Chapter 6
The First Cell Cycle of the *Caenorhabditis elegans* Embryo: Spatial and Temporal Control of an Asymmetric Cell Division

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Abstract Throughout the development of an organism, it is essential that the cell cycle machinery is fine-tuned to generate cells of different fate. A series of asymmetric cell divisions leads to lineage specification. The *Caenorhabditis elegans* embryo is an excellent system to study various aspects of the early embryonic cell cycle. The invariant nature of the rapid cell divisions is the key feature for studying the effects of small perturbations to a complex process such as the cell cycle. The thorough characterization of the asymmetric first cell division of the *C. elegans* embryo has given great insight on how the oscillations of the cell cycle coordinate with the cytoplasmic rearrangements that ultimately lead to two developmentally distinct daughter cells.

6.1 Introduction

The mechanisms underlying the duplication and division of cells have stimulated research ever since Virchow postulated that cells can only come from preexisting cells (omni cellulae e cellula) in 1858. Earlier, in 1839, Schleiden and Schwann had recognized the importance of cells as the basic building blocks of life. From 1970s onward, groundbreaking work was done to understand the regulation of the cell cycle in genetically tractable eukaryotes, such as yeast, where many of the key players in the cell cycle were first described (Hartwell et al. 1970, 1974; Nurse et al. 1976; Nurse and Thuriaux 1980). The characterization of genes essential for a cell to divide was complemented by the discovery of temporally regulated protein synthesis, which was made possible by working in biochemical accessible organisms such as frog and sea urchin embryos (Masui and Markert 1971; Evans et al. 1983). With these two methods at hand, the model of the eukaryotic cell cycle based on the activity of cyclin dependent kinases (CDKs) was established. Over the years,

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many details of the regulatory pathways were described in a variety of organisms, and it has been found that the core regulatory principles are extremely well conserved among eukaryotic species. The discovery of the cell cycle machinery is reviewed in a great series of Nobel Prize lectures (Hartwell 2002; Hunt 2002; Nurse 2002). The question remaining is how the cell cycle regulators can be adopted throughout development to give rise to different tissues. Before we can comprehend the role of the cell cycle in cell fate regulation, we first need to understand how the cell cycle machinery mediates the morphological changes a cell undergoes during an asymmetric cell division.

6.1.1 Temporal Control: The Idea About a Cell Cycle Clock

The eukaryotic cell cycle is often described as a clock, with the different phases of the cell cycle occurring at certain times. Each clock is made up of an oscillator that defines the time periodicity, a controller that corrects the frequency of the oscillator, a counter that translates the oscillations to a more convenient unit, and an indicator that tells the time. In the cell cycle clock, the expression of cyclins sets the oscillations, a checkpoint or entrainment mechanism acts as a controller, the CDKs correspond to the counter, they translate the oscillations of the cyclins to biochemical changes in the cell, and the morphology of the cell tells us in which phase the cell cycle is, it tells the time. In most cell types, different species of cyclins oscillate at an internal frequency, and a mechanism is required to entrain the oscillations to activate the CDKs in the proper order. In some cell types, such as yeast, strong checkpoints were observed that are able to halt the cell cycle clock and restart it only when the oscillations are synchronized again. In other systems, there is only evidence for a subtle controller that can coordinate events but is not able to halt all aspects of cell division. Because of the differences observed in various cell types, different models were proposed on how a minimalist cell cycle clock could work. We discuss three models that imply different controller mechanisms.

The first model on cell cycle regulation was based on results from genetic studies in yeast (Nasmyth 1996; Stern and Nurse 1996). This model suggests that an increasing activity of a certain CDK will reach a threshold level at which it crosses a checkpoint (Fig. 6.1a). Recently, it has been shown for human cells that mitotic events are indeed ordered by their dependence on different thresholds of CDK activity (Gavet and Pines 2010). This view of the cell cycle is termed the ratchet model as each checkpoint acts as a switch from one phase to the next by irreversible protein degradation (reviewed by Reed 2003). These switches are called checkpoints because the cell will halt every aspect of cell division until the switch has been triggered (Kastan and Bartek 2004). An alternative hypothesis is that the cell cycle is composed of a set of independent clocks that are coordinated by checkpoints (Fig. 6.1b). This idea was initially proposed for the cell cycle of the prokaryote *Escherichia coli* (Jones and Donachie 1973). Nordström et al. (1991) supported this view of the *E. coli* cell cycle and suggested that the cell cycle of

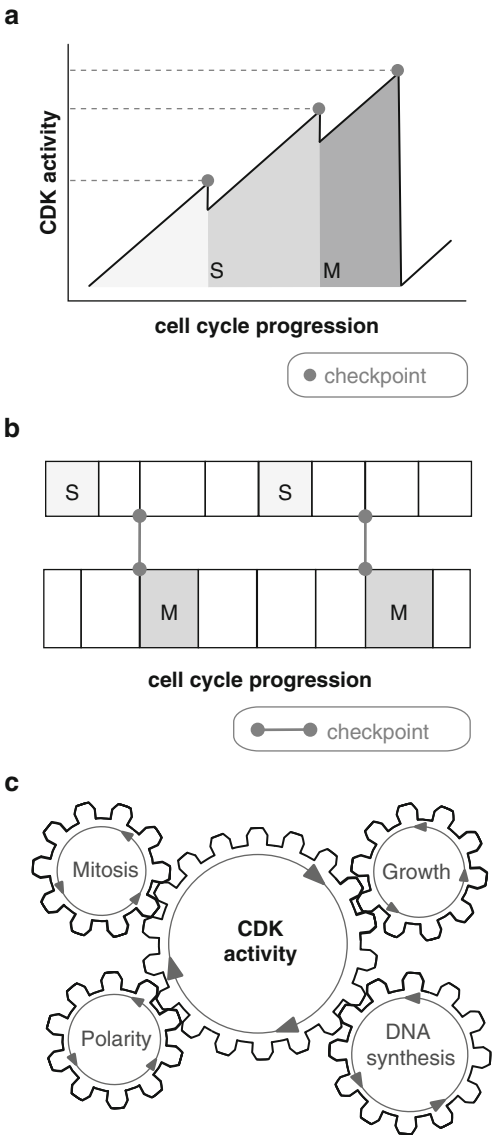

Fig. 6.1 Schematic illustrations of cell cycle clock models. (**a**) The ratchet model assumes that the phases of the cell cycle are initiated by overcoming thresholds of CDK activity. The threshold is passed by satisfying a checkpoint. This is a point of no return, ensuring that every event only happens once per cell cycle (Stern and Nurse 1996). (**b**) The model of independent clocks proposes that the different phases of the cell cycle run in parallel and are kept in synchrony by the activity of cell cycle checkpoints (Boye and Nordström 2003). (**c**) The phase-lock model is based on independent clocks that are entrained by the oscillations of a master regulator (Lu and Cross 2010)

higher organisms is also regulated by multiple independent clocks (Boye and Nordström 2003). In their model, the checkpoints act to synchronize events that run in parallel and do not necessarily depend on the same signals. The third model is the only one that does not depend on strict checkpoints as a controller unit. Lu and Cross (2010) proposed a phase-lock model for the cell cycle of a eukaryote, the budding yeast *Saccharomyces cerevisiae*. Phase locking presumes the presence of multiple independent oscillators, which run in parallel at their own frequencies.

