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Coupling the cell cycle to development

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The core machinery that drives the eukaryotic cell cycle has been thoroughly investigated over the course of the past three decades. It is only more recently, however, that light has been shed on the mechanisms by which elements of this core machinery are modulated to alter cell cycle progression during development. It has also become increasingly clear that, conversely, core cell cycle regulators can play a crucial role in developmental processes. Here, focusing on findings from *Drosophila melanogaster* and *Caenorhabditis elegans*, we review the importance of modulating the cell cycle during development and discuss how core cell cycle regulators participate in determining cell fates.

Introduction

Extensive studies have led to a thorough understanding of the core mechanisms that drive the eukaryotic cell cycle (Box 1). It has also become increasingly clear that these core mechanisms are modulated during development. Such modulation is important, for instance, during *Drosophila* eye organogenesis, in which cell cycle synchronization is crucial for photoreceptor fate determination. Interestingly, recent findings demonstrate that components of the cell cycle machinery can in turn regulate development independently of their roles in cell cycle progression. For example, the cell cycle regulators Polo-like kinase 1 (PLK-1) and PLK-2 contribute to fate determination in early *C. elegans* embryos by phosphorylating proteins involved in the establishment of cell polarity. Overall, these and other observations underscore the tight coupling between the cell cycle and development.

In this review, we first provide an overview of the core features of the eukaryotic cell cycle. We then discuss mechanisms by which the cell cycle is modulated in different developmental settings, from the early embryo through to terminal cell differentiation. Finally, we consider how cell cycle regulators can in turn impart cell fate during development. The focus of this review is on *D. melanogaster* and *C. elegans*, two organisms in which the coupling of cell cycle progression and development has been well studied, with other systems included where appropriate. For additional information, we refer readers to reviews on the cell cycle that cover related material, including the link between cell cycle progression and cell growth or cancer, as well as the role of cell cycle modulation in plant development (see Barton et al., 2006; De Veylder et al., 2007; Giacinti and Giordano, 2006; Johnson and Degregori, 2006; Leevers and McNeill, 2005; Potter and Xu, 2001; Stanger, 2008).

The core eukaryotic cell cycle

The core eukaryotic cell cycle, which operates in most somatic cells, is composed of a synthesis (S) phase, a mitotic (M) phase and two intervening gap phases (G1 and G2; see Box 1). The core engines that drive the eukaryotic cell cycle consist of protein heterodimer complexes, each containing a cyclin and an associated kinase moiety. This group of kinases is referred to as cyclin-dependent kinases (Cdks), as kinase activity requires the presence of the cyclin (reviewed by Malumbres and Barbacid, 2009). In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, a single Cdk drives the cell cycle by binding to stage-specific cyclins (Hartwell et al., 1974; Nurse et al., 1976) (Table 1). In higher eukaryotes, it was initially posited that distinct Cdks regulate specific cell cycle phases or transitions (reviewed by Malumbres and Barbacid, 2009). This view was derived primarily from experiments with cultured mammalian cells, in which individual Cdks were depleted or inactivated. From such studies, Cdk4-Cyclin D and Cdk6-Cyclin D were implicated in committing a cell to the G1/S phase transition, Cdk2-Cyclin E in the initiation of S phase and Cdk2-Cyclin A in its completion, as well as in S phase exit. Furthermore, Cdk1-Cyclin A and Cdk1-Cyclin B were proposed to promote the onset of M phase, with Cdk1-Cyclin B also driving other mitotic processes. However, mouse knockout experiments targeting Cdk loci have demonstrated that Cdk1 is the only Cdk essential for survival in this species (Santamaria et al., 2007) (Table 1). Although dispensable for survival, Cdk4, Cdk6 and Cdk2 are required for cell cycle progression in specific mouse cell types (Berthet et al., 2003; Malumbres et al., 2004; Martin et al., 2003; Moons et al., 2002; Ortega et al., 2003; Rane et al., 1999). For example, Cdk4 controls the proliferation of pancreatic β cells and pituitary lactotrophs (Martin et al., 2003; Moons et al., 2002; Rane

Box 1. The core cell cycle in eukaryotes

A complete core eukaryotic cell cycle comprises four phases: the synthesis (S) phase, the mitotic (M) phase, and the two intervening gap phases, G1 and G2 (see figure). During S phase, the DNA is replicated, whereas during M phase, the replicated genetic material is segregated into the two resulting daughter cells. A fifth phase, known as G0, is a quiescent state that ensues when cells withdraw from the cell cycle under unfavorable growth conditions or upon terminal differentiation. The core engines that drive the progression through the eukaryotic cell cycle are complexes of cyclins and cyclin-dependent kinases (Cdks). For simplicity, the particular Cdks that regulate each cell cycle transition are not illustrated in the figure (see Table 1 for more information), and a single generic name is assigned to the cyclin species that typically regulate each cell cycle transition.

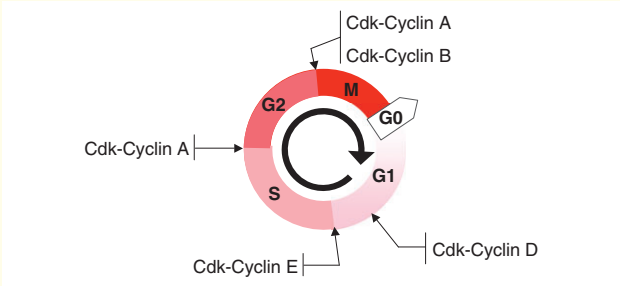

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Table 1. Essential Cdks in select species, their cyclin counterparts and the cell cycle phase or transition they primarily regulate

Organism	Cdks	Cyclin counterparts	Functions
S. cerevisiae	Cdc28	Cln1,2,3	G1
		Clb5,6	G1/S
		Clb3,4	G2/M
		Clb1,2	M
S. pombe	Cdc2	Cig1,2	G1/S
		Cdc13	G2/M and M
C. elegans	Cdk4 (CDK-4)	Cyclin D	G1/S
	Cdk2 (CDK-2)	Cyclin E	G1/S
	Cdk1 (NCC-1)	Cyclin B1,3	G2/M and M
D. melanogaster	Cdk2	Cyclin E	G1/S
	Cdk1	Cyclin A	G2/M
		Cyclin B,B3	G2/M
M. musculus	Cdk1	Cyclin D1,2,3	G1/S
		Cyclin E1,2	G1/S
		Cyclin A1,2	G2/M
		Cyclin B1,2,3	G2/M

et al., 1999), Cdk6 that of hematopoietic cells (Malumbres et al., 2004) and Cdk2 that of germ cells (Ortega et al., 2003). In contrast to the situation in the mouse, two Cdks are essential for survival in *Drosophila*: Cdk2, which is needed for S phase progression; and Cdk1, which is needed for M phase (Lane et al., 2000; Stern et al., 1993) (Table 1). Whereas Cdk4 is largely dispensable for cell proliferation in *Drosophila*, it is nevertheless required for cell growth, as Cdk4 mutant embryos develop into small adults (Meyer et al., 2000). Further specialization is encountered in *C. elegans*, in which all three Cdks are essential (Table 1). Here, CDK-4 is required for the G1/S transition (Boxem and van den Heuvel, 2001; Park and Krause, 1999), CDK-2 for S phase progression (Cowan and Hyman, 2006) and the Cdk1 NCC-1 for the onset of M phase (Boxem et al., 1999). Overall, although there is a strict functional specialization among Cdks in some metazoan organisms, substantial functional redundancy exists in other cases.

