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Review

The cell cycle and development: Lessons from *C. elegans*

David S. Fay *

University of Wyoming, College of Agriculture, Department of Molecular Biology, Dept 3944, 1000 E. University Avenue, Laramie, WY 82071, USA

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Abstract

The invariant developmental cell lineage of *Caenorhabditis elegans* (and other similar nematodes) provides one of the best examples of how cell division patterns can be precisely coordinated with cell fates. Although the field has made substantial progress towards elucidating the many factors that control the acquisition of individual cell or tissue-specific identities, the interplay between these determinants and core regulators of the cell cycle is just beginning to be understood. This review provides an overview of the known mechanisms that govern somatic cell growth, proliferation, and differentiation in *C. elegans*. In particular, I will focus on those studies that have uncovered novel genes or mechanisms, and which may enhance our understanding of corresponding processes in other organisms.

Keywords: Cell cycle; *Caenorhabditis elegans*; Development; Review

Contents

1. Introduction to *C. elegans* development

Just how a typical worm goes about generating 558 cells during embryonic development as well as an additional 401 somatic cells (plus ∼2000 germ cells) during its four postembryonic larval stages has been a subject of great interest for nearly 40 years. Aiding researchers in this ambitious goal has been the worm itself, both through its amenability to genetic approaches as well as its quasi-fixed or "hard-wired" developmental lineage. Briefly, during the first ∼1.5 h of embryogenesis, six "founder" cells are generated that will subsequently give rise to all cell types within the embryo [1]. The specific timing of founder cell establishment ranges from ∼30 min (in the case of AB) to ∼90 min (for D and P4; Fig. 1). Three of the founder cells will produce differentiated cell types of a single class, e.g. E, from which the intestine is derived. Three others

* Tel.: +1 307 766 4961; fax: +1 307 766 5098.
E-mail address: davidfay@uwyo.edu.

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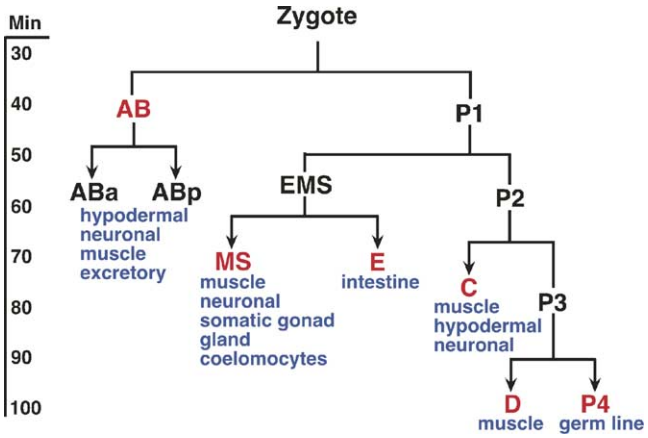

Fig. 1. Cell lineage of the early *C. elegans* embryo. Founder cells (red) and their derivatives (blue) are indicated along with the approximate timing of divisions after fertilization (at 25 °C). Left and right placements of daughter cells indicate their relative anterior and posterior locations within the embryo, respectively. Adapted from [1].

will generate diverse cell types, such as AB, whose descendents include skin, neurons, and muscle cells. After hatching, 53 cells produced during embryogenesis (termed "blast cells") will undergo subsequent rounds of division over the course of four larval stages (designated L1–L4) to generate the cell types and structures associated with the adult animal [2]. Two other embryonically derived cells (Z2 and Z3) are responsible for populating the germ line.

In examining the lineage of *Caenorhabditis elegans*, three things become apparent: (1) the relative timing of all (somatic) cell divisions is invariant; (2) the orientation planes of the cell divisions (with respect to the major animal axes) are also highly reproducible; and (3) the ultimate fates assumed by individual cells are invariant and correlate with the specific position of a cell within the greater lineage. As described below, by altering any single aspect of the lineage, other characteristics of the lineage may experience consequences. For example, by changing the plane of cell division, differentiation may be affected because of the abnormal segregation of cell fate determinants. In addition, by shifting cell fates (most commonly through loss of gene function), the subsequent timing of cell divisions will typically (and predictably) be altered. It is important to note that whereas the fixed lineage of *C. elegans* is suggestive of a model that could rely exclusively on the activities of asymmetrically segregated differentiation factors, in fact, cell signaling and inductive events play a major role in determining developmental outcomes during early embryogenesis, as well as later during postembryonic development. It is the invariance of the lineage with respect to both timing and orientation that leads to a reproducible pattern of cell-cell contacts, thereby ensuring consistency of the inductive events.