In contrast to the former model, these clocks depend on each other, they act like slave clocks that are entrained by a master clock (Fig. 6.1c). In this model, each peripheral oscillator could be responsible for a different phase of the cell cycle. In eukaryotic cells, the CDK/cyclin system is the most likely candidate to provide a reliable oscillator capable of synchronizing the peripheral clocks and ensuring a stable order of events (Pomerening et al. 2003).

It is possible to distinguish these three models experimentally. Perturbation of the first and second model could lead to a complete arrest of the cell. Distinct events could only be decoupled by disruption of the checkpoint mechanisms. If non-checkpoint proteins are perturbed, the second and third model could result in a deceleration of the cell cycle but only the third model could lead to the decoupling of events as observed in many embryonic systems, such as the first cell cycle of the *Caenorhabditis elegans* embryo (see Sect. 6.1.3).

6.1.2 An Elegant Model: C. elegans

The free-living, bacterivorous nematode *C. elegans* is an excellent model organism for the genetic analysis of cell cycle regulation during embryonic development. The first two rounds of cell division are highly reproducible in their temporal and spatial sequence (Fig. 6.2). All consecutive embryonic divisions have been characterized (Deppe et al. 1978). Any discrepancy from the stereotypical cell divisions can be

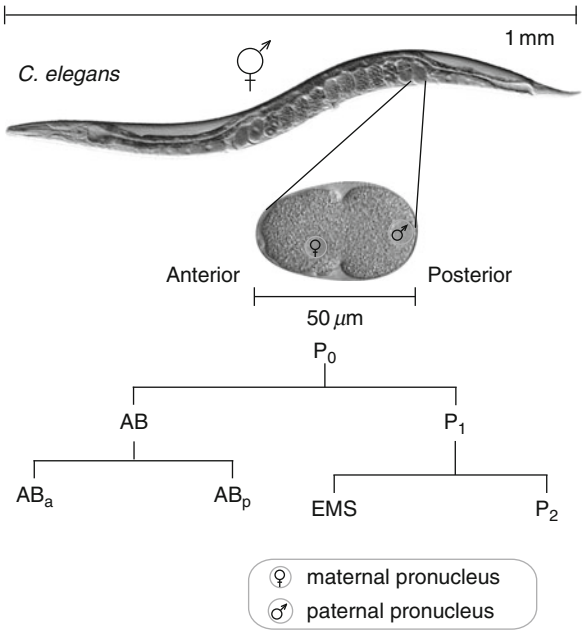

Fig. 6.2 Nomarski micrographs of a *C. elegans* hermaphrodite and a one-cell stage embryo (P_0). The lineage up to the four-cell stage is depicted. The cell divisions of P_0 and P_1 are asymmetric. The division of P_1 is initiated later than the symmetric division of AB

detected as a phenotype if the system is experimentally compromised. Another advantage as a genetic model organism comes from its mode of reproduction. Genetic homogeneity can be achieved by self-fertilization of the hermaphrodites, while genetic crosses can be set up with males that are infrequently occurring in the population (Brenner 1974; Sulston and Brenner 1974). Two breakthroughs for *C. elegans* as a model organism came in 1998 when it became the first animal to have its genome fully sequenced (*C. elegans* Sequencing Consortium 1998). At the same time, RNA interference was adopted as a tool to deplete cellular protein levels in *C. elegans* (Fire et al. 1998) and opened the possibility of genome wide screens (Fraser et al. 2000; Gönczy et al. 2000; Sönnichsen et al. 2005). The early embryo proved to be ideal for studying the effects of small perturbations such as single gene knockdowns. The size (roughly 50-μm long, with a diameter of 30 μm) and transparency of the *C. elegans* embryo make it easily accessible for light microscopy. This is a clear advantage for studying the morphological changes that a cell undergoes throughout the cell cycle. Individual proteins can be fluorescently tagged, and their distribution can be followed through the cell cycle by time-lapse microscopy. Through recent technological advance in the generation of transgenic lines, it is promising that the activity of any gene or promoter of interest can be tracked throughout the development of the worm (Sarov et al. 2006; Frøkjaer-Jensen et al. 2008; Murray et al. 2008). The first division of the *C. elegans* embryo is asymmetric, providing an opportunity to study the coordination between the CDK/cyclin rhythms and the series of polarization events leading to the segregation of cell fate determinants. Many players essential to the complex process of asymmetric cell division were uncovered by using the *C. elegans* embryo as a model organism.

6.1.3 Regulation of the Embryonic Cell Cycle in C. elegans

The first divisions of the *C. elegans* embryo are fast and do not require growth of cells. The cell cycle in the early embryo is only comprised of consecutive rounds of DNA synthesis (S phase) and cell division during mitosis (M phase), with no gap phase until the 28-cell stage (Edgar and McGhee 1988). Although the cell cycles of the embryo progress in a highly stereotypical fashion, it is clear that various processes essential to cell division can be uncoupled. The fact that endoreduplication cycles, the duplication of DNA without segregation of sisters, exist in later stages in the development of the embryo suggests that various aspects of cell division can be separated (van den Heuvel 2005). Specific experimental disturbance confirmed that the individual steps in the cell cycle in *C. elegans* are not strictly dependent on each other. If one aspect of the cell cycle like chromosome segregation is artificially blocked in the one-cell embryo, it does not prevent a later phase of the cell cycle; the cytokinesis furrow will still dissect the cell even though it has to cut through the DNA and spindle (van der Voet et al. 2009). Another example is that DNA and centrosome duplication proceed normally in the absence of cytokinesis (Chase et al. 2000). In a classic experiment, Schierenberg and Wood (1985)

showed that enucleated blastomeres of the one-cell embryo divided with typical timing. These examples indicate that at least the nuclear and the cytoplasmic cell cycle can be uncoupled to a certain extent in the early embryo.

