) required for regulation of cell proliferation are also shown, although how Bazooka regulates cell cycle progression remains unknown. Note that some proteins are known to be required only in one model system of asymmetric divisions (e.g., Aurora-A) or may regulate cell cycle in different ways in different cellular contexts (e.g., Prospero). See text for details.

What is the mechanism of asymmetric expression of *CycE* mRNA in the neuronal, but not glial, precursor? Does this asymmetric expression reflect asymmetric localization of CycE protein or *CycE* RNA that involves some of the known proteins required for asymmetric distribution of cell fate determinants in neuroblasts and GMCs? The subcellular localization of *CycE* protein or RNA in NB6-4 lineage remains uncharacterized, although it was reported that CycE protein does not form a cytoplasmic crescent during GMC4-2a division in the NB4-2 lineage [65].

Interestingly, other components of the cell cycle machinery (Cdc2/Cdk1, Cyclin A, Dacapo, E2f and Retinoblastoma-family protein (Rbf), a potent inhibitor of E2F target genes [103,104]) appear to play only a minor role [102] suggesting a critical role for CycE in regulating cell fate in segment-specific lineages. In summary, Cyclin E is necessary and sufficient for the specification of neuronal versus glial fate in the NB6-4 lineage and this function appears to be independent, at least in part, of its more established role in regulating cell proliferation. We expect that such segment-specific modulation of cell cycle regulators by homeotic genes is likely to be a general developmental mechanism to increase regional diversity of cell types in organisms with a segmented body plan.

8. Final remarks

Despite recent progress in our understanding of how cell cycle and asymmetry machineries cooperate (Fig. 4), it is obvious that we have barely scratched the surface of the problem and many questions remain unanswered. The connection between cell cycle and asymmetric division was probably an unexpected discovery. It has long been known that asymmetry proteins are dynamically expressed during mitosis and that their subcellular localization is cell cycle-dependent. However, few thought that the two machineries will share key regulators, like Cdc2/Cdk1 (e.g., in asymmetric neuroblast divisions in the CNS) and Aurora-A (in SOP lineage in the PNS) kinases. It is not yet clear if Cdc2/Cdk1 and Aurora-A play a more general, universal role—in regulating asymmetric divisions in other model systems. Although both are required for asymmetric localization of cell fate determinants, establishing polarity of division requires neither Cdc2 nor Aurora-A. However, the axis of polarity has to be coordinated, prior to cell division, with the body axes implying some unknown developmental and cell cycle controls.

Our knowledge about the role of cell cycle in regulating asymmetric divisions remains fragmented and spotty. Largely, it is due to the fact that almost all cell cycle regulators implicated in asymmetric divisions have been identified fortuitously in unrelated screens for mutations that affect neuroblast, GMC or SOP lineages. There were no systematic attempts to analyze function of key cell cycle regulators using either loss-of-function mutations or RNA interference *in vivo*. On the other hand, numerous genetic screens for mutations required for asymmetric division of

neuronal progenitors led to identification of only a handful of cell cycle regulators. Is the interface between cell cycle and asymmetric division machineries quite simple and involves few key regulators? We know that Cdc2 is one of them, but we have very little understanding how it links the two machineries. It was noted that Inscuteable has several putative Cdc2 phosphorylation sites. However, these sites appear to reside in regions of the protein not required for its function in asymmetric divisions as assessed in overexpression paradigms [105,106]. Future studies are likely to reveal not only the identity of the molecular link that allows cross-talk between cell cycle and asymmetric division, but also much more about the role of asymmetry proteins (Prospero, Bazooka and others) in regulating cell cycle progression.

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