In addition to the actions of cell fate determinants and instructive signaling molecules, more global controls exist to guide the relative timing of postembryonic cell divisions. Such regulation is provided primarily by the heterochronic genes (so named for their striking defects in developmental timing), which include a number of micro RNAs as well as their presumptive targets. Mutations in the heterochronic genes lead to juxtapositions of developmental events, such that divisions typical of the L2 stage may occur during L1, or to patterns that are characteristic of one stage being reiterated throughout multiple stages, producing the equivalent of a developmental stutter [3]. Of particular significance is the implication that heterochronic genes must ultimately interface with cell cycle regulators to control both re-entry into and withdrawal from the cell cycle [4,5]. The exact mechanism by which this occurs, however, remains largely unsolved (also see below).

Components of the dauer pathway, the core portion of which includes an insulin-like signaling pathway, provides a necessary degree of flexibility to postembryonic development, allowing animals to temporarily withdraw from the normal course of development in times of food shortage or other environmental challenges [6]. Similar to the situation for heterochronic genes, entry into the dauer state (following L2) must necessarily be coordinated with cell cycle regulators, such that cells withdraw from the cell cycle and remain quiescent until re-entry into the L3 stage.

2. Control of embryonic cell fates and divisions

Prior to the isolation of informative mutations, several early studies suggested a role for asymmetrically distributed cytoplasmic determinants in governing the duration of individual cell cycles [7,8]. Using several different manipulation techniques, it was found that nuclei within a common cytoplasm divide synchronously, whereas enucleated cells continue to cycle (based on surface contractions) with a timing that is characteristic of their normal lineage. Furthermore, the duration of cell cycles can be greatly altered by the introduction of cytoplasm from cells with different inherent periodicities. Notably, these studies failed to detect alterations in the timing of blastomere divisions based solely on changes in nuclear-to-cytoplasmic ratios [7].

Initial studies also demonstrated that lineage-specific differences in cell cycle lengths could be attributed solely to disparities in the duration of S phase; the early cycles of *C. elegans*, like those of *Drosophila*, lack detectable gap phases [9]. True gap phases are first observed in the daughters of the E cell, and this delay (which corresponds with gastrulation) is dependent on embryonic transcription [10]. More recently, it has been reported that the duration of S phase in the two-cell-stage blastomeres AB and P1, depends on the actions of several conserved checkpoint genes including *C. elegans* homologs of Chk1 and ATM/ATR [11]. In the absence of checkpoint function, normal differences in the timing of these divisions were substantially, although not completely, abrogated. This study provided further evidence that the longer cell cycle associated with P1 may be the indirect effect of the smaller size of P1 relative to AB; a phenomenon that is

controlled in the first cell cycle by the asymmetric position of the mitotic spindle apparatus. The authors speculate that a cytoplasmic factor required for DNA replication may therefore be limiting in P1, leading to activation of the S-phase checkpoint and a delay in division.

Complementing these studies have been forward and reverse genetic analyses to identify early-acting factors and control mechanisms [12–15]. In general, these fall into three broad categories: (1) factors that directly (or indirectly) implement cell fates by controlling the expression of specific genes and proteins; (2) factors that regulate the asymmetric distribution of maternally-derived cell fate determinants belonging to the first category; and (3) factors that control the orientation of the early division planes (through regulation of the mitotic spindle position). Typically, mutations in genes from the first category lead not only to gross transformations in cell fates but also to concomitant changes within the lineage producing these aberrant fates. In other words, the pattern of the altered cell divisions often mimics that of the normal lineage that would produce this tissue in the wild type. This phenomenon is also characteristic for transformations of the postembryonic lineages, and these results indicate that cell cycle regulators often take their cue from factors controlling cell fate and differentiation.

It also follows that mutations affecting division planes and the distribution of maternal determinants would lead to the missexpression of genes that implement cell fate [13,16–18]. Such defects generally result in gross perturbations of normal cell division and differentiation patterns. In some instances, this can result in pseudo-hyperproliferative phenotypes, such as mutations in *par-1* that give rise to embryos with >800 cells [14]. In addition, alterations in the activity of genes that globally control transcription, such as histone acetylase, can also lead to hyperproliferation along with the absence of many differentiated cell types [19]. Nevertheless, the actions of such genes on cell cycle control are in most instances quite indirect.