Furthermore, these experiments indicate that the checkpoint mechanisms present in the embryo are not sufficient to halt all aspects of cell cycle progression. The lack of definite checkpoints in the one-cell *C. elegans* embryo makes it an ideal model system to study the finer details of the cell cycle. In other systems with strong checkpoint mechanisms, most perturbations of the cell cycle will lead to a checkpoint dependent arrest and give rise to a number of phenotypes limited by the number of checkpoints. Thus, studying the cell cycle in *C. elegans* has given great insight into some peripheral regulatory mechanism, but the system that synchronizes all aspects of the cell cycle is still poorly understood. In this review, we discuss various features of the first cell division of the *C. elegans* embryo individually and also emphasize steps that coordinate the different processes essential to the cell cycle.

6.2 The Nuclear and Centrosome Cycle

6.2.1 Meiosis and DNA Synthesis Phase

Before fertilization, the *C. elegans* oocyte is arrested at diakinesis of meiosis I in the gonad of the hermaphrodite. The oocytes are kept in a quiescent state by the activity of CKI-2, an inhibitor of the S-phase initiating CDK-2/cyclinE complex (Buck et al. 2009). The inhibition of CDK-2 by CKI-2 also seems to be the mechanism that eliminates the maternal centrosomes (Kim and Roy 2006). It is important that the set of centrosomes, which enters the oocyte at fertilization together with the paternal nucleus, will form the only microtubule-organizing center in the one-cell embryo. Upon fertilization, the activation of the oocyte triggers several events. First, meiosis I and II proceed, resulting in the extrusion of two polar bodies. Next an incompletely understood centrosome signal initiates a change in cortex activity at the site of sperm entry, leading to polarity establishment (see Sect. 6.3.1). Proper timing of this centrosome signal requires an active CDK-2/cyclinE complex (Cowan and Hyman 2006). From the activation of the oocyte onward, the CDK-2 complex is thought to be constantly active during early embryonic development (Fig. 6.3a) (Brodigan et al. 2003). The onset of cytoplasmic streaming by the asymmetric activation of the actomyosin cortex slightly precedes the migration of the maternal pronucleus toward the paternal pronucleus (Fig. 6.3b). Polarity establishment coincides with the DNA synthesis phase of the cell cycle (Edgar and McGhee 1988). Before the pronuclei migrate toward each other, the haploid chromosomes within both nuclei are replicated. The DNA damage checkpoint is silenced in the one-cell embryo (Holway et al. 2006). Stalling DNA replication delays cell cycle progression in the first cell division, which leads to defects in the

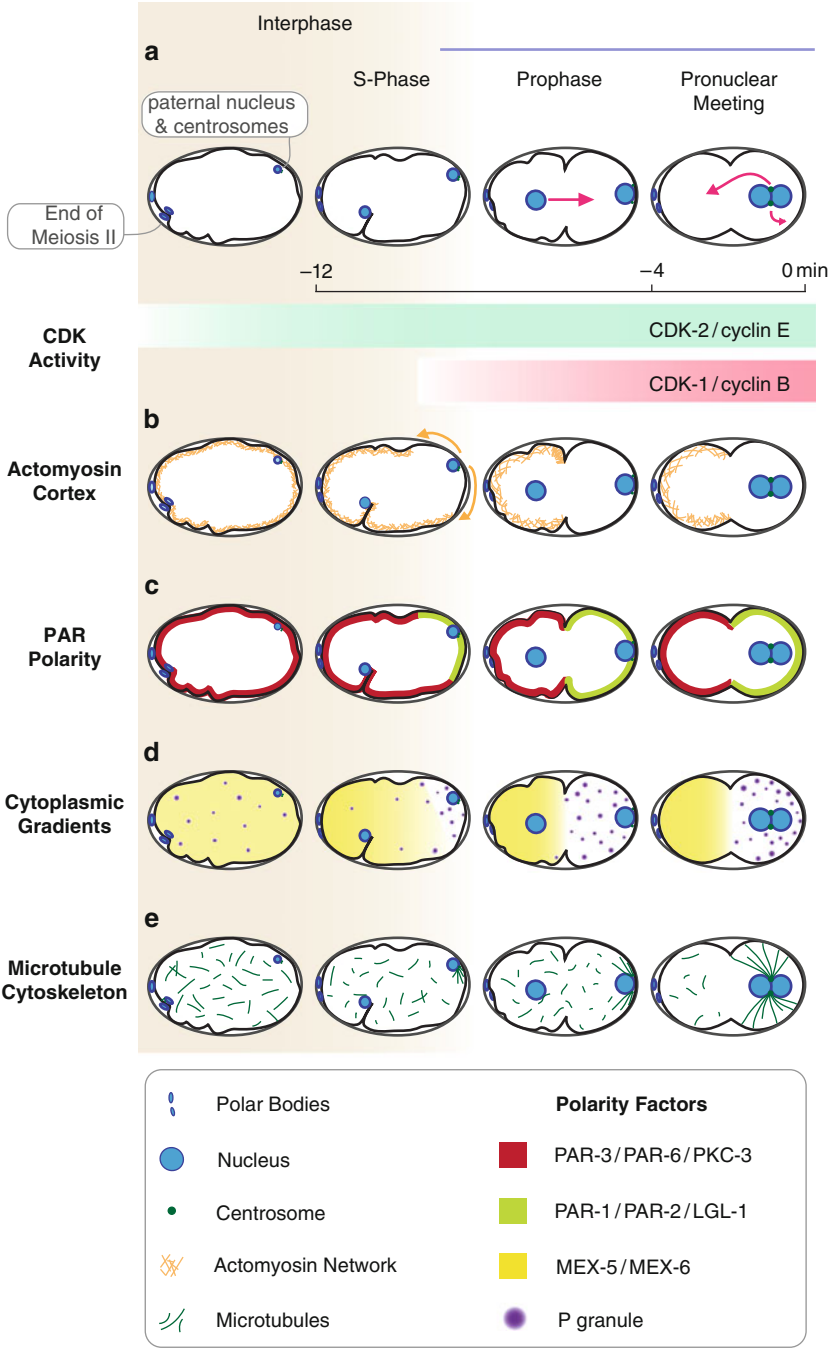

Fig. 6.3 (*continued*)

