Mitotic Cell Division in *Caenorhabditis elegans*

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ABSTRACT Mitotic cell divisions increase cell number while faithfully distributing the replicated genome at each division. The *Caenorhabditis elegans* embryo is a powerful model for eukaryotic cell division. Nearly all of the genes that regulate cell division in *C. elegans* are conserved across metazoan species, including humans. The *C. elegans* pathways tend to be streamlined, facilitating dissection of the more redundant human pathways. Here, we summarize the virtues of *C. elegans* as a model system and review our current understanding of centriole duplication, the acquisition of pericentriolar material by centrioles to form centrosomes, the assembly of kinetochores and the mitotic spindle, chromosome segregation, and cytokinesis.

KEYWORDS Cell division; Centrosome; Cytokinesis; Kinetochore; Mitosis; Spindle assembly; WormBook

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CELL division in the early *Caenorhabditis elegans* embryo alternates between rapid S phases and mitosis without intervening gap phases, a common feature during early animal embryogenesis; most studies of cell division in *C. elegans* have focused on the one-cell zygote (Figure 1). During fertilization, the sperm brings not only a haploid genome, but also a pair of centrioles into the oocyte, which lacks centrioles. Subsequently, the two sperm-derived centrioles recruit pericentriolar material (PCM) from the oocyte cytoplasm to form centrosomes that can nucleate microtubules and separate to be positioned on either side of the paternal pronucleus. The oocyte and sperm-derived pronuclei are initially located at opposite ends of the embryo, but then migrate toward each other as chromosomes condense, meeting near the posterior pole. After the pronuclei meet, the nuclear–centrosome complex moves to the center while rotating to align with the embryo's long axis (Figure 1). The nuclear envelope then breaks down as the mitotic spindle assembles, with asymmetric elongation during anaphase contributing to a posterior displacement of the spindle. Thus, when the cleavage furrow bisects the mitotic spindle during cytokinesis, an asymmetric division results (Figure 1).

C. elegans offers compelling advantages for dissecting the molecular mechanisms governing mitotic cell division. It is easy to cultivate, its short life cycle (only 3 days at 25°) makes for fast genetics, and its transparency and the large size (50 × 20 μm) and rapid division (~20 min) of the one-cell stage embryo make it amenable to live (Gönczy *et al.* 1999) and fixed imaging, as well as correlative light microscopy and electron tomography (O'Toole *et al.* 2003; Pelletier *et al.* 2006; Srayko *et al.* 2006; Schlaitz *et al.* 2007; Laband *et al.* 2017; Redemann *et al.* 2017). Moreover, cell divisions in *C. elegans* are highly stereotyped, providing a rich context for quantitative analysis.

A powerful molecular genetic toolkit facilitates investigation of cell division in *C. elegans*. The syncytial germline contains an array of meiotic nuclei that are transcriptionally active and produce the gene products that are loaded into oocytes, and RNA interference (RNAi) efficiently and selectively depletes them. One can then quantitatively monitor defects in the stereotyped sequence of events that normally occur during the first cell division (Fraser *et al.* 2000; Gönczy *et al.* 2000; Piano *et al.* 2000; Kamath *et al.* 2001; Maeda *et al.* 2001; Kamath *et al.* 2003; Gunsalus *et al.* 2005; Sönnichsen *et al.* 2005; Green *et al.* 2011). Genome-wide RNAi screens have shown that ~2500 protein-coding genes, out of the ~20,000 that comprise the *C. elegans* genome, are essential for embryo production or viability (Kamath *et al.* 2003; Sönnichsen *et al.* 2005). Phenotypic profiling by videomicroscopy has identified ~600 genes essential for early embryonic cell divisions (Sönnichsen *et al.* 2005). Importantly, temperature-sensitive (TS) alleles isolated by forward genetic screens (O'Connell *et al.* 1998; Encalada *et al.* 2000; O'Rourke *et al.* 2011) have been found for ~200 of these essential cell division genes. With TS alleles, gene requirements can be assessed at temperatures that only partially compromise gene function. Furthermore, many TS alleles are fast-acting, such that gene products can be inactivated within a few minutes to define multiple requirements and TS periods (Severson *et al.* 2000; O'Rourke *et al.* 2011; Davies *et al.* 2014; Davies *et al.* 2017). Yeast two-hybrid screens have identified interactome networks that facilitate the identification and analysis of genetic pathways (Li *et al.* 2004; Boxem *et al.* 2008), and the application of auxin to rapidly degrade