3. Core regulators of the cell cycle in *C. elegans*

In addition to the indirect effectors described above, approaches using forward and reverse genetics have identified cellular and developmental roles for a number of conserved cell cycle components. However, because of the robust maternal expression of many cell cycle regulators (as well as their presumptive S- and M-phase targets), homozygous mutants may remain unaffected during embryogenesis and do not show defects until later stages of larval development, at which time maternal stores become depleted. One example is the presumptive Cdk1 homolog, *ncc-1*, which encodes one of six *cdc-2*-related kinases in *C. elegans*. *ncc-1* homozygous mutants (derived from a heterozygous mother) hatch as viable embryos but generally fail to complete any further cell divisions. In contrast, RNAi inactivation of *ncc-1* results in a depletion of *ncc-1* mRNA in the germline, and embryos consequently arrest at the one-cell stage as a result of incomplete meiotic maturation [20]. Similarly, mutations in the cyclin E homolog, *cye-1*, also lead to defects in postembryonic cell divisions (also see below), and like *ncc-1*, a role during embryogenesis is only revealed after RNAi depletion of germline *cye-1* [21]. In contrast, loss of function (by RNAi or mutation) in either the *C. elegans* Cdk-4 homolog or cyclin D leads to early larval arrest but fails to affect embryogenesis, indicating that embryonic divisions are not regulated by a classical G1/S-phase restriction point [22,23].

Negative regulators of cell cycle progression in *C. elegans* include Cdk inhibitors, the retinoblastoma protein, a *cdc-25*-like phosphatase, mediators of ubiquitin-dependent cyclin degradation, and a number of novel proteins (also see below). The two CIP/KIP family members in *C. elegans* (*cki-1* and *cki-2*) are encoded by adjacent genes, and inactivation of *cki-1* leads to excessive divisions during both embryonic and larval development [24,25]. Correspondingly, overexpression of *cki-1* leads to a block in division at the G1/S-phase transition [24]. Reporter analyses indicate that *cki-1* levels are elevated in G1-arrested blast derivatives and in cells that have undergone terminal differentiation [24]. Interestingly, *cki-1* expression is itself positively regulated by several known heterochronic genes, and *cki-1* inactivation results in the premature division of vulval precursor cells (VPCs), a defect also observed for the heterochronic mutant and positive regulator of *cki-1*, *lin-14* (for a discussion of VPC divisions, see below) [24]. Thus, *cki-1* may serve as a link between the cell cycle machinery and global regulators of developmental timing.

Work from my laboratory, together with studies from another group, has in recent years shown a role for the *C. elegans* retinoblastoma protein homolog, LIN-35, in cell cycle control. The genome of *C. elegans* encodes for a single ancestral Rb family member (in contrast to vertebrates which contain three family members), and animals harboring null alleles of *lin-35* are viable and show no systematic defects in cell division control or differentiation [26,27]. A function for *lin-35* in G1/S-phase control is indicated by the ability of *lin-35* mutations to enhance the hyperproliferative phenotype of *cki-1* loss-of-function (LOF) and to partially rescue the postembryonic division defects of cyclin D and Cdk4 mutants [22]. In addition, *lin-35* also shows a synthetic hyperproliferation phenotype with mutations in *fzr-1*, the *C. elegans* homolog of the anaphase promoting complex (APC) specificity component, Cdh1/fizzy related [27]. *lin-35* also enhances the hyperproliferative phenotype of *lin-23* mutants; *lin-23* encodes an F-box protein and constituent of the Skp1–Cullin–Rbx1–F-box (SCF) complex [27]. Both the APC and SCF complexes function as (E3) ubiquitin ligases and are known to promote the degradation of G1-type cyclins (A and E, respectively). Our results support a model whereby the observed synthetic phenotypes result from the inactivation of two principal mechanisms for regulating cyclin levels, transcriptional repression (via LIN-35) and targeted destruction (via FZR-1 and LIN-23). In the absence of either pathway alone, sufficient regulation can be brought about to

keep G1 cyclin levels within permissible limits. However, in the absence of both pathways, cyclin levels exceed a critical threshold resulting in abnormal cell cycle re-entry.