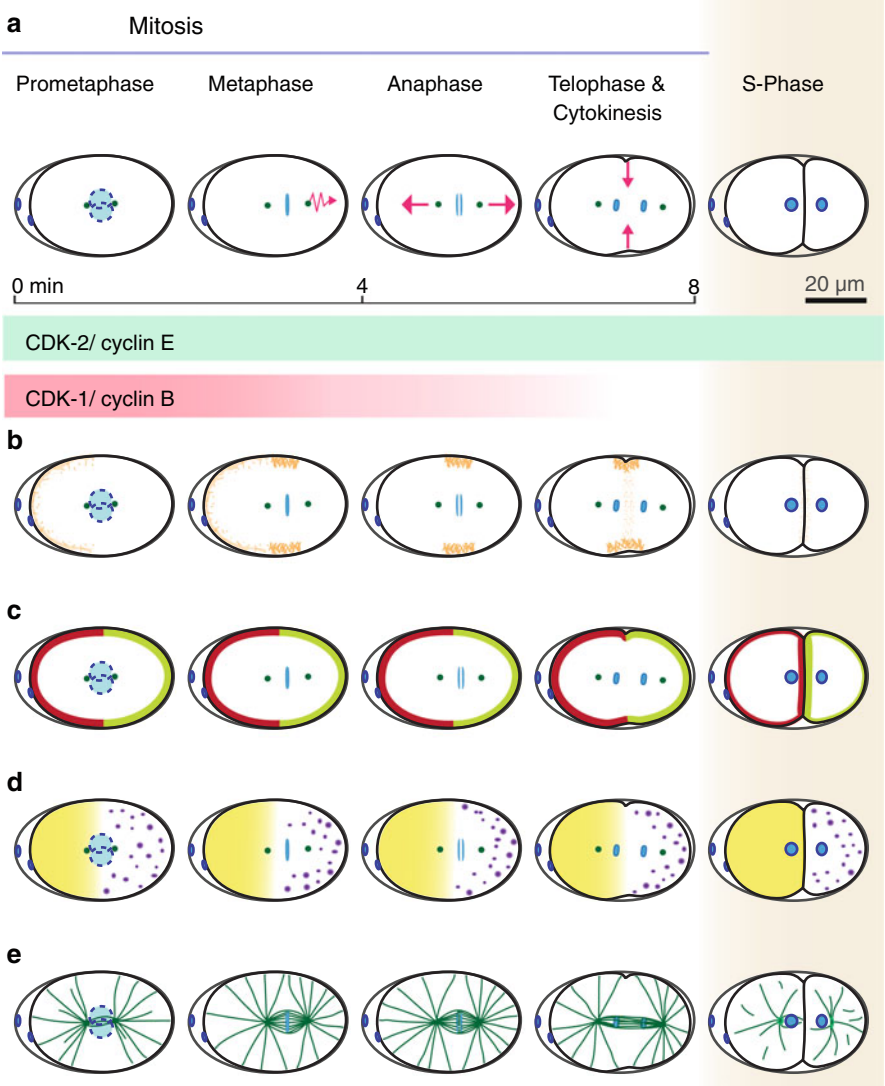

Fig. 6.3 Outline of the mitotic cell cycle in the one-cell *C. elegans* embryo. (**a**) The nuclear cycle and the activities of the cyclin dependent kinases are depicted. Timing is relative to prometaphase at 20°C. (**b**) The dynamics of the cortical actomyosin network (*orange*). (**c**) The cortex of the embryo is patterned by the anterior (*red*) and posterior (*green*) PAR proteins. (**d**) The distribution of the cytoplasmic cell fate determinants is downstream of PAR polarity. The somatic determinants MEX-5/-6 (*yellow*) segregate to the anterior and the germline specific P granules (*purple*) to the posterior. (**e**) The microtubule cytoskeleton (*green*) reorganizes into the mitotic spindle to segregate the sister chromatids. The anterior of the embryo is to the *left*, the posterior to the *right*. *Arrows* indicate motion

asymmetric cell division and hence it is not a true checkpoint (Encalada et al. 2000). The lack of a delay mechanism in case of DNA damage ensures that the cell cycle timing is not disrupted and S-phase completes before pronuclei meet in the posterior of the cell. DNA damage checkpoint activation becomes apparent in the two-cell embryo (see Sect. 6.6).

6.2.2 Prophase and Prometaphase

The increase of CDK-1 activity during mitotic prophase is accompanied by chromosome condensation in the nuclei (Hachet et al. 2007; Portier et al. 2007). The activation of CDK-1 in the early embryo is dependent on the phosphatase CDC-25.1 (Ashcroft et al. 1999; Rivers et al. 2008). At the same time, daughter centrioles are nucleated off the existing centrioles within the centrosomes (reviewed by Müller-Reichert et al. 2010). The mitotic kinase Aurora B (AIR-2) recruits the condensin complex to the chromosomes and is required for the phosphorylation of histone H3 during prophase, which temporally regulates DNA condensation (Kaitna et al. 2002; de Carvalho et al. 2008). Upon phosphorylation, the *C. elegans* homologue of CENP-A, centromere histone H3 (HCP-3), relocalizes from the center of the chromosomes to their surface, a process called centromere resolution. HCP-3 decorates almost the entire length of the chromosomes, typical for the establishment of holocentric kinetochores, which assemble around the centromeres (Moore et al. 2005). At the time of pronuclear meeting, the polo-like kinase PLK-1 can be detected at the centrosomes and has been implicated in promoting centrosome maturation (Chase et al. 2000). After pronuclear meeting, the nucleocentrosomal complex migrates to the middle of the cell and rotates. Timely regulation of nuclear envelope break down (NEBD) requires the cell cycle regulators PLK-1, CDC-25, and CDK-1 (Chase et al. 2000). Furthermore, it has been shown that the association of centrosomes with the nuclei is critical for timely NEBD (Hachet et al. 2007; Portier et al. 2007). Centrosomes are thought to act as signaling hubs that coordinate certain aspects of cell cycle progression. The maturation of centrosomes by Aurora A kinase (AIR-1) is essential for the simultaneous dissociation of the nuclear envelopes of both pronuclei (Hannak et al. 2001; Hachet et al. 2007; Portier et al. 2007). The coupling of centrosome maturation to nuclear envelope breakdown ensures that both sets of chromosomes are located between the spindle poles to be efficiently captured by spindle microtubules during prometaphase (Fig. 6.3e).