Regulating Cdk-Cyclin activity

The activity of Cdks is tightly regulated. As mentioned above, an essential step for Cdk activation entails the binding of a cyclin to the Cdk kinase moiety. In general, cyclins are synthesized periodically prior to the stage at which their activity is required and degraded thereafter. Such periodic oscillation ensures unidirectional cell cycle progression. As is the case for Cdks, there can be substantial functional redundancy among cyclins. In *S. pombe*, for example, the G2/M and M cyclin Cdc13 can drive the cell cycle in the absence of G1/S cyclins (Fisher and Nurse, 1996). Similarly, in *S. cerevisiae*, the overexpression of the G1/S cyclin Clb5 rescues cells that lack all three G1 cyclins under certain growth conditions (Epstein and Cross, 1992). Cyclin D and Cyclin E are also dispensable for survival in the mouse (Geng et al., 2003; Kozar et al., 2004). In *Drosophila*, as anticipated from the requirements of their respective Cdk counterparts, the absence of the Cdk4 partner Cyclin D results in smaller adults (Emmerich et al., 2004). By contrast, the Cdk2 partner Cyclin E is essential for S phase (Knoblich et al., 1994), and the Cdk1 partners Cyclin A and Cyclin B for M phase (Knoblich and Lehner, 1993; Lehner and O'Farrell, 1990). The requirements for cyclins mirror those for their Cdk counterparts in *C. elegans* as well. Thus, the CDK-4 partner CYD-1 is essential for the G1/S transition (Boxem and van den Heuvel, 2001; Park and Krause, 1999), the CDK-2 partner CYE-1 for S phase (Cowan and Hyman, 2006) and the B type Cyclins for M phase (Cowan and Hyman, 2006; Sonneville and Gönczy, 2004).

Besides binding to a cyclin subunit, Cdk activation requires its phosphorylation by a Cdk-activating kinase to fully open the Cdk catalytic cleft (reviewed by Kaldis, 1999). Despite the requirement of this phosphorylation event, Cdk-cyclin complexes are kept inactive by binding to a Cdk kinase inhibitor (CKI) and/or through inhibitory phosphorylation by the Wee1 and Myt1 kinases (reviewed by Malumbres and Barbacid, 2005; Pines, 1999). Cdk-cyclin activation is triggered once CKI is released and/or the inhibitory phosphate groups are removed through the action of Cdc25 phosphatase family members. Importantly, the initial activation of Cdk-cyclin results in the phosphorylation and thus in the further activation of positive regulators such as Cdc25, thereby generating a robust positive-feedback loop that irreversibly activates Cdk-cyclin.

Apart from these general mechanisms that regulate most Cdks, specific Cdks can be regulated by additional components. For example, Cdk1-Cyclin B is activated by the serine/threonine protein kinases Aurora A and by Polo-like kinase 1 (Plk1). Aurora A, in conjunction with its partner proteins Ajuba and Bora (Hirota et al., 2003; Hutterer et al., 2006), is thought to promote Cdk1-Cyclin B activity by phosphorylating and thereby activating Cdc25B (Dutertre et al., 2004). Plk1 activates Cdk1-Cyclin B by phosphorylating Cyclin B and Cdc25, thus activating them, as well as by phosphorylating Myt1, thus inactivating it (Inoue and Sagata, 2005; Jackman et al., 2003; Kumagai and Dunphy, 1996; Nakajima et al., 2003; Qian et al., 2001; Roshak et al., 2000). Overall, the above examples underscore the notion that the activity of Cdk-cyclin complexes is tightly regulated.

Progression through the core cell cycle

The further modulation of Cdk-cyclin complex activity allows for the proper progression through the core eukaryotic cell cycle. During G1, cells assess whether to commit to S phase based, notably, on the availability of growth factors and nutrients (reviewed by Vermeulen et al., 2003). When this commitment is made in mammalian cells, Cdk4-Cyclin D and Cdk6-Cyclin D phosphorylate the tumor-suppressor retinoblastoma protein (Rb). Whereas unmodified Rb sequesters the transcription factor E2F, Rb phosphorylation results in the release of E2F, which induces the transcription of genes required for the G1/S transition and for S phase, including Cyclin E and Cyclin A. The Cdk2-Cyclin E complex further phosphorylates Rb, whereas E2F stimulates its own transcription, which together create a positive-feedback loop that promotes S phase entry. During S phase, a checkpoint operates to block cell cycle progression whenever replication forks are stalled or DNA is damaged, and ensures that progression resumes only after such problems have been fixed. This S phase checkpoint relies on a signaling cascade that includes the PI3-related kinases Ataxia Telangiectasia Mutated (ATM) and ATM-related (ATR), as well as the effector kinases Checkpoint Kinases 1 and 2 (Chk1 and Chk2) (reviewed by Sancar et al., 2004). These effector kinases prevent cell cycle progression by phosphorylating and thereby modifying the activity of substrates that impinge on Cdk-cyclin activity, including Cdc25. A related checkpoint operates during G2 to ensure that the DNA is intact and fully replicated prior to entry into mitosis. Upon Cdk-Cyclin A and Cdk-Cyclin B activation, cells enter mitosis (reviewed by Lindqvist et al., 2009). The degradation of Cyclin A during prometaphase enables progression to metaphase (den Elzen and Pines, 2001), whereas the degradation of Cyclin B during the

metaphase to anaphase transition leads to Cdk1 inactivation and, consequently, to exit from mitosis. In summary, the precisely orchestrated modulation of Cdk activity is necessary for orderly progression through the core eukaryotic cell cycle.

Cell cycle modulation during development

In this section, we discuss how the core cell cycle is modulated as development unfolds. We first consider the simplified cell cycle devoid of gap phases that characterizes the rapidly dividing cells of early embryos. We then discuss how the complexity of the cell cycle is increased by the introduction of a G2 phase at the mid-blastula transition (MBT). Thereafter, we illustrate how the regulation of the G1/S transition can be modulated during organogenesis, and also how endoreplication cycles can contribute to this process by generating polyploid tissue. We conclude this section by considering a situation in which a developmental program is terminated by permanent exit from the cell cycle. An overview of the different types of cell cycle mentioned in this section and of the principal components that regulate them is given in Fig. 1.

Modulating the transition between S and M phase during early embryogenesis

Whereas most proliferating cells have cell cycles in which S and M phases are separated by G1 and G2 phases, this is not the case in the early embryos of many species, in which the two gap phases are lacking (Fig. 1A). In these early embryonic cycles, cell cycle regulators are contributed maternally and used gradually until the mid-blastula transition (MBT), which marks the onset of broad transcription from the zygotic genome. The gradual decrease of maternally contributed components has consequences for cell cycle progression during early embryogenesis. For instance, in the syncytial *Drosophila* embryo, the duration of S phase (interphase) increases steadily after cycle 7, whereas that of M phase remains constant (Ji et al., 2004). This steady increase in interphase duration parallels the gradual decrease in the maternal pool of Cyclin B (Edgar et al., 1994), presumably reflecting the fact that increasingly more time is needed to accumulate sufficient Cyclin B to enter mitosis. Accordingly, interphase duration is already lengthened at cycle 6 in embryos with only a single copy of *CycB*, as opposed to the normal two copies present in the wild type, and is lengthened only at cycle 9 in embryos harboring four copies of *CycB* (Ji et al., 2004). Interphase duration in wild-type embryos increases further in cycles 10-13, correlating with a further decline in Cyclin B, as well as a decrease in Cyclin A levels (Edgar et al., 1994). The progression from cycle 10 to cycle 13 is severely delayed in embryos heterozygous for *CycB* and *CycA*, and is accelerated in embryos with six copies of *CycB*, which suggests that the levels of Cyclin B and Cyclin A set interphase duration during these cycles (Crest et al., 2007; Edgar et al., 1994). However, reducing the copy number of *CycB* from two to one does not significantly delay mitotic entry further at cycle 12 in embryos depleted of Cyclin A and Cyclin B3, although mitosis is abortive in such embryos (McCleland et al., 2009). Whereas these findings establish that the levels of Cyclin B are not sufficient to set interphase duration under these conditions, it remains possible that Cyclin B levels are upregulated in such embryos to compensate for the loss of Cyclin A and Cyclin B3. Overall, it is clear that the levels of maternally contributed mitotic cyclins are crucial for determining interphase duration in early *Drosophila* embryos.