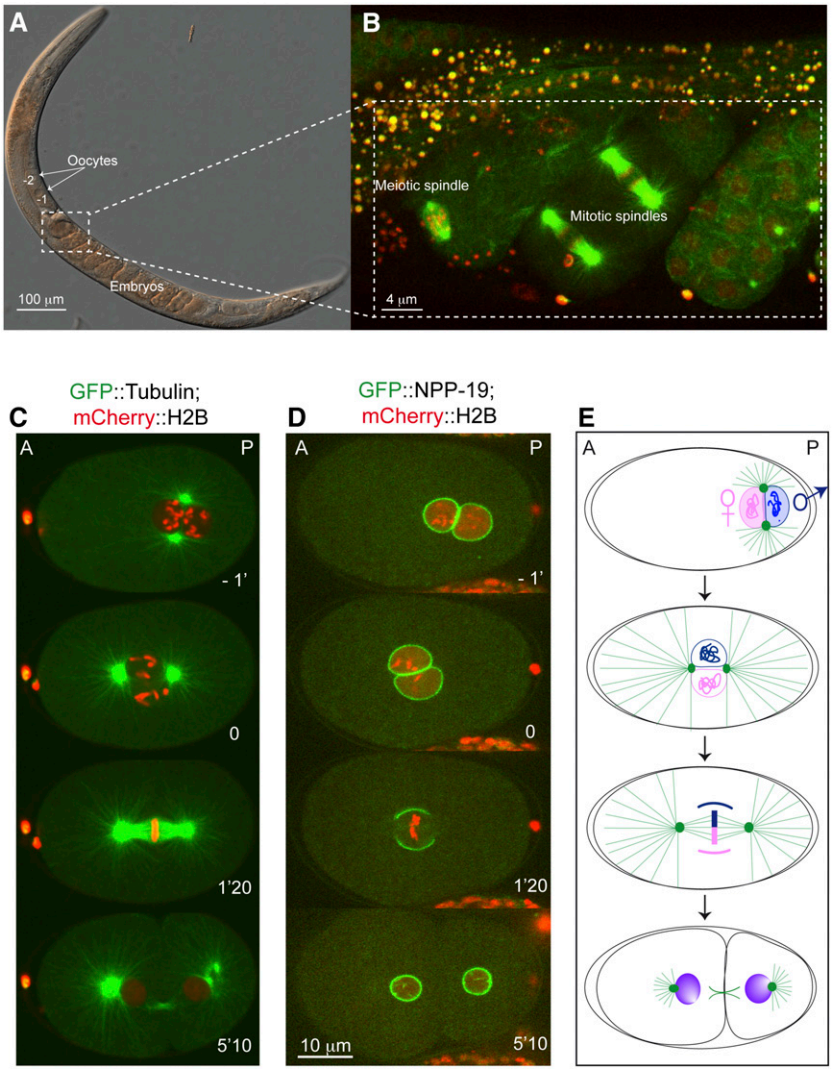

Figure 1 Mitotic cell division in the early *C. elegans* embryo. (A) Differential interference contrast (DIC) image of an adult worm. The positions of the two most mature oocytes relative to the spermatheca are indicated by white arrows and labeled -1 and -2; embryos within the uterus are also indicated. Bar, 100 μm. (B) Live image of embryos in the uterus of an adult worm expressing GFP-tagged β-tubulin (green) and mCherry-tagged H2B (red) to visualize oocyte meiotic and embryonic mitotic spindles. (C) Frames from *in vivo* live imaging of mitotic embryos expressing GFP-tagged β-tubulin (green) and mCherry-tagged H2B (red), or (D) GFP::NPP19 (green) and mCherry::H2B (red), to mark microtubules, chromosomes, and the nuclear envelope, respectively. In these and other figures, anterior (A) is to the left and posterior (P) is to the right. Bar, 10 μm. (E) Accompanying schematics (microtubules in green, paternal and maternal chromosomes in blue and pink, respectively). Time in minutes relative to Nuclear Envelope Breakdown NEBD at time = 0.

degron-tagged proteins in transgenic animals has expanded conditional loss-of-function approaches (Zhang *et al.* 2015). Finally, genome editing approaches including MosSCI (Frøkjaer-Jensen *et al.* 2008) and CRISPR/Cas9 (Dickinson and Goldstein 2016; Paix *et al.* 2016), in conjunction with RNAi- or degron-mediated depletion of endogenous wild-type gene products, have enabled powerful gene replacement strategies that facilitate functional probing of specific domains and residues within evolutionarily conserved proteins. All of this information about genome sequence, gene structure and function, RNAi phenotypes, the availability of TS alleles, and more are available at the WormBase database (www.wormbase.org) (R. Y. N. Lee *et al.* 2018).