The *C. elegans* genome encodes four members of the *cdc25* phosphatase gene family [28]. Recently, two groups have identified gain-of-function (GOF) mutations in one of the family members (*cdc-25.1*) that specifically lead to supernumerary divisions of the E (intestinal) lineage during embryogenesis [29,30]. Conversely, loss of *cdc-25.1* function leads to early defects in meiosis and mitosis and in the reduced mitotic proliferation of the germline [31,32]; see [33] for a review of proliferation control of the germline. In contrast to fission yeast, where *cdc25* acts at the G2/M-phase transition, *cdc-25.1* appears specifically to promote entry into S phase [30]. The extra E cell divisions in *cdc-25.1* mutants are distinguishable from those caused by LOF in *cki-1*. For example, there are significant differences in the timing of the extra divisions in the two single mutants and *cki-1*; *cdc-25.1* double mutants have a partially additive phenotype. Therefore, *cki-1* and *cdc-25.1* may be active at different points in the cell cycle, or it could be that one or both does more than simply regulate CDK activity. The functions of the other *cdc25* family members are currently unknown.

As a conclusion to this section, it is worth noting that for the hyperproliferative mutants described above, terminal differentiation into the proper somatic cell types always seems to follow in the wake of the excess division cycles, indicating that although differentiation is delayed, it is not ultimately prevented. This is in apparent contrast to mutations that result in germline hyperproliferation [33–35], and probably reflects both underlying genetic redundancies and the inherent difficulty of completely derailing a hardwired developmental lineage.

4. The cell cycle, division, and differentiation

4.1. Dependence and independence

Although the differentiation of individual cell types is tightly correlated with specific patterns of division in the wild type, a number of studies indicate that terminal differentiation for many cell types does not necessarily require a complete execution of the normal lineage. For example, in two-cell-stage embryos treated with the cleavage inhibitor cytochalasin B, P1 was found to express (after extended incubation) at least one marker of gut cell differentiation [36]. These studies were complicated, however, by the ability of cytochalasin B-treated embryos to continue nuclear division cycles, leading to multinucleate cells of indeterminate developmental age. A more controlled study using aphidicolin (which blocks DNA synthesis) found that intestinal markers were expressed only in those embryos where the replication inhibitor was applied after the generation of the intestinal progenitor cell, E [9]. Furthermore, gut cell differentiation occurred only if the E cell had first been allowed to undergo some period of DNA synthesis, suggesting that entry into S phase (or exit from G1) was necessary for terminal differentiation to be initiated (perhaps as a result of changes in chromatin states following replication). For the expression of epidermal and muscle differentiation markers, resistance to DNA replication inhibitors occurs at ∼140 and 180 min, respectively, well before terminal divisions and the onset of normal differentiation by these cell types. The precise timing of differentiation commitment in these and other embryonic lineages has, however, not been well explored.

Complementary studies have also been carried out using mutations that produce variable cell cycle defects in postembryonic lineages. In several studies, neuronal development was observed to occur in the absence of complete division cycles [37,38]. Interestingly, these neurons typically displayed the differentiation characteristics of only one of the possible descendents, indicating that certain fates may be either intrinsic or dominant. In addition, the dominance of a particular fate (for a given blocked division) was in part dependent on the specific mutant used, suggesting that differences in the precise stage of cell cycle arrest may affect developmental outcomes [38]. Our own studies on the effects of cell cycle perturbations on vulval development are also consistent with proper lineal differentiation occurring in mutants with either reduced or excessive divisions [21]. Furthermore, our studies suggest that a differentiation timing mechanism might be set in motion following the initial induction of vulval cell fates. The timer we observed did not rely on counting cell divisions but rather appeared to measure the passage of time. Similar observations have been made in a number of diverse systems, and it has been suggested that such timers may depend on the progressive expression of Cdk inhibitors [39–42].

A notable exception to the above findings was a recent study showing that the production of extra distal tip cells (DTCs; a cell type associated with the somatic gonad) in *cki-1* mutants does not result from extra divisions of preexisting DTCs [43]. Rather, the origin of the extra DTCs was deduced to be the result of cell fate transformations by several other cells types within the somatic gonad lineage. This finding is in contrast to the extra DTCs produced in *lin-35*; *fzr-1* mutants, which arise from preexisting DTCs [27]. The former study indicates that *cki-1* may have roles in tissue-specific differentiation outside of cell cycle control or that perturbations in the cell cycle can result in some cases in cell fate transformations.