Besides mitotic cyclins, the checkpoint components ATR (also known as Mei-41) and Chk1 (also known as Grapes, Grp) are the principal regulators of interphase duration in cycles 11-13 of

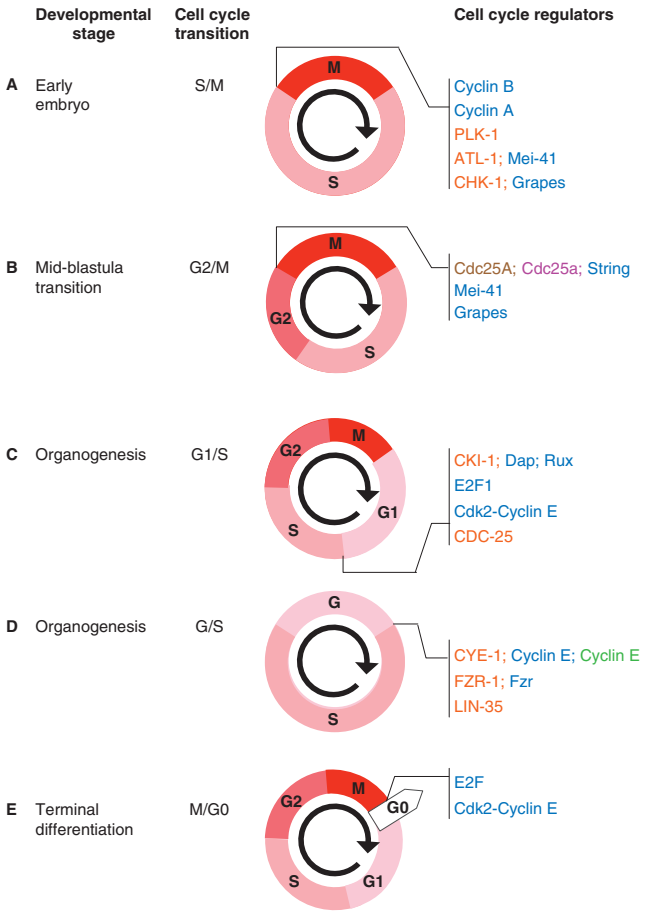


Fig. 1. Modulation of the cell cycle during development. Schematic of the key developmental stages, cell cycle transitions and principal cell cycle regulators discussed in the main text. Homologous cell cycle regulators are listed on the same line. Proteins from *Drosophila* are shown in blue, *C. elegans* in orange, *Xenopus* in brown, zebrafish in purple and mammals in green. (**A**) In the early embryo of many species, the cell cycle is driven by maternal components and alternates between S and M phases. In *Drosophila*, this embryonic cycle is regulated notably by Cyclin B and Cyclin A, as well as by Mei-41 (ATR) and Grapes (Chk1); in *C. elegans*, important regulators of the embryonic cycle include the Polo-like kinase PLK-1, as well as ATL-1 (ATR) and CHK-1 (Chk1). (**B**) At the mid-blastula transition (MBT), a broad switch from maternal to zygotic transcription is initiated and a G2 phase is introduced into the cell cycle in many species. The phosphatase Cdc25 is important for regulating MBT in *Drosophila*, *Xenopus* and zebrafish. (**C**) Later during development, when organs are forming, a G1 phase is introduced into the cell cycle in certain lineages, such as the intestine of *C. elegans* and the developing eye of *Drosophila*. The regulation of the G1/S transition in intestinal cells of *C. elegans* requires the Cdk inhibitor (CKI) CKI-1 and CDC-25, whereas that in the developing eye of *Drosophila* requires the CKI Dacapo (Dap) and the CKI-like Rux, as well as the transcription factor E2F and Cdk2-Cyclin E. (**D**) In some instances of organogenesis, endoreplication cycles are present, in which successive S phases take place without intervening M phases. Endoreplication in *Drosophila*, *C. elegans* and mammalian cells requires Cyclin E. Besides Cyclin E, endoreplication in *Drosophila* also requires the APC activator Cdc20/Fizzy-related (Fzr), whereas endoreplication in *C. elegans* also requires FZR-1 and the tumor suppressor retinoblastoma (Rb) protein LIN-35. (**E**) Upon terminal differentiation, cells often exit the cell cycle (G0). Terminal differentiation during *Drosophila* eye and wing development requires suppression of the activity of E2F and Cdk2-Cyclin E.

Drosophila embryos (Sibon et al., 1999; Sibon et al., 1997). In the wild type, Mei-41 and Grp are strongly activated in cycles 11-13, presumably in response to an increased nuclear:cytoplasmic ratio, which titrates out limiting maternal components required for DNA replication (Sibon et al., 1999). As a result, interphase duration is lengthened in the wild type, but not in *mei-41* or *grp* mutant embryos. Experiments in which the copy number of *CycB* is altered in *mei-41* or *grp* mutant embryos indicate that the checkpoint is already functional prior to cycle 11, but that its activity is normally masked by high maternal Cyclin B levels (Crest et al., 2007). In summary, whereas the slight reduction in Cyclin B levels in cycles 7-9 has a modest impact on cell cycle progression, the more drastic reduction of Cyclin B and Cyclin A in cycles 11-13, together with the strong activation of Mei-41 and Grp, significantly increases interphase duration. Therefore, developmental cues regulate cell cycle progression during early *Drosophila* embryogenesis through the availability of maternal components.

Checkpoint components also regulate interphase duration in early *C. elegans* embryos. The first cleavage division of *C. elegans* embryos is unequal, giving rise to a larger anterior blastomere, AB, and a smaller posterior blastomere, P_1 (Fig. 2A). Interphase duration differs between these cells, with AB entering mitosis before P_1. This difference is regulated by developmental anteroposterior (AP) polarity cues established by the partitioning defective (PAR) proteins and associated components, including actin and non-muscle myosin 2. When these components are absent, the two blastomeres enter mitosis synchronously. Two mechanisms mediate the difference in interphase duration between AB and P_1. The first mechanism involves the checkpoint proteins ATL-1 (ATR) and CHK-1 (Chk1) (Brauchle et al., 2003). When ATL-1 and CHK-1 are depleted by RNA interference (RNAi), interphase duration is shortened slightly in AB, but more so in P_1, leading to a ~40% reduction in the difference of interphase duration. Why should ATL-1 and CHK-1 activity differ between the two blastomeres? This might stem from the unequal distribution of CHK-1, which associates with ribonucleoprotein P granules and is thus enriched in P_1 (Kim et al., 2007). Alternatively, the preferential checkpoint activation in P_1 could stem from the first cleavage division being unequal (Brauchle et al., 2003). By analogy with the situation in cycle 11-13 *Drosophila* embryos, one or several maternally contributed components might be limiting for DNA replication in early *C. elegans* embryos. If such components were uniformly distributed in the one-cell stage, the smaller P_1 would inherit fewer of them than the larger AB. As the same amount of DNA must be synthesized in the two blastomeres, preferential ATL-1 and CHK-1 activation would occur in P_1 to ensure complete DNA replication. Compatible with this hypothesis, the difference in interphase duration between the two blastomeres is reduced by ~40% when the first division is made equal in the absence of polarity defects (Brauchle et al., 2003). Such a reduction would not be expected if preferential checkpoint activity in P_1 relied on asymmetric CHK-1 distribution because, in the absence of polarity defects, P granules and thus presumably also CHK-1 are still enriched in P_1. Given that equal cell division reduces the time difference to the same extent as ATL-1 or CHK-1 depletion, and that the combined depletion of these components in embryos undergoing an equal first division does not have a significant further impact (Brauchle et al., 2003), it appears that differential checkpoint activation occurs largely through a size-dependent mechanism.