6.2.3 Metaphase and Anaphase

After NEBD, the spindle microtubules attach to the kinetochores on the chromosomes and arrange the condensed DNA onto the metaphase plate. Premature

chromosome segregation is prevented by cohesin, which holds the sister chromatids together. Cell cycle dependent activation of the catalytic function of separase cleaves the cohesin molecules all along the chromosomes and allows segregation of sister chromatids at the onset of anaphase. Two of the ultimate targets of the Anaphase Promoting Complex/Cyclosome (APC/C), an ubiquitin E3-ligase, are the inactivation of CDK-1 as well as the activation of separase (McCarthy Campbell et al. 2009). A study by van der Voet et al. (2009) has shown that association of CDK-1 with cyclinB1 is required for full condensation of chromosomes and for proper alignment at the metaphase plate, while cyclinB3 (CYB-3) is essential for the segregation of sister chromatids. The direct target of the CDK-1/CYB-3 complex has not yet been identified. Interestingly, inhibition of chromosome segregation by CYB-3 depletion does not activate a checkpoint to prevent cytokinesis. The activation of separase not only leads to sister chromatid segregation but also plays a role in centriole separation and regulation of spindle dynamics (Sullivan et al. 2001; Tsou et al. 2009). In *C. elegans*, the catalytic activity of separase is essential for sister chromosome separation, while it has a noncatalytic function in the completion of cytokinesis. Bembenek et al. (2010) described a separase mutant, which successfully segregates chromosomes but leads to cytokinesis failure. This indicates, for the first time, that the various functions of separase during exit of mitosis are likely to depend on different cell cycle signals.

Following successful sister chromatid segregation and initiation of cytokinesis, the nuclear envelopes reform in the two daughter cells during telophase. Centriole and centrosome dynamics in the one-cell *C. elegans* embryo have recently been reviewed (Müller-Reichert et al. 2010). For a review on nuclear envelope dynamics, see Gorjánácz et al. (2007). After telophase, the two-cell embryo is ready for a new division cycle, which is equipped with one nucleus and two centrioles per cell.

6.3 Asymmetric Cell Division: PAR Polarity

The first cell division of the *C. elegans* embryo is asymmetric, producing one larger anterior cell and one slightly smaller posterior cell (reviewed by Cowan and Hyman 2004, 2007; Munro and Bowerman 2009). Making two cells with different volumes is not the only challenge of the asymmetric cell division in the one-cell embryo. In order for the embryo to develop into an adult organism, cell fate determinants need to be segregated into one or the other half of the cell before the first division. The anterior cell will give rise to purely somatic tissue. The posterior cell will go on to give rise to the germline of the adult organism, among other tissues, and needs to inherit the germ cell factors, which are initially distributed throughout the oocyte. The prerequisite for an asymmetric cell division is the polarization of the parent cell. Asymmetry is initiated by the segregation of anterior and posterior PAR (partitioning defective) domains (Fig. 6.3c). Polarization of the one-cell embryo requires the PDZ domain proteins PAR-3 and PAR-6, as well as the atypical protein

kinase C (PKC-3) to form the anterior domain. The serine threonine kinase PAR-1, the ring-finger protein PAR-2, and the tumor suppressor LGL-1 establish the contrasting posterior domain (Hoege et al. 2010). The PAR-3/PAR-6/PKC-3 complex will thereafter be called the anterior PAR-6 domain, and the localization of PAR-1, PAR-2, and LGL-1 will be referred to as the posterior PAR-2 domain. The cytoplasmic proteins PAR-5, a member of the regulatory 14-3-3 protein family and PAR-4, a serine-threonine kinase, are also required for stable polarity domains in the embryo although their functions are less well understood.

Once the PAR polarity is established, it is essential to keep the cell polarized to ensure the accurate distribution of other factors, which ultimately gives rise to daughter cells with different fate. On the one hand, the PAR polarity regulates cortical factors to position the spindle slightly posterior before cytokinesis, leading to two differently sized daughters. On the other hand, it signals to cytoplasmic components that segregate the fate determinants, ensuring that the daughters will form different types of tissue.

6.3.1 Polarity Establishment Phase

In the *C. elegans* embryo, the onset of polarity is triggered by the sperm, which enters the oocyte in the process of fertilization. The site of sperm entry designates the posterior of the embryo (Goldstein and Hird 1996). The one-cell embryo continues to be unpolarized during meiosis. At meiosis II, the PAR-2 complex is weakly associated with the cortex. At the end of meiosis II, PAR-2 falls off the cortex and PAR-6 decorates the whole cortex (Fig. 6.3c). The onset of anterior–posterior (AP) polarity is not apparent until the start of mitotic prophase when the cortex contractility becomes asymmetric and PAR-2 forms a posterior domain, thereby replacing PAR-6 in this region (Cuenca et al. 2003). The mechanism by which sperm entry initiates polarity establishment half an hour after sperm–egg fusion is incompletely understood. The asymmetric activation of the actomyosin network has been suggested as an important factor for the timely regulation of polarity onset. A spatial cue that is dependent on the centrosome is thought to lead to the exclusion of the activating RHO-1 guanine exchange factor (GEF) ECT-2 (Cowan and Hyman 2006; Motegi and Sugimoto 2006). The GAP (GTPase activating protein) CYK-4 has also been proposed to play a role in the asymmetric activation of the cortex (Jenkins et al. 2006). In this model, the regulation of RHO-1 activity through spatial localization or exclusion of RHO inhibitors or activators has been proposed to induce the retraction of the active actin cytoskeleton toward the anterior. However, due to a lack of tools to image the activity level of RHO-1, this model remains controversial. The cortical actomyosin flow from the posterior pole correlates with the displacement of the PAR-6 domain toward the anterior, allowing the complementary PAR-2 domain to establish on the noncontractile cortex at the posterior (Munro et al. 2004).

6.3.2 Polarity Maintenance Phase

The contractility of the anterior cortex ceases as the nuclei migrate toward the center of the cell. The boundary between the cortical polarity domains needs to be maintained at the center of the cell until mitotic metaphase. While RHO-1 is the major regulator of the actomyosin network during polarity establishment, maintaining two stable domains depends on the interaction between the anterior PAR proteins and the rho family GTPase CDC-42 (Schonegg and Hyman 2006; Kumfer et al. 2010). The active form of CDC-42 interacts with PAR-6 and is enriched on the anterior cortex, possibly by the opposing activity of the GAP CHIN-1 localized at the posterior cortex (Gotta et al. 2001; Aceto et al. 2006; Kumfer et al. 2010). At the cortex, PAR-6 is thought to interact with the kinase PKC-3, which can phosphorylate PAR-2 to keep it off the anterior cortical domain (Hao et al. 2006). It has been proposed that PKC-3 is able to phosphorylate PAR-1, while PAR-1 phosphorylates the anterior protein PAR-3, constructing a feedback loop. This model is based on cell polarity studies in other organisms, mainly *Drosophila melanogaster* (Benton and St Johnston 2003; Hutterer et al. 2004). The *Drosophila* polarity model can be mapped onto *C. elegans*, considering that the *C. elegans* specific polarity protein PAR-2 performs the same function as LGL in flies (Hoege et al. 2010). The other polarity proteins discussed here are conserved. The ability of the anterior complex to phosphorylate the components of the posterior PAR complex and vice versa is at the heart of mutual antagonism, the process that is thought to allow maintenance of the polarity boundary. The cytoplasmic 14-3-3 protein PAR-5 functions to ensure a sharp boundary between the anterior and posterior cortical complexes but mechanism by which it does so is not yet clear. 14-3-3s are known to bind to phosphorylated proteins, and it has been speculated that they are able to remove phosphorylated PAR proteins from the cortical region, thereby increasing the turnover of proteins at the cortex.