4.2. Control of vulval cell fate specification

During the L3 stage of postembryonic development, three of the six adjacent vulval precursor cells (termed P3.p–P8.p) are reproducibly induced to acquire vulval cell fates [44]. This event prompts the three most centrally located VPCs (relative to the inducing cell) to take on either a 1° (P6.p) or 2° (P5.p and P7.p) vulval cell fate; the two fates are distinguishable by their lineage patterns as well as by several expression markers (Fig. 2A) This precise pattern of cell fate specification is

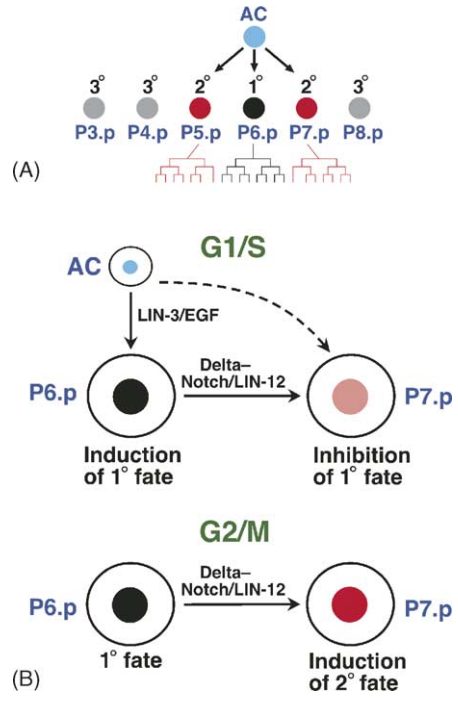

Fig. 2. Summary of vulval induction events. (A) Normal differentiated fates of the six VPCs after multi-step induction has taken place. The inducing anchor cell (AC) is depicted at top. The lineage patterns of P5.p, P6.p, and P7.p following induction are indicated below. (B) Model for a two-step VPC induction process that is separated by cell cycle phases (Adapted from [48]). Black nuclei (P6.p) indicate the 1° fate; red nuclei (P.7.p), the 2° fate. The intermediate pink nuclei (P7.p) indicate an induced vulval cell with a bias towards the 2° fate. Because of space considerations, the mirror induction of P5.p by P6.p has been omitted. For additional information, please see the text.

accomplished by two temporally separated inductive events, each involving distinct signaling pathways. Initially, an EGF-like ligand (LIN-3; secreted by a gonadal cell just dorsal to P6.p) triggers a graded response of Ras/Map kinase signaling in P5.p–P7.p with highest activation levels occurring in P6.p [45]. This leads to P6.p adopting a 1° fate, while P5.p and P7.p initially acquire indeterminate vulval fates. Next, P6.p activates Notch (LIN-12) signaling in the adjacent cells, P5.p and P7.p, (via several Delta family ligands) leading to these cells adopting a 2° fate [46]. The outlying VPCs (P3.p, P4.p, and P8.p) normally fail to receive either signal and instead assume a default epidermal fate (termed the 3° fate). Studies from several laboratories indicate that cell cycle position of the VPCs during these induction events is important for the determination of vulval cell identities [47,48]. Namely, acquisition of the 1° fate (via LIN-3) must normally occur prior to onset of M phase of the first VPC cell cycle, most likely during late G1 and S phases. In addition, lateral signaling (via LIN-12) during the first S-phase cycle by P6.p prevents P5.p and P7.p from adopting a 1° fate. Thus by late S phase of the first VPC cell cycle, the 1° fate is normally determined and cannot be reversed. These studies are also consistent with our own findings that an extended G1/S phase in *cye-1* mutants renders VPCs more susceptible to induction by Ras/Map kinase signaling [21]. (An apparent exception to these findings is that in animals that overexpress LIN-3, acquisition of the 1° fate can be extended to later time points [47].)

In contrast, the formal acquisition of 2° fates by P5.p and P7.p (also via LIN-12) appears to occur only after the completion of S phase, during G2/M of the first cell cycle, or early in the subsequent cycle. Thus the timing of 1° and 2° cell fate acquisition is temporally separated as a result of distinct differences in the competence of VPCs in G1/S versus G2/M to respond to instructive cues [48]. This temporal sequencing of cell fate choices was suggested to provide a means by which to coordinate the selection of different fates by cells with multiple potentials. This would in turn help to ensure the production of a functional organ by preventing more than one cell from acquiring the 1° fate.