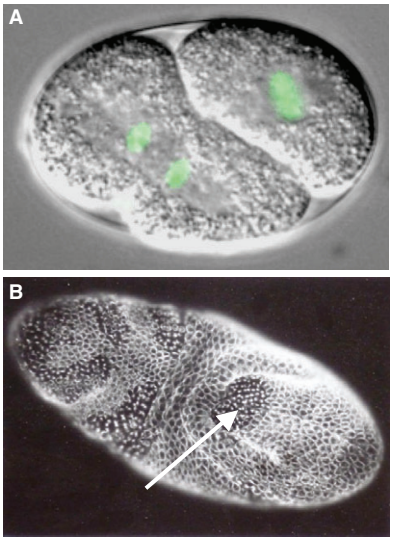


Fig. 2. Lineage-specific cell cycle progression during *C. elegans* and *Drosophila* embryogenesis. (**A**) A two-cell stage *C. elegans* embryo that carries a GFP-Histone 2B fusion protein imaged using dual time-lapse differential interference contrast (DIC) and fluorescence microscopy. The DIC signal and the GFP signal (pseudocolored in green) are overlaid. The embryo is ~50 μm long. AB (left) and P_1 (right) exhibit different cell cycle timing: while AB is already in anaphase, as indicated by the separation of sister chromatid pairs, P_1 is still in metaphase. Anterior is to the left. (**B**) A dorsal view of a cycle 14 *Drosophila* embryo stained with anti β-tubulin antibodies. The embryo is ~500 μm long. The arrow points to a mitotic domain, recognizable by the fact that all cells within this domain have a mitotic spindle, in contrast to the surrounding cells in which the β-tubulin signal is cytoplasmic. Anterior is to the left. Figure courtesy of Victoria Foe (Center for Cell Dynamics, University of Washington).

Given that ATL-1 and CHK-1 regulate only ~40% of the time difference in interphase duration between AB and P_1, at least one additional, size-independent mechanism must account for the remaining difference. The existence of such a mechanism has also been indicated by microsurgery experiments showing that AB enters mitosis earlier, even when made smaller than P_1 through the removal of cytoplasmic material (Schierenberg and Wood, 1985). This second mechanism appears to involve PLK-1, a positive regulator of Cdk-Cyclin B activity. PLK-1 is distributed asymmetrically in two-cell stage embryos, with more protein accumulating in AB than in P_1 (Budirahardja and Gönczy, 2008; Chase et al., 2000; Nishi et al., 2008; Rivers et al., 2008). The asymmetric distribution of PLK-1 is independent of cell size (Budirahardja and Gönczy, 2008), but is regulated by AP polarity cues (Budirahardja and Gönczy, 2008; Nishi et al., 2008; Rivers et al., 2008). Experiments in which PLK-1 is mildly depleted by RNAi, which bypasses earlier requirements for PLK-1 during meiosis and the first mitosis, establish that under these conditions, interphase duration is lengthened only in P_1; as a result, the difference in interphase duration between the two blastomeres is increased (Budirahardja and Gönczy, 2008). Additional experiments indicate that this effect of PLK-1 is independent of ATL-1 and CHK-1 (Budirahardja and Gönczy, 2008). These findings raise the question as to which substrates PLK-1 phosphorylates to promote entry into mitosis. CDC-25 has been proposed as a candidate in this context (Rivers et al., 2008). Nuclear CDC-25 also accumulates asymmetrically in two-cell stage embryos, with more protein present in AB, an asymmetry that is regulated by AP polarity cues and PLK-1. Moreover, the phenotype upon partial CDC-25 depletion resembles that observed upon partial PLK-1 depletion, with the difference in interphase duration

increasing between AB and P_1 (Rivers et al., 2008). However, other PLK-1 substrates probably also promote mitotic entry in the wild type, as P_1 enters mitosis with less nuclear CDC-25 than does AB. Such substrates might reside at centrosomes, where PLK-1 is enriched (Chase et al., 2000), especially considering that centrosomes dictate the timing of mitotic entry in one-cell stage *C. elegans* embryos (Hachet et al., 2007).

In summary, developmental AP polarity cues regulate the differential timing of interphase duration in two-cell stage *C. elegans* embryos through two mechanisms. First, they ensure an unequal first division, which results in preferential ATL-1 and CHK-1 activation in P_1. Second, AP polarity cues ensure the presence of more PLK-1 in AB. Together, these two mechanisms couple AP polarity cues with cell cycle progression during early development. What is the significance of such coupling? One plausible answer is that it ensures the proper cell-cell contacts that are crucial for cell fate determination. Because of the asynchrony in mitotic entry at the two-cell stage, the surrounding eggshell invariably pushes one of the AB daughter cells, ABp, to occupy a posterior position in the embryo. As a result, ABp is juxtaposed to P_2, the posteriorly located daughter cell of P_1, a position that is crucial for enabling P_2 to induce dorsal fates among the descendants of ABp through Notch (GLP-1) signaling (reviewed by Gönczy and Rose, 2005).

Modulating the transition from G2 to M phase at the MBT

Whereas the cell cycle oscillates between S and M phases in the early embryos of many species, gap phases are typically introduced at the MBT, which marks the onset of broad zygotic transcription (Fig. 1B). For example, in *Drosophila* embryos, a G2 phase is introduced at cycle 14 after the degradation of two maternal Cdc25 transcripts, String (Stg) and Twine, at the end of cycle 13 (Edgar and Datar, 1996). The resulting reduced Cdk1 activity is essential to initiate robust zygotic transcription at cycle 14. When Cdk1 inactivation is incomplete, as in *mei-41* or *grp* mutant embryos, interphase duration remains short, and zygotic transcription is severely impaired (Sibon et al., 1999; Sibon et al., 1997). Cdk1 inactivation, rather than extended interphase duration, is essential for zygotic transcription, as reduced Cyclin A and Cyclin B levels in *mei-41* mutants, which are expected to lower Cdk1 activity, rescue zygotic transcription, but not interphase duration (Sibon et al., 1999). The RNA-binding protein Smaug (Smg) is a broad regulator of the switch from maternal to zygotic transcription and notably ensures the degradation of maternal Stg transcripts at the end of cycle 13 (Benoit et al., 2009). Smg recruits the deadenylase complex, which removes poly(A) tails from maternal transcripts, thus targeting them for degradation (Semotok et al., 2005; Semotok et al., 2008; Zaessinger et al., 2006). Smg inactivation leads to maternal transcript stabilization and to the inhibition of zygotic transcription. In addition, Smg inactivation shortens the interphase duration of cycles 11-13 by somehow interfering with DNA checkpoint activation (Benoit et al., 2009). Smg modulates *Stg* mRNA through its requirement for the zygotic transcription of *fruhstart* (*frs*; *Z600* – FlyBase), which itself promotes *Stg* mRNA degradation (Grosshans et al., 2003). Overall, Smg plays an indirect role in controlling cell cycle timing, presumably by promoting checkpoint activation and *frs* transcription. Furthermore, Smg regulates the timing of cellularization, a process in which membranes form to encase each previously syncytial nucleus and that coincides with the MBT (Benoit et al., 2009). Whereas low Smg levels delay cellularization, excess Smg does not accelerate the process, despite cells terminating division earlier at the end of mitosis 12. Therefore, factors other than Smg are limiting for MBT timing. Moreover, these observations imply that a developmental clock, rather than the number of cell divisions, determines MBT timing. Accordingly, the depletion of all mitotic cyclins (Cyclin A, Cyclin B and Cyclin B3) by RNAi, which also results in the premature termination of cell division, does not alter the timing of cellularization either (McCleland and O'Farrell, 2008).