If polarity establishment was blocked in early prophase by inhibiting cortical contractility, there is an alternative mechanism to form the two opposing domains during the maintenance phase (Zonies et al. 2010). The signal initiating the accumulation of PAR-2 on the posterior cortex at this time point in the cell cycle has yet to be revealed. The expansion of the PAR-2 domain in the absence of strong cortical flows is dependent on the activity of CDC-42 (Zonies et al. 2010). This indicates that the antagonistic interaction between the posterior and anterior PAR complexes allows the PAR-2 domain to push back the PAR-6 domain. The position of a PAR boundary established in the absence of flows would, therefore, depend on the balanced activity between the antagonistic complexes. On the basis of our current understanding of PAR polarity, it is likely that the redistribution of the PAR domains during early prophase is due to passive transport by cortical flows while the delayed onset of polarity in the absence of strong cortical contractility as well as the maintenance of the established domains is due to an active mechanism involving mutual antagonism between anterior and posterior PARs.

6.3.3 Repositioning the PAR Boundary to Match the Cytokinesis Furrow

The final position of the PAR polarity boundary correlates with the site of the asymmetric position of the cytokinesis furrow (Schenk et al. 2010). Just before the sister chromatids segregate to opposite poles of the one-cell embryo at anaphase, the spindle is displaced slightly toward the posterior (see Sect. 6.5.3) (Labbé et al. 2004; McCarthy Campbell et al. 2009). At metaphase, the shift in spindle position induces a reorganization of the actomyosin cortex (Werner et al. 2007). The formation of the cytokinesis furrow depends on a spatial activation of RHO-1, which is required to assemble the actomyosin based contractile ring. During the establishment phase, positioning the boundary between the anterior and the posterior PAR complexes can be artificially adjusted by changing the activity of RHO-1 (Schonegg et al. 2007; Werner et al. 2007).

The exact position of the polarity boundary at cytokinesis is important, since the PAR proteins ultimately mediate the distribution of cytoplasmic cell fate determinants.

6.4 Cytoplasmic Segregation of Cell Fate Determinants

The PAR protein complexes pattern the cortex and thereby establish the polarity of the embryo; however, this is not sufficient to form daughter cells with different fates. The segregation of cytoplasmic germline factors from determinants of the somatic lineage in the one-cell embryo is essential for proper development. The exclusive inheritance of P granules, germline specific ribonucleoprotein (RNP) aggregates, and free germline proteins to the posterior daughter, conditions the cells to follow their fate (Strome and Wood 1982). The differential segregation of fate determining factors leads to the induction of specific cell cycle control in the two-cell embryo (Budirahardja and Gonczy 2008; Rivers et al. 2008).

6.4.1 A Gradient in MEX Proteins Determines the Somatic Lineage

Protein and RNA segregation in the cytoplasm of the one-cell embryo is dependent on PAR polarity and on an anterior–posterior gradient of MEX (muscle excess) proteins (Schubert et al. 2000; Cuenca et al. 2003). As the PAR-6 complex retracts after polarity onset, it cosegregates the active form of MEX-5 and MEX-6 (MEX-5/-6) toward the anterior (Fig. 6.3d). These CCCH finger proteins are required for the asymmetric distribution of all cell fate determinants, and they inhibit the expression of germline factors in the somatic daughter cells. The MEX proteins

were identified in a mutant screen for aberrant muscle cells (Schubert et al. 2000). The observed defect was that the muscle specific transcription factor SKN-1 was not restricted to the posterior daughter cell, which implied the regulatory role of the MEX proteins on generating cytoplasmic asymmetry. The activity of MEX-5/-6 is regulated in a cell cycle dependent manner. Maternally provided MEX-5 is evenly distributed in the oocyte, so are the germline specific zinc-finger protein PIE-1 and the P granules (Schubert et al. 2000; Cuenca et al. 2003). At meiosis II, CDK-1 activates the DYRK kinase MBK-2, which subsequently marks oocyte specific proteins for degradation (Stitzel et al. 2006; Cheng et al. 2009). For a discussion of the oocyte-to-embryo transition and the regulation of MBK-2, see Parry and Singson (2011). The cell cycle dependent activation of MBK-2 leads to a priming phosphorylation of MEX-5/-6 (Nishi et al. 2008). During polarity establishment, PLK-1 and PLK-2 bind to the primed MEX-5/-6 and activate them. As a result of active MEX-5/-6 being sequestered to the anterior half, PIE-1 and other germline factors get restricted to the posterior half of the one-cell embryo (Nishi et al. 2008). Interestingly, PLK-1 segregates to the anterior half together with MEX-5/-6, which ultimately leads to an unequal inheritance of PLK-1/-2 to the daughters. The next three divisions of the P lineage continue to be asymmetric, producing one somatic and one germline daughter (Fig. 6.2). These divisions of the germline blastomere show that MEX-5/-6 and PLK-1 are always enriched in the somatic daughter cell. MEX-5/-6 abundance diminishes in somatic daughters after cell division (Schubert et al. 2000), while PLK-1 protein levels stay high in somatic cells (Nishi et al. 2008), indicating that MEX-5/-6 are only transiently required to deplete the somatic cytoplasm of germline factors, while high levels of PLK-1 might be required for the regulation of the somatic cell cycle. In the posterior, MEX-5/-6 was shown to assemble with P granules (Schubert et al. 2000; Tenlen et al. 2008). The association of determinants of somatic tissues with P granules could be relevant by ensuring that minimal amounts of somatic determinants are inherited by the P lineage.