Some cell division traits in *C. elegans* are unusual compared to other model systems. In particular, *C. elegans* has holocentric chromosomes. Rather than being restricted to a limited region of the chromosome (the centromere), the kinetochores that mediate attachment to spindle microtubules run along the entire length of each sister chromatid (Dernburg 2001; Melters *et al.* 2012). Other distinctive properties include relatively small and simple centrioles that nucleate the microtubule-organizing centers called centrosomes, incomplete nuclear envelope breakdown during mitosis, and a nearly exclusive reliance on centrosome-mediated nucleation of microtubules during mitotic spindle assembly. Nevertheless, work in *C. elegans* continues to inform our general understanding of animal cell division.

Centriole Duplication and Centrosome Maturation: Ensuring Fidelity in Bipolar Mitotic Spindle Assembly

Mitotic spindle assembly requires a collaboration between two spindle poles and the chromosomes that are ultimately segregated into daughter cells. The spindle poles are organized by two centrosomes, microtubule-organizing centers that each have a pair of orthogonally oriented centrioles at their core (Figure 2). Centrioles direct the assembly of an attached matrix of PCM that nucleates and anchors microtubules. Across species, centrioles are cylindrical structures roughly 100–400 nm long and 100–250 nm wide (Figure 2A). Their distinguishing feature is a ninefold symmetric