After the global switch from the maternal to the zygotic genome has occurred under the control of Smg, entry into mitosis at cycle 14 is dictated by zygotic *stg* expression. This occurs at different times and in different domains of the embryo at cycle 14, owing to developmental cues that act on a rich array of cis-acting elements within the *stg* promoter (Edgar and O'Farrell, 1989; Lehman et al., 1999). Importantly, these *stg* expression domains predict the pattern of the mitotic domains that follow at the end of cycle 14 (Fig. 2B) (Edgar and O'Farrell, 1989; Foe, 1989). Whereas *stg* zygotic mutant embryos arrest in the G2 phase of cycle 14, ectopic *stg* expression during cycles 14-16 induces premature mitotic entry and partial embryonic lethality (Edgar and O'Farrell, 1990). Together, these results indicate that Stg levels are limiting for mitotic entry in cycles 14-16, and also that alterations in division timing might contribute to defective embryogenesis.

Similar to the situation in *Drosophila*, a G2 phase is introduced at the MBT in zebrafish and *Xenopus* embryos (Dalle Nogare et al., 2008; Shimuta et al., 2002). Cdc25 homologs also regulate the G2/M transition in these systems, albeit with slight differences in the underlying mechanisms compared with *Drosophila*. Cdc25 overexpression in zebrafish or abnormal Cdc25 stabilization in *Xenopus* results in embryonic lethality, further underscoring the importance of proper cell cycle regulation during embryogenesis (Dalle Nogare et al., 2008; Shimuta et al., 2002). Besides Cdc25, Cyclin E has also been proposed to control the MBT in *Xenopus*. The decrease in maternal Cyclin E pools correlates with MBT timing (Howe and Newport, 1996), whereas Cdk2-Cyclin E inhibition through the injection of a truncated form of the Cdk inhibitor Xic1 delays cell cycle progression and MBT (Hartley et al., 1997). However, Cyclin E depletion using antisense oligonucleotides does not affect MBT timing (Slevin et al., 2005), but as Cyclin E mRNA might not have been completely eliminated in this study, it remains to be ascertained whether Cyclin E plays a role in controlling MBT timing in *Xenopus*. Interestingly, Cyclin E protein levels decrease independently of transcription, protein synthesis, cell cycle progression and nuclear:cytoplasmic ratio, all of which affect MBT timing. Therefore, if the decrease of maternal Cyclin E protein were crucial for this transition, it could be thought of as being an integral part of an MBT clock (Howe and Newport, 1996).

Overall, a conserved strategy at the MBT entails the introduction of a G2 phase through the modulation of Cdk-cyclin activity. The duration of the cell cycle does not appear to have a direct impact on this developmental transition, as, for instance, zygotic transcription initiates in a timely manner even when interphase duration is shortened in *Drosophila* embryos (Sibon et al., 1999; Takada et al., 2007). Instead, the introduction of a G2 phase at this stage could reflect the need to inactivate Cdk1 to allow the initiation of zygotic transcription.

Modulating the G1 to S phase transition during organogenesis

As development proceeds, the cell cycle becomes increasingly more complex and usually comprises all four phases of the core cell cycle (Fig. 1C). As is the case in tissue culture cells, the G1/S transition

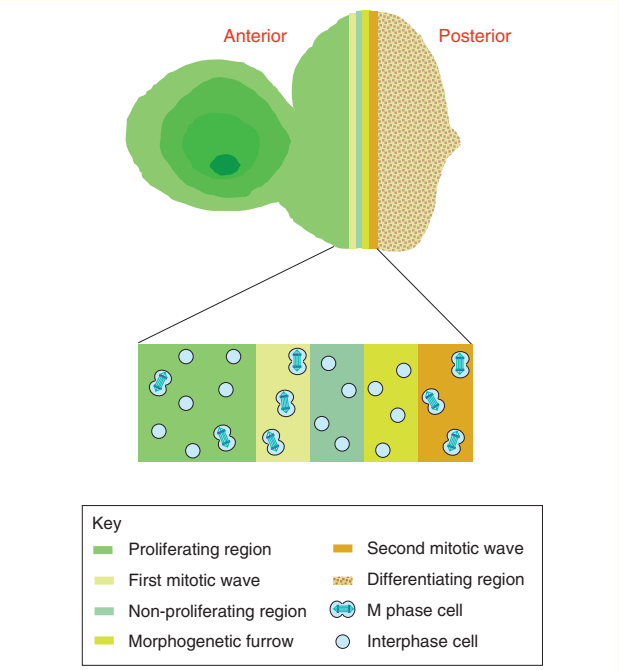
now becomes a prime target for regulation by developmental cues, as illustrated below for organogenic processes in *C. elegans* and *Drosophila*.

The *C. elegans* intestine is formed by descendants of the founder cell E, which divides four times during embryogenesis to give rise to 16 cells. Four of these cells divide once more to generate the final 20 intestinal cells that are present at the time of hatching. The number of cells in the E lineage is regulated by two parallel mechanisms acting at the G1/S transition, which involve CDC-25 and CKI-1, respectively. Regarding the first mechanism, maternal CDC-25 is present until the 28-cell stage in wild-type embryos and targeted thereafter for degradation by the β-TrCP protein LIN-23, an F-box protein that is part of an Skp–Cullin–F-box (SCF) E3 ubiquitin ligase (Hebeisen and Roy, 2008). When CDC-25 is stabilized, as is the case in two gain-of-function (gf) *cdc-25* mutant alleles, additional rounds of proliferation occur in the E lineage, generating a total of ~38 intestinal cells (Clucas et al., 2002; Kostic and Roy, 2002). Despite this increase, intestinal function does not appear to be affected, which indicates that some variability in final cell number is tolerated in this instance (Clucas et al., 2002). CDC-25 regulates E lineage proliferation by promoting the G1/S transition, as the partial depletion of CYE-1 in *cdc-25* gf animals suppresses the formation of additional intestinal cells (Kostic and Roy, 2002). The reason why CDC-25 stabilization specifically affects the E lineage might be that zygotic LIN-23 is highly expressed in all tissues except the intestine, which might therefore be particularly sensitive to increased CDC-25 levels (Hebeisen and Roy, 2008). The second mechanism acting at the G1/S transition involves CKI-1, which binds to and inactivates CDK-2-Cyclin E (Fukuyama et al., 2003; Hong et al., 1998). CKI-1 depletion results in extra cycling, with ~29 intestinal cells at the time of hatching. Simultaneous CDC-25 stabilization and CKI-1 depletion further increases this number to ~45, which suggests that the two proteins act via distinct mechanisms (Kostic and Roy, 2002).

Another interesting developmentally regulated G1/S transition occurs during *Drosophila* eye organogenesis. During the third larval stage, a wave of differentiation sweeps across the eye imaginal disc (see Box 2 for an overview of this process). The front of this wave is marked by a depression in the disc epithelium called the morphogenetic furrow (MF). Anterior to the MF, cells undergo a first mitotic wave that is synchronized by Decapentaplegic (Dpp) and Hedgehog (Hh) signaling (Escudero and Freeman, 2007). These developmental cues presumably act upon *Stg* expression, as *Stg* RNA is upregulated in these cells in a Dpp- and Hh-dependent manner (Escudero and Freeman, 2007; Thomas et al., 1994). Subsequently, cells arrest in G1 in the non-proliferating region (NPR) through the action of the same signaling molecules. Cell cycle arrest in the anterior part of the NPR is regulated by Dpp through the downregulation of Cyclin E levels and E2F activity (Escudero and Freeman, 2007), whereas arrest in the posterior part of the NPR is controlled by Hh, through the upregulation of expression of the CKI Dacapo (Dap) and the repression of E2F expression (Avedisov et al., 2000; Escudero and Freeman, 2007; Thomas et al., 1997). Arrest in the posterior part of the NPR is further regulated by the CKI-like protein Roughex (Rux), which targets Cyclin A for degradation and also inhibits Cdk2-Cyclin A activity (Avedisov et al., 2000; Thomas et al., 1997). After a second mitotic wave, the major player that controls the subsequent G1/S transition is Cdk2-Cyclin E (Sukhanova and Du, 2008). Moreover, certain components of the Notch signaling pathway play a role in this context (Baonza and Freeman, 2005; Firth and Baker, 2005; Sukhanova and Du, 2008). Removing either the Notch receptor itself or the transcription factor Suppressor of Hairless [Su(H)] prevents S phase entry. Whereas Su(H) controls this G1/S transition by regulating Dap expression (Sukhanova and Du, 2008), the mechanism by which Notch functions here remains to be clarified, but might entail Cyclin A modulation, as Cyclin A levels are reduced in *Notch* mutant cells (Baonza and Freeman, 2005). The appropriate modulation of cell cycle progression is crucial for pattern formation during *Drosophila* eye organogenesis. For instance, in *rux* mutant imaginal discs, cells do not arrest in G1 in the posterior part of the NPR, which results in the defective organization and fate determination of the R8 photoreceptor cell (Thomas et al., 1994).