5. Novel contributions

5.1. The cullins

Perhaps the most notable contribution of *C. elegans* research to the cell cycle field was the discovery in 1996 of the evolutionarily conserved family of cullins. The first cullin mutant to be identified, *cul-1*, exhibited hyperproliferation of most or all postembryonic blast lineages, including cells of the germline [49]. Since then, cellular and developmental functions have been ascribed to an additional three of the six family members in *C. elegans*. Biochemically, cullins serve as scaffolds for SCFs and ECSs (Elongin C-Cul2-SOCS box). Both complexes function as multisubunit ubiquitin ligases (E3s), whose targets include a diverse set of proteins. Based on the large number of predicted Skp1-like and F-box proteins in *C. elegans*, the potential for many distinct E3 isoforms, each with unique sets of targets, is high [50–52]. In the case of *cul-1*, one of its targets is likely to include cyclin E, since loss of cyclin E function can partially suppress *cul-1*-associated hyperproliferation [21].

Other cullin family members have been shown to function in a wide spectrum of developmental processes. For example, *cul-2* is required in germ cells to promote transition through G1/S [53]. In the absence of *cul-2* function, CKI-1 accumulates at high levels in germ cells, leading to a G1 arrest. This phenotype can be partially suppressed by a reduction in CKI-1 levels using RNAi. This particular function for CUL-2 is somewhat surprising given that the human homolog of CUL-2 associates with the VHL tumor suppressor protein, implying a negative role for CUL-2 in cell growth or proliferation in humans [54]. *cul-2* mutants are also unable to complete meiosis (due to a failure to degrade cyclin B1) and are slow or defective at multiple steps in mitosis, a phenotype that probably stems from a requirement for CUL-2 in promoting chromosome condensation [53,55,56]. In addition to these functions, *cul-2* is necessary for the proper establishment of embryonic polarity (through the localized

targeting of PAR-2 for destruction), and for the degradation of germline-associated proteins in somatic cells during early embryogenesis [55,57]. Similarly, *cul-3* is also required for multiple cellular processes including the regulation of mitotic spindle position following the meiosis to mitosis transition, the control of actomysin contractility during cytokinesis, and progression through S phase [58,59]. Recently, it was shown that a BTB-domain-containing adapter protein (MEL-26) serves a bridging function between CUL-3 and its target substrate MEI-1/katanin [60,61]. This finding suggests that BTB proteins may commonly function as substrate adapters for CUL-3-E3 ligases.

CUL-4 was recently shown to be required for genome stability through the regulation of the DNA-replication licensing factor, CDT-1 [62]. *cul-4* mutant animals arrest in early larval development with undivided but highly polyploid blast cells (up to 100C). The increased ploidy was found to be due to re-replication, whereby replication origins continue to fire and cells remain fixed in S phase. Lowering the gene dosage of the *C. elegans* Cdt1 ortholog substantially reduced the re-replication defects of *cul-4* mutants. In addition, antibodies to CDT-1 revealed that CUL-4 is required for the downregulation of CDT-1 following the progression of cells through S phase. It is currently unknown if CDT-1 is a direct target for ubiquitination by the CUL-4-SCF complex.

5.2. New dogs old tricks and old dogs new tricks

One unlikely source for new cell cycle components has come from the identification of genes involved in the specification of vulval cell fates (also see above). In the 1980s, the Horvitz laboratory used powerful genetic screens to uncover two genetically redundant classes of vulval cell fate determinants, collectively referred to as the SynMuv genes [63]. Whereas single mutants in either the A or B class of SynMuv genes failed to show pronounced defects, animals containing double mutations in both an A and B class gene displayed a striking multivulval (Muv) phenotype (reviewed by [64]). This phenotype occurs when >3 VPCs adopt vulval fates and is also observed for a number of mutations that augment Ras pathway signaling (also see above). Through the cloning of class B SynMuv genes, several conserved regulators of cell cycle progression were identified, including *lin-35*/Rb and *efl-1*/E2F [26,65]. More recently, Boxem and van den Heuvel [66] tested 18 of the known class B SynMuv genes for the ability to suppress division defects in cyclin D mutants. Interestingly, five class B genes (in addition to *lin-35*) tested positive for cell cycle roles including *efl-1*/E2F, its binding partner *dpl-1*/DP, and three novel genes, one of which, *lin-9*, has clear homologs in vertebrates [67]. While the precise role of *lin-35*/Rb and other Class B genes in vulval cell fate determination is currently unknown, a working model is that LIN-35 (acting in a complex with class B genes such as E2F, histone deacetylase, and NURD components) acts to antagonize Ras signaling through transcriptional repression. At present, the mutual regulatory targets of the Ras pathway and the Class B SynMuv genes remain to be elucidated.