6.4.2 Spatial Regulation of Protein Mobility Generates Segregation of Cytoplasmic Components

We are slowly starting to understand how the partitioning of the anterior and posterior PAR domains influences the antagonistic distribution of cytoplasmic factors. As for the cell cycle in general, the differential distribution of cytoplasmic components also greatly depends on the activity and spatial distribution of kinases and phosphatases. The PAR-1/PAR-4 dependent phosphorylation status of MEX-5/-6, for example, has been reported to be important for the asymmetric segregation to the anterior (Tenlen et al. 2008). It has also been shown that cytoplasmic flows, generated during polarity establishment, are neither sufficient nor necessary to segregate cell fate determinants. The current models for the distribution of cytoplasmic factors are based on spatial regulation of the mobility of these factors in the

embryo (Tenlen et al. 2008; Brangwynne et al. 2009; Daniels et al. 2010). An alteration in mobility can be brought by phosphorylation. Tenlen et al. (2008) proposed that phosphorylation of MEX-5 at a specific site would enhance its mobility in the posterior increasing the likelihood that it crosses the boundary into the anterior cytoplasm. In the anterior, the mobility of MEX-5 is reduced, limiting its chance to move back to the posterior. This model proposes that a difference in diffusive mobility would drive the AP gradient of MEX-5. It is still unclear if the asymmetric distribution of MEX-5 requires asymmetric binding sites to improve its enrichment in the anterior (Tenlen et al. 2008; Daniels et al. 2010). In the case of the RNP rich P granules, a slightly different diffusion based model has been proposed. Brangwynne et al. (2009) illustrate that P granules behave like liquid droplets in the cytoplasm, and hence they can be in a dissolved, or in a condensed form. In this model, the MEX-5 dependent dissolution of P granules in the anterior increases the mobility of proteins and RNAs, while the condensation into droplets in the posterior serves to decrease the mobility of soluble components. The segregation of cell fate determinants, achieved by differential diffusion mechanisms, is dependent on polarity establishment of the cortical PAR proteins.

6.5 Microtubule Dynamics Throughout the Cell Cycle

The mitotic spindle has to faithfully orchestrate the segregation of chromosomes to the daughter cells. In order to do so, the structure of the microtubule network needs to be dramatically altered throughout the cell cycle (Fig. 6.3e). For proper development of an organism, it is essential that the shape of the spindle correlates with the size of cells (Hara and Kimura 2011). The length of the mitotic spindle at metaphase of the early *C. elegans* embryo is regulated by the size of the centrosomes (Greenan et al. 2010).

6.5.1 Interphase and Prophase

In the one-cell embryo, the microtubules exhibit the fastest growth rates at interphase and early prophase but they stay short and lack directionality (Srayko et al. 2005). Upon centrosome maturation in late prophase, the microtubule network in the cell drastically alters its structure. Long, microtubules emanate from centrosomes, forming asters that span the whole cell soon after pronuclear meeting. Microtubules are thought to anchor to the cell cortex where force is generated to pull the centrosomes anterior toward the center of the cell and then rotate along the AP axis (Park and Rose 2008). This process also centers the pronuclei, which are tightly attached to the centrosomes (reviewed by Gorjánácz et al. 2007).

6.5.2 Prometaphase

At this stage of the cell cycle, the centrosomes are fully mature and induce the disintegration of the nuclear envelope. In the early stages of nuclear envelope breakdown, fenestrae form in the membrane, allowing the nucleoplasm to mix with the cytoplasm and microtubules to grow toward the chromosomes (Gorjánácz et al. 2007; Müller-Reichert et al. 2010). The mitotic spindle is established by a biased growth of microtubules toward the chromosomes. This process depends on the small GTPase RAN-1 but not on de novo microtubule formation at the kinetochores (Srayko et al. 2005). For more details on the functions of RAN gradients in meiosis and mitosis, see Kaláb et al. (2011). The assembly of a microtubule rich spindle between the centrosomes ensures efficient capture of kinetochores and alignment of the chromosomes on the metaphase plate.

6.5.3 Posterior Displacement of the Spindle During Metaphase and Anaphase

Before the onset of anaphase, the whole spindle starts rocking and is displaced toward the posterior, preparing for the asymmetric division of the one-cell embryo. The regulation and mechanisms of spindle displacement in *C. elegans* has been a focus of intense research over the last decade. The asymmetric positioning of the spindle places the cytokinesis furrow off center resulting in two daughter cells of different size. Displacement of the spindle is tightly linked to anaphase onset and both processes depend on the inactivation of CDK-1 (McCarthy Campbell et al. 2009). *C. elegans* has become the prime model to study the forces that are exerted on a spindle during mitosis, as Grill et al. (2001) introduced the technique of local microtubule severing by directed laser light. With this method, distinct sections of the spindle microtubules were cut or centrosomes were disintegrated, which allowed the indirect determination of forces by measuring the rates at which the remaining parts of the spindle move. Application of this method allowed the dissection of the pathway leading to an asymmetric cell division. The cell cycle components that activate the force-generating complex (FGC) on the cortex, which promotes spindle displacement, are largely unknown. The phosphatase PPH-6 has been implicated in the localization of the FGC and in the timing of force generation during anaphase (Afshar et al. 2010). As mentioned above, cortex-generated forces mediate the centration of the nucleocentrosomal complex during prophase. The force generating Gα (GOA-1 and GPA-16) signaling complex acts downstream of the PAR polarity and is redistributed from an anterior enrichment at prophase to a posterior cortical localization at metaphase (Park and Rose 2008). Microtubule-based forces and an asymmetric position of the spindle can only be achieved upon proper localization of Gα and its associated proteins GPR-1/-2 (G protein regulator) and the coiled-coil protein LIN-5 (Colombo et al. 2003; Gotta et al. 2003;

Srinivasan et al. 2003). LIN-5 can bind to both the Gα/GPR complex as well as the microtubule motor dynein, thereby anchoring the astral microtubules to the cortex and achieving force generation (Couwenbergs et al. 2007; Nguyen-Ngoc et al. 2007). At anaphase, the cortex is patterned with low GPR-1/-2 and LIN-5 at the anterior domain, a band of LET-99 slightly posterior to the center of the cell and a posterior domain enriched in GPR-1/-2 and LIN-5 (Colombo et al. 2003; Bringmann et al. 2007; Wu and Rose 2007; Krueger et al. 2010). In order to move the spindle, the FGC needs to link the microtubules to the cortex or membrane of the embryo. It has been shown recently that the actomyosin network at the cortex stabilizes the anchoring of the FGC at the membrane (Redemann et al. 2010). By weakening of the actomyosin network, single force generating events could be observed, as microtubules were able to pull membrane invaginations toward the centrosomes. Spindle severing experiments, analysis of centrosome movements as well as observation of individual force generation events came to the conclusion, that the asymmetric distribution of force generators results in a net asymmetry of cortical pulling forces that enables the posterior positioning of the spindle at metaphase and anaphase (Grill et al. 2003; Pecreaux et al. 2006; Redemann et al. 2010).