Taken together, these findings in *C. elegans* and *Drosophila* illustrate how the proper modulation of the G1/S transition can be crucial for pattern formation during organogenesis.

Box 2. Cell cycle progression during development of the eye imaginal disc in *Drosophila* third instar larvae



Well ahead (left in figure) of the morphogenetic furrow (MF), cells progress asynchronously through the cell cycle. Just ahead of the MF, cells become synchronized in mitosis during the first mitotic wave and are then held in G1 in a non-proliferating region. Behind the MF, cells either directly differentiate into photoreceptors or enter a final synchronous S phase, which is followed by a second mitotic wave and subsequent differentiation.

Endoreplication: accumulating DNA during organogenesis

There are several instances during animal development where cells become polyploid (i.e. have more than two sets of chromosomes) owing to endoreplication cycles in which successive S phases occur without intervening mitoses (Fig. 1D). Endoreplication is thought to be important for ensuring increases in gene expression, metabolic output or improved resistance against DNA damage through the presence of multiple copies of each gene (reviewed by Edgar and Orr-Weaver, 2001; Lilly and Duronio, 2005).

During *Drosophila* development, cells in many tissues, including the gut, fat body, Malpighian tubules, trachea and salivary glands, initiate endoreplication cycles. These cycles are regulated mainly by Cdk2-Cyclin E activity. The *CycE* gene is transcribed prior to the onset of endoreplication cycles and is required for this process (Knoblich et al., 1994). As expected, factors that regulate Cyclin E levels or Cdk2-Cyclin E activity control endocycles. This is the case for E2F, which promotes *CycE* transcription (Duronio and O'Farrell, 1995; Sauer et al., 1995), as well as for Dap, which inactivates Cdk2-Cyclin E, and the F-box protein Archipelago, which degrades Cyclin E (de Nooij et al., 2000; Hong et al., 2003; Moberg et al., 2001). Cdk2-Cyclin E activity oscillates during endocyling, and *CycE* mRNA oscillates in endocycling gut and larval tissues (Duronio and O'Farrell, 1995), whereas Cyclin E protein oscillates in endocycling ovarian nurse cells (Lilly and Spradling, 1996). Cyclin E negatively regulates its own transcription (Sauer et al., 1995), probably by promoting Dap accumulation (de Nooij et al., 2000). The oscillation of Cdk2-Cyclin E activity is important for endocycling. Indeed, continuous Cyclin E overexpression blocks endoreplication, presumably because a reduced Cdk2-Cyclin E activity after S phase is necessary for assembling pre-replication complexes anew (Follette et al., 1998; Weiss et al., 1998). Apart from Cdk2-Cyclin E oscillation, endocycles also require a reduction in several mitotic regulators, including Cdk1, Cyclin A, Cyclin B and Stg (Sauer et al., 1995), which can occur either at the transcriptional or the post-translational level (Narbonne-Reveau et al., 2008).

Cyclin E is also a crucial regulator of endocycles in *C. elegans*. At the end of the first larval stage, the posterior intestinal cells undergo a single nuclear division (karyokinesis), which is followed by one endoreplication cycle at each larval molt, ultimately generating cells with a 32N DNA content (where N is the haploid number of chromosomes). The switch between karyokinesis and endoreplication is regulated by the Rb protein LIN-35 through the suppression of *cye-1* transcription (Ouellet and Roy, 2007), as well as by the APC cofactor Cdc20/Fizzy-related-1 (FZR-1), presumably through Cyclin E degradation (Fay et al., 2002). When either LIN-35 or FZR-1 is compromised, CYE-1 levels increase, which triggers additional rounds of karyokinesis that result in supernumerary intestinal nuclei (Fay et al., 2002; Ouellet and Roy, 2007). Whereas the initial switch from karyokinesis to endoreplication requires the reduction of CYE-1 levels, similar to the situation in *Drosophila*, high CYE-1 levels are required for subsequent endoreplication cycles (Fay and Han, 2000).

The crucial role of Cdk2-Cyclin E in endoreplication extends to mammalian cells. During mouse trophoblast giant cell (TGC) endoreplication, levels of Cyclin E and Cyclin A oscillate, with protein levels being highest at the onset of S phase (MacAuley et al., 1998). Cyclin E1 and E2 are required for these endocycles, which do not occur in mice that lack both components (Parisi et al., 2003). In addition, protein levels of the Cdk2-Cyclin E inhibitor $p57^{Kip}$ also oscillate (Hattori et al., 2000), which suggests that endoreplication in TGCs requires oscillating Cdk2-Cyclin E activity.

What is the link between endoreplication and development? A study in *Drosophila* showed that failure in endoreplication is accompanied by larval growth defects (Pierce et al., 2004). In *C. elegans*, the function of intestinal cell endoreplication has been proposed to be important for maintaining intestinal structure (reviewed by Kipreos, 2005). The nematode intestine is a long tube composed of a single cell layer, which might not efficiently withstand the rounding and shear forces that could accompany mitosis.

Exiting the cell cycle upon terminal differentiation

Terminal differentiation at the end of a particular developmental program is often characterized by permanent withdrawal from the cell cycle (Fig. 1E). This is the case, for instance, during *Drosophila* eye and wing development. Here, the dual repression of E2F, along with the inactivation of Cdk4-Cyclin D and Cdk2-Cyclin E, is essential for cell cycle exit (Buttitta et al., 2007). The single overexpression of the positive regulators E2F, Cyclin D or Cyclin E, as well as the single depletion of the negative regulators Rb and DP, which counteracts E2F activity, merely delays cell cycle exit. By contrast, cells never exit the cycle upon the dual overexpression of E2F and Cyclin E, which indicates that the concurrent downregulation of E2F as well as Cdk4-Cyclin D and Cdk2-Cyclin E is essential for cell cycle exit. In contrast to the situation in cycling cells, there is no positive-feedback loop between E2F-DP and Cdk2-Cyclin E in this case (Buttitta et al., 2007). As the two mechanisms are independent, they both must be repressed to allow cell cycle exit. How such independence is achieved, and which developmental cues trigger repression, requires further investigation. Terminal differentiation still takes place in the eye and the wing when cell cycle exit is prevented (Buttitta et al., 2007), which indicates that this particular process is dispensable for at least some aspects of pattern formation.

From the examples discussed in this section, it is clear that the cell cycle is modulated in an intricate manner during the entire course of development, from the early embryo to terminal cell differentiation. As mentioned, analogous cell cycle transitions are regulated by homologous proteins in different species, which indicates a broad evolutionary conservation of the underlying mechanisms. As discussed above, the modulation of cell cycle progression is often crucial for developmental processes, and faulty coupling with the cell cycle can have dire consequences.