The role of *lin-35* in vulval cell fate specification suggests a novel and non-cell-cycle-associated function for Rb family members in *C. elegans*. In addition to this, our laboratory has uncovered several unique developmental roles for *lin-35* in genetic screens for novel synthetic phenotypes [27]. In one case, LIN-35 was shown to functionally collaborate with a conserved SWI/SNF family member (XNP-1/ATR-X) to control the generation of several lineally-connected cell types in the somatic gonad [68]. In addition to this, we have identified an unexpected role for LIN-35 during organogenesis of the *C. elegans* pharynx [69]. Specifically, LIN-35 appears to control an early step in pharyngeal morphogenesis that requires a shift in the apical/basal polarities of several pharyngeal epithelial cell precursors. Several other pathways were also identified that act redundantly (and presumably in parallel) with LIN-35 in this process, including a conserved E2 ubiquitin conjugating enzyme and its E3 ligase partner ([69,70] and X. Qiu and D.S. Fay unpublished observations). We are currently working towards the identification of the target substrate(s) of these pathways. However, our genetic and phenotypic analyses strongly indicate that these new functions for LIN-35 are unconnected to its established role in cell cycle control. Another “cell cycle” gene for which a non-cell cycle role has been recently ascribed is the SCF complex component, LIN-23 (also see above). In addition to a hyperproliferation phenotype, strong LOF mutations in *lin-23* lead to defects in axon outgrowth in a subset of *C. elegans* neurons [71,72]. Interestingly, a partial LOF allele of *lin-23* was identified that displays only neuronal defects, indicating that this mutation effectively uncovered two distinct functions of LIN-23 [72].

Several recent studies have also identified new functions for CDC-14, a protein phosphatase previously shown to be required in budding yeast for mitotic exit [73]. In one analysis, CDC-14 was found to be required for the maintenance of G1 arrest in quiescent blast cells during larval development [74]. Several lines of experimental evidence point to a model whereby CDC-14 functions upstream of CKI-1 to control its stability in quiescent cells. Furthermore, CDC-14 activity may itself be regulated through its subcellular localization. CDC-14 was observed to cycle between the cytoplasm and nucleus in interphase and mitotic cells and is similarly segregated to the cytoplasm and nucleus in quiescent and post-mitotic cells, respectively [74]. In a separate line of work, CDC-14 was found to act in apparent opposition to Cdk1 to control binding of the mitotic spindle by ZEN-4, a mitotic kinesin-like protein [75]. Earlier studies on CDC-14 also detected an association with ZEN-4 at the mitotic spindle, and RNAi of *cdc-14* was observed to cause defects in cytokinesis [76]. These later findings are, however, somewhat complicated by issues of strain background, as complete LOF mutations in *cdc-14* (in an otherwise wild-type background) failed to produce the same defects [74].

5.3. Control of body size

The isolation of *C. elegans* mutants with altered body sizes (both larger and smaller) has provided insights into the pathways and mechanisms that regulate cell and organismal mass. Whereas it has been shown in many systems that organismal size more often correlates with variations in cell number than changes in cell size (mice and elephants have equivalently sized cells), this does not appear to be the case for *C. elegans*. A number of mutants showing "small" (Sma) and "long" (Lon) phenotypes have been isolated and these animals appear to contain equivalent numbers of somatic cells when compared with wild type [77–80]. Such findings indicate that for organisms with invariant developmental lineages (and that do not retain somatic division potential into adulthood), changes in organismal size can be attributed to changes in cell size. Curiously, although Ras activity has been shown to promote cell growth in other organisms including *Drosophila*, Ras appears to have no role in the growth or proliferation of somatic cells in *C. elegans* [81,82]. A recent study of Ras pathway mutants in *Oscheius tipulae*, a related species of nematode, suggests that Ras may, however, control cell proliferation in this organism [83]. Another major regulator of cell growth in mammals and flies, c-Myc, does not appear to be encoded by the worm genome.