6.5.4 The Role of the Central Spindle During Anaphase

The destruction of cyclin B by the APC/C deactivates CDK-1 at anaphase onset. As discussed, this leads to the segregation of sister chromatids and centrioles (see Sect. 6.2.3), and ultimately evokes cytokinesis. It also induces a remarkable change in spindle morphology. As the chromosomes separate, the central spindle forms between the opposite poles and limits the rate of spindle elongation (Grill et al. 2001). For a functional central spindle, the nonkinetochore microtubules need to form stable bundles. Bundling of microtubules requires the activity of the kinesin-like protein ZEN-4 (CeMKLP-1) and the microtubule-associated protein SPD-1 (Verbrugghe and White 2004). ZEN-4 is inactivated prior to anaphase onset by an inhibitory CDK-1 mediated phosphorylation. Upon anaphase onset, the phosphatase CDC-14 removes the inhibitory modification that prevented the association of ZEN-4 with microtubules (Mishima et al. 2004). Localization of ZEN-4 to the central spindle depends on Aurora B and the inner centromere protein ICP-1 (Kaitna et al. 2000; Severson et al. 2000). ZEN-4 functions in the centralspindlin complex (ZEN-4 and CYK-4), which not only stabilizes the central spindle but is also required for the midzone to signal to the cytokinesis furrow and for the completion of cytokinesis (Bringmann and Hyman 2005). The capacity of the central spindle to position the contractile actomyosin ring is most likely through the ability of CYK-4 to recruit the RHO-1 activator ECT-2, which in turn recruits myosin to pattern the cortex around the spindle midzone (Werner et al. 2007).

6.5.5 *Two Consecutive Signals Determine the Cleavage Plane of the Embryo*

It has been established that two successive signals position the cytokinesis furrow in the one-cell embryo (Dechant and Glotzer 2003; Bringmann and Hyman 2005; Werner et al. 2007). Both signals are mediated by the microtubule cytoskeleton. The first signal comes from an interaction between the astral microtubules and the cortex. The separation of the asters at anaphase onset temporally couples chromosome segregation with the initiation of the cytokinesis furrow (Lewellyn et al. 2010). The ingression of the furrow is mediated by the constriction of an actomyosin ring. Furrow ingression based on the astral signal alone is faster than wildtype, indicating that the central spindle sends a retardation signal to the actomyosin network. The activity of the myosin light chain NMY-2 determines the speed of furrow ingression (Bringmann and Hyman 2005). This indicates that the second furrow-positioning signal, which depends on the central spindle, is able to correct the position of the cleavage plane by influencing actin dynamics.

6.6 The Differential Segregation of Cell Cycle Regulators Determines Cell Cycle Timing and Cell Fate of the Daughter Cells

The development of an organism with many different tissues requires the differentiation of the stem cell-like, one-cell embryo into various cell lineages. The asymmetric first division determines the future developmental program of the whole worm as it leads to the segregation of germline factors to one daughter exclusively. The specific timing of the cell cycle is tightly linked to cell fate (Bao et al. 2008; Lange et al. 2009). Schierenberg and Wood demonstrated in 1985 that the lineage specific timing in the *C. elegans* embryo is dependent on the nature of the cytoplasm and not a differential control by the nuclei. As discussed above, the asymmetric first division gives rise to two cells with distinct cytoplasmic protein compositions (see Sect. 6.4).

The larger anterior, somatic cell AB divides around 2 min prior to the smaller, posterior germline progenitor P_1. It is known that PLK-1, CDC-25, and CDK-1 are required for timely breakdown of the nuclear envelope in the first mitosis of the embryo (Chase et al. 2000). These cell cycle regulators have been implicated in mediating the differential timing between the AB and the P_1 cell, as the embryo goes from the two- to the four-cell stage (Fig. 6.2). From this set of cell cycle regulators, only PLK-1 is distributed in an asymmetric fashion. It has been proposed that the kinase PLK-1 promotes the nuclear accumulation of the phosphatase CDC-25. Activation of CDC-25 triggers CDK-1 function, leading to DNA condensation and the initiation of mitosis. At this point, it is not clear if PLK-1 activity is required upstream of CDK-1 or if these two kinases function in parallel. Sixty-percent

of the difference between the cell cycle timing of AB and P_1 can be accounted for by the unequal distribution of PLK-1 (Budirahardja and Gonczy 2008; Rivers et al. 2008). The kinase PLK-1 is enriched in the somatic AB cell, giving rise to a faster import of CDC-25 into the nucleus of the anterior cell. It has also been shown that the posterior cell is more sensitive to the levels of PLK-1 and CDC-25, while it seems that there is an excess of these cell cycle regulatory proteins in the anterior daughter. It is plausible that the timing of cell division in the early embryo is regulated by a balance between PLK-1, CDC-25, and CDK-1, with different emphasis on one of these proteins in different lineages. Bao et al. (2008) have shown that CDC-25 levels are rate limiting for the cell division timing of P_3 and the E lineage but not for any other lineage in the developing worm. The remaining 40% in the difference in cell cycle timing between AB and P_1 comes from a differential activation of the DNA replication checkpoint (Brauchle et al. 2003). The regulation of cell cycle timing in a DNA checkpoint dependent manner is specific to the germline precursors. It is reasonable that DNA replication is monitored more carefully in cells that give rise to the germline to ensure healthy offspring.

6.7 Conclusion

The development of the nematode *C. elegans* is highly reproducible (Sulston and Horvitz 1977; Deppe et al. 1978). The exact timing and stereotypic order of events in the one-cell *C. elegans* embryo allow for the precise description of the major morphological changes during a cell division cycle. The detailed characterization of different events with high time resolution encourages new studies on possible links between seemingly independent parts of the cell cycle and provides a base for further hypothesis driven research. The continuous advance in the genetic tools available for *C. elegans* provides opportunities to answer long-standing questions such as which mechanism regulates the cell cycle clock and how is an asymmetric cell division coordinated with the cell cycle.

The mapping of cell divisions and cell fates throughout the worm's development is the perfect prerequisite to study the regulation of cell cycle control throughout its development. It has been recognized that the temporal regulation of the cell cycle is critical for cell fate determination as each lineage has its own rhythm of divisions. The challenge is the identification of the key regulators for the individual steps of the cell cycle and of development without loosing sight of the complexity of cell division and cell–cell interactions. It will be interesting to learn how the cell cycle, as we understand it today, is modified to produce cells with different developmental fate.

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