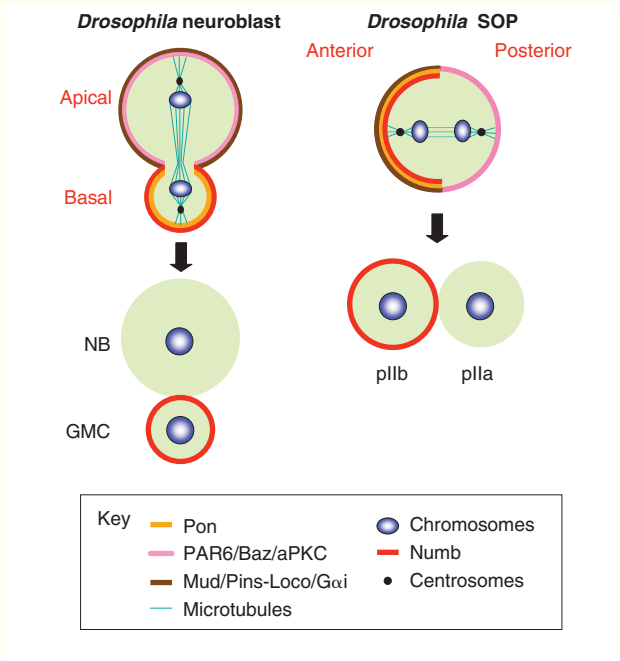
Core cell cycle regulators contribute to fate determination

It has become increasingly clear that apart from their functions in the cell cycle, core cell cycle regulators can also play a role in fate determination. In this section, we discuss such developmental roles of the cell cycle regulators Cdk1, Aurora A and Polo/PLK-1.

Studies of the *Drosophila* central and peripheral nervous systems (CNS and PNS, respectively) have been instrumental in uncovering the mechanisms that govern asymmetric cell division in metazoan organisms (see Box 3 for an overview, including of the principal proteins). Mutant screens have revealed that core components of the cell cycle machinery are important for fate determination in these cases, independently of their role in cell cycle progression. One cell cycle gene with such dual functions is *Cdk1* (also known as *cdc2*) (Tio et al., 2001). Embryos that carry the dominant-negative $cdc2^{E51Q}$ or the temperature-sensitive $cdc2^{ts4x}$ mutant allele exhibit a mislocalization of proteins important for asymmetric cell division, as well as spindle positioning defects, which often results in the symmetric division of the neuroblast (NB) (Tio et al., 2001). Temporally restricted Cdc2 inactivation using the $cdc2^{ts4x}$ allele indicates that the protein acts during mitosis. The failure to localize fate determinants is apparently not caused by a cell cycle progression delay, as *stg* mutant cells first blocked in G2 and then rescued by inducible Stg expression divide asymmetrically (Chia et al., 2001). The mechanism by which Cdc2 dictates NB asymmetric division is not understood in detail, but probably entails the phosphorylation of polarity proteins known to be crucial for asymmetric cell division. Cdc2 also plays a role in the asymmetric

division of the PNS sensory organ precursor (SOP) (Fichelson and Gho, 2004). However, in contrast to its function in NBs, in this case Cdc2 appears to be important through its contribution to cell cycle progression. Indeed, when Cdc2 activity is reduced by overexpressing the inhibitor Dwee1 or Dmyt1 together with the inducer of Stg degradation Tribbles (Trbl), cell cycle progression is delayed, and both daughter cells adopt the same fate (Fichelson and Gho, 2004).

Box 3. Asymmetric cell division in *Drosophila* neuroblast and SOP cells



The embryonic *Drosophila* CNS (left in figure) is derived from neuroblasts (NBs), progenitors that divide asymmetrically, giving rise to another NB and to a ganglion mother cell (GMC) (reviewed by Chia et al., 2008; Gönczy, 2008; Knoblich, 2008). Two apical complexes crucial for NB asymmetric division are coupled through the adaptor protein Inscuteable (not shown). The first complex comprises Par6, Bazooka (Baz; also known as Par3) and atypical protein kinase C (aPKC). This complex excludes fate determinants from the apical cortex through the aPKC-mediated phosphorylation and local inactivation of the tumor suppressor Lethal giant larvae (Lgl). Active Lgl at the basal cortex recruits the adaptor Miranda, which in turn recruits the GMC fate determinants Prospero, Brain tumor and Staufen to the basal cortex that is inherited by the GMC (not shown in figure). In parallel, the Notch antagonist Numb and Partner of Numb (Pon) also become localized to the basal cortex. The second complex comprises the Gα protein $G\alpha_i$, the GoLoco proteins Partner of Inscuteable (Pins) and Locomotion defects (Loco), as well as the coiled-coil protein Mushroom body defective (Mud). This complex plays a major role in mediating spindle positioning, thus ensuring robust cleavage plane orientation orthogonal to the apical-basal axis.

The asymmetric division of the PNS (right in figure) sensory organ precursor (SOP) cells of the *Drosophila* PNS gives rise to an anterior cell, pIIb, and a posterior cell, pIIa. Signaling from pIIb results in the activation of Notch in pIIa and thereby in the correct allocation of cell fate. Although the mechanisms that underlie asymmetric divisions in NB and SOP are related, Insc is not present in SOP cells, such that Pins, $G\alpha_i$, Pon and Numb localize to the anterior cortex, whereas Baz and aPKC localize to the posterior side.

The molecular link by which a core cell cycle regulator imparts cell fate during the asymmetric division of NBs and the SOP has been uncovered in the case of Aurora-A (Aurora – FlyBase) (Berdnik and Knoblich, 2002; Lee et al., 2006; Wang et al., 2006; Wirtz-Peitz et al., 2008). Aurora-A phosphorylates Par6 that is associated with atypical protein kinase C (aPKC) and the tumor suppressor lethal giant larvae (Lgl) (Wirtz-Peitz et al., 2008). Such phosphorylation of Par6 leads to the release of aPKC, which can then phosphorylate Lgl, triggering its dissociation from the posterior cortex of the SOP. In turn, this enables the binding of Bazooka (Baz; Par3) to a Par-6–aPKC complex. Baz then recruits the Notch antagonist Numb, allowing its phosphorylation by aPKC. As a result, Numb is released into the cytoplasm, and cortical Numb distribution is restricted to the anterior side, which is inherited by the pIIb daughter cell (see Box 3). Compromising Aurora-A function results in Numb being distributed around the entire cell cortex and in its segregation into both daughter cells, which consequently adopt the same fate. Aurora-A employs an analogous mechanism to regulate NB asymmetric division (Wirtz-Peitz et al., 2008). Compromising Aurora-A function in this case also results in symmetric Numb distribution, which gives rise to two NBs, instead of one NB and one ganglion mother cell (GMC), and eventually to NB tumor formation (Wirtz-Peitz et al., 2008).

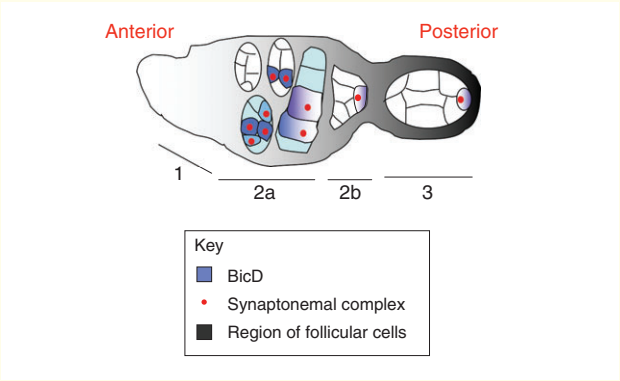
Another positive regulator of the G2/M transition that plays a crucial role in NB asymmetric division is Polo kinase (Wang et al., 2007). Polo is required for phosphorylation of the Numb adaptor protein Partner of Numb (Pon), which mediates Numb localization to the basal cell cortex (Wang et al., 2007). In addition, Polo is needed for apical aPKC localization and for correct spindle positioning. Similar groups of proteins are mislocalized in *aur-A* and *polo* mutant NBs (Wang et al., 2007; Wang et al., 2006). Furthermore, as Aurora-A regulates PLK1 activity in human cells (Macurek et al., 2008; Seki et al., 2008), it is plausible that Aurora-A regulates asymmetric cell division via Polo in *Drosophila*. However, recombinant Aurora-A can phosphorylate Par6 on its own (Wirtz-Peitz et al., 2008), and recombinant Polo can likewise phosphorylate Pon without Aurora-A (Wang et al., 2007). It will be interesting to investigate whether Polo activation requires Aurora-A in vivo.