One unique mechanism by which *C. elegans* may control body size is through the regulation of cellular ploidy. During development, a sheet of epithelial cells (termed the hypodermis) forms the outer layer of the animal. By adulthood, the majority of hypodermal nuclei are located within several large multinucleate cells that form by cell fusion events during embryonic and larval development [2,84]. The largest of these syncytial cell types, *hyp-7*, contains ∼133 nuclei. Interestingly, most nuclei of *hyp-7* undergo multiple rounds of endoreduplication (DNA synthesis without karyokinesis) in larvae and adults, leading to an average nuclear ploidy of ∼10C by late adulthood [85,86]. In studies on body size regulation in nematodes from the order Rhabditida (which includes *C. elegans*), Flemming et al. found a striking correlation between body size and hypodermal DNA content (a measure of both cell number and nuclear ploidy), such that species containing greater hypodermal DNA contents generally achieved larger adult sizes [86]. While this study did not establish a direct causal relationship between hypodermal DNA content and body size, it has been observed that mutations that compromise DNA replication in late-stage larvae, also display a reduction in body size ([21]; and D.S.F, unpublished observations). Moreover, in *C. elegans*, an increase in ploidy from diploid to triploid results in increased body size [87].

Several groups have subsequently examined hypodermal DNA contents in characterized *C. elegans* mutants where adult body size has been altered. In a number of cases, a direct correlation was observed between hypodermal ploidy and body size, particularly in mutants that affect TGF-β pathway signaling [86,88,89]. For example, loss of function in *lon-1*, a known target for repression by TGF-β in *C. elegans*, leads to an increased size and higher ploidy of hypodermal nuclei, whereas overexpression of *lon-1* in the hypodermis results in a reduction in body size and a decrease in ploidy [89]. One current model is that TGF-β, secreted by neuronal cells along the major body axis, may directly signal neighboring hypodermal nuclei to undergo endoreduplication [89,90]. However, other mutations that lead to similar defects in body size (including both larger and smaller animals) do not show concomitant effects on the ploidy of hypodermal nuclei, or for that matter, on total hypodermal DNA content [86,88]. Thus hypodermal ploidy (or DNA content) per se is not likely to be a direct determinant of body size, but may reflect a mechanism to maintain an optimal nuclear-to-cytoplasmic ratio in growing syncytial cells.

Most recently, a role in the regulation of body size was identified for the *C. elegans* p53 homolog, *cep-1* (A.K. Jolliffe and W.B Derry, personal communication). CEP-1 has previously been shown to function in the DNA-damage response that leads to apoptosis as well as in chromosome segregation during meiosis [91,92]. In addition, *cep-1* mutations are sensitive to a number of environmental stresses including reduced oxygen concentration (hypoxia; [92]). One outcome of hypoxia is that *cep-1* mutants (but not wild type) display a significant reduction in body size. Interestingly, inactivation of the *C. elegans* homolog of human Krit1, a disease gene associated with vascular abnormalities, results in a synthetic "small" phenotype with *cep-1* mutants, even in the presence of normal oxygen concentrations. Together, these findings suggest a novel role for CEP-1/p53 in coordinating cell size and the adaptation to low oxygen.

The precise relationship between *cep-1* and other size-control pathways in *C. elegans* is currently unclear. In genetic epistasis tests, the reduced size of *cep-1* Krit1 double mutants was found to be dominant to the hypertrophic effects normally conferred by mutations in *lon-1*. As *lon-1* is a known transcriptional target of TGF-β signaling [89], this result suggests that *cep-1* may act either downstream or in parallel to the TGF-β pathway. In addition, *cep-1* Krit1 double mutants show synthetic lethality with certain SMAD pathway genes (e.g., *sma-3*), further suggesting that these pathways may function in parallel to carry out overlapping functions, including the regulation of body size.

6. Concluding remarks

Until recently, the relative contribution of *C. elegans* studies to the cell cycle field has been somewhat minimal compared with that of yeast or even *Drosophila*; however, a current survey reveals a trend of steadily increasing impact. Moreover, as the focus of cell cycle studies shifts from single cells to developing organisms, the worm will undoubtedly occupy a place at the forefront of this emerging discipline. Future challenges include connecting the actions of cell fate determinants to core components of the cell cycle machin-

ery, developing a better understanding of the networks linking global regulators of development to cell cycle control, identifying additional conserved cell cycle components through forward genetics, and continuing to uncover the diverse developmental and cellular functions (including those unconnected to cell cycle regulation) of many "cell cycle" genes.

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