Polo is also used during *Drosophila* oogenesis to determine cell fate specification (see Box 4 for an overview of early *Drosophila* oogenesis). However, in this case, the role of Polo in fate determination might be a consequence of its function in regulating meiotic cell progression (Mirouse et al., 2006). Throughout the progressive determination of the oocyte, the distribution of Polo mimics that of synaptonemal complexes (SC), which mark cells that are undergoing meiosis (Mirouse et al., 2006). Whereas a partial loss of Polo function delays meiosis, increased Polo levels induce more cells to enter meiosis, which suggests that Polo levels are limiting for meiotic entry (Mirouse et al., 2006). Furthermore, a reduction of Polo function also delays the polarized distribution of the oocyte determinant Bicaudal D (BicD) (Mirouse et al., 2006). Although this delay in polarized BicD distribution might be secondary to the meiotic delay, Polo co-immunoprecipitates with BicD, which raises the possibility that Polo plays a more direct role in BicD distribution (Mirouse et al., 2006). Proteins needed for oocyte fate in turn play a role in Polo distribution. BicD, together with Egalitarian (Egl), a subunit of the dynein motor complex, transports various cargos, one of which is Polo (Mirouse et al., 2006). Therefore, developmental cues might in turn impinge on a core cell cycle regulator.

In *C. elegans* embryos, Polo-like kinases participate in cell fate determination in a manner that appears to be distinct from their role in promoting mitotic entry. Their role in cell fate determination is

Box 4. Oogenesis in the *Drosophila* germarium

In *Drosophila*, oogenesis begins in region 1 of the germarium, where germ line stem cells divide to ultimately form a cyst of 16 cells interconnected by cytoplasmic bridges (reviewed by Huynh and St Johnston, 2004). When this cyst enters region 2a, oocyte fate determinants, including Bicaudal D (BicD), become localized in two of these 16 cells, which are then converted into pro-oocytes. At the same time, synaptonemal complexes form along chromosomes as these pro-oocytes enter meiosis. In region 2b, one pro-oocyte is selected to become the oocyte; the oocyte maintains BicD localization and remains in meiosis, whereas the other 15 cells ultimately differentiate into so-called nurse cells, which support oocyte growth. Figure adapted, with permission, from Mirouse et al. (Mirouse et al., 2006).



exerted through the regulation of the related CCCH finger motif-containing proteins MEX-5 and MEX-6 (collectively referred to as MEX-5/6) (Nishi et al., 2008). MEX-5/6 mediate the degradation of several germ plasm proteins from somatic cells, including that of the transcriptional repressor PIE-1 (DeRenzo et al., 2003; Schubert et al., 2000). In the absence of MEX-5/6, cell fates are altered, ultimately giving rise to dead embryos that contain excess muscle (Schubert et al., 2000). The Polo-like kinases PLK-1 and PLK-2 interact with MEX-5/6, which leads to MEX-5/6 phosphorylation and presumably activation (Nishi et al., 2008). The partial combined depletion of PLK-1 and PLK-2 by RNAi results in phenotypes similar to those observed following MEX-5/6 inactivation, for instance in a failure of PIE-1 degradation from somatic blastomeres. The regulation of MEX-5/6 activity by PLK-1 and PLK-2 appears to be independent of the role of these Polo-like kinases in cell cycle progression, because the partial depletion of PLK-1 without that of PLK-2 appears to affect cell division just like their combined depletion, but does not significantly affect PIE-1 distribution (Nishi et al., 2008). Therefore, Polo-like kinases in *C. elegans* appear to impart cell fate independently of their role in promoting cell cycle progression.

Developmental cues also appear to impinge on cell cycle regulators in this case. The fate determinant MEX-5 regulates the asymmetric distribution of PLK-1 (Nishi et al., 2008), and MEX-5 and PLK-1 physically interact. Hence, the anterior restriction of MEX-5 distribution, which occurs in response to developmental AP polarity cues, concomitantly leads to PLK-1 enrichment in the embryo anterior. Although polarity establishment remains normal when MEX-5 is depleted owing to the partially redundant MEX-6 (Schubert et al., 2000), PLK-1 asymmetry is significantly reduced (Nishi et al., 2008). The fact that some PLK-1 asymmetry remains suggests that a player other than MEX-5 also participates in this process. A plausible candidate is MEX-6 itself, which also interacts with PLK-1 (Nishi et al., 2008). The depletion of MEX-6 alone does not alter PLK-1 asymmetry, but the double depletion of MEX-5 and MEX-6 results in a completely symmetric PLK-1 distribution (Budirahardja and Gönczy, 2008; Nishi et al., 2008; Rivers et al., 2008); this could, however, be an indirect effect of the role of MEX-5 and MEX-6 in polarity establishment (Schubert et al., 2000). Apart from regulating PLK-1 distribution, MEX-5 and MEX-6 have also been suggested to control PLK-1 levels (Rivers et al., 2008).

Taken together, these findings demonstrate that cell cycle regulators can control developmental decisions by directly regulating the expression, activity and/or the distribution of fate determinants.

Conclusions

Cell cycle progression is fundamentally intertwined with metazoan development as the zygote develops into a mature multicellular organism with a large number of differentiated cells. Here, we have reviewed mechanisms by which the regulation of core components of the cell cycle machinery modulates the sequence and the pace of cell cycle progression during the course of development. It is clear that in some systems, such modulation is crucial for the proper execution of developmental processes; for example, during *Drosophila* eye development, in which altered cell cycle progression interferes with photoreceptor differentiation. In other cases, such as in the early *C. elegans* embryo, proper cell cycle modulation is thought to be crucial for the correct positioning of daughter cells so as to enable proper cell-cell communication. Similarly, certain developmental decisions might require cells to be in a particular stage of the cell cycle, as seen during vulval development in *C. elegans* (Ambros, 1999; Wang and Sternberg, 1999). For instance, LIN-12 determines the primary or the secondary fate of the vulval precursor cells prior to the end of S phase, whereas it determines the tertiary or the secondary fate after the completion of S phase. Along similar lines, it has been postulated that the changes in cell shape during mitosis might be incompatible with morphogenic movements, which might explain the necessity for carefully timing mitotic entry in cycle 14 *Drosophila* embryos (Foe, 1989). It will be interesting to further investigate the necessity of altering cell cycle pace during developmental processes.

Recent work on asymmetric division in the *Drosophila* nervous system and in the early *C. elegans* embryo has revealed that core components of the cell cycle machinery are also harnessed to directly impart cell fate. Similarly, glial fate determination in *Xenopus* retinoblasts requires a cell cycle-independent role of the CKI Xic1 (Ohnuma et al., 1999). Conversely, developmental cues can modulate the distribution and/or the activity of core cell cycle regulators, as exemplified by the importance of MEX-5/6 function for PLK-1/2 distribution in *C. elegans*. Therefore, it appears that a mutual relationship between cell cycle regulators and cell fate determinants generates a robust positive-feedback loop that ensures the tight coupling between the cell cycle and development.

Despite important progress in recent years, much remains to be discovered in this area of research. One important path will be to further elucidate the developmental consequences of cell cycle modulations, which can be experimentally challenging owing to the reciprocal relationships mentioned above. Another important line of work entails the study of additional model systems to investigate the extent to which mechanisms identified in model organisms such as *C. elegans* and *Drosophila* are evolutionarily conserved. Together, these and other approaches should help to shed light on the processes that govern the fascinating coupling between the cell cycle and development.

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