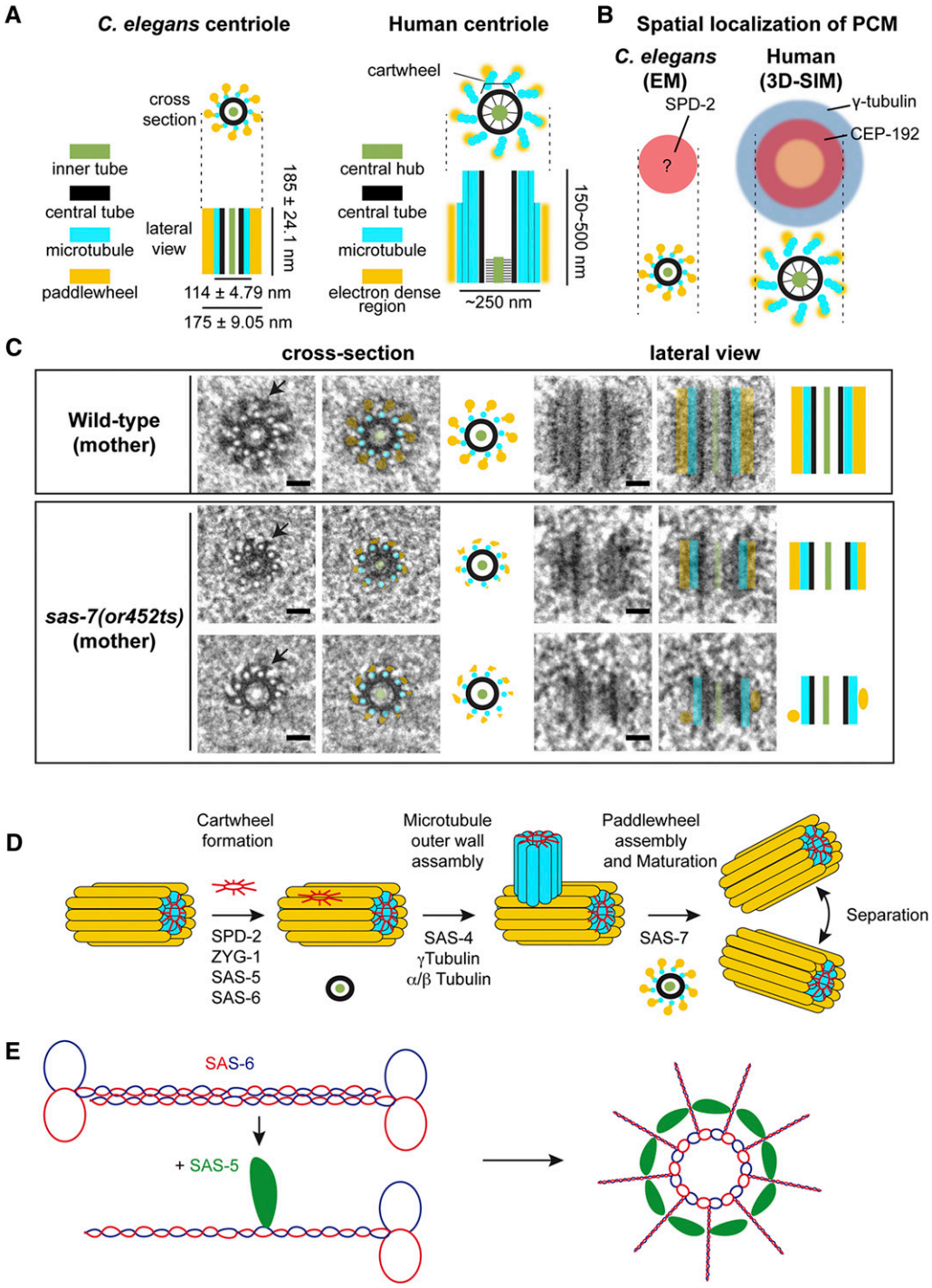

Figure 2 Centriole architecture. (A) Schematic illustration of *C. elegans* and human centriole architecture. (B) Spatial localization of PCM components. For *C. elegans*, results from immunogold electron microscopy staining data are shown. Most of the SPD-2 signal accumulated within a 200 nm diameter range (Pelletier *et al.* 2004). Human data are based on 3D-SIM images (Sonnen *et al.* 2012). SPD-2 and its human homolog Cep192 localize at the interface between centriole and PCM, and SPD-2 is potentially a component of the paddlewheel in *C. elegans* (loss of SPD-2 from centriole in sas-7 mutants coincides with the loss of paddlewheel structure). Modified from Sugioka *et al.* (2017). (C) Cross-section and lateral view of wild-type and *sas-7* mutant centrioles. Overlay indicates an interpretation of structures. Arrows indicate wild-type and defective sas-7 mutant paddlewheel structures. Bars, 50 nm. Modified from Sugioka *et al.* (2017). (D) Schematics showing the steps in the assembly and maturation of the daughter centriole along with the factors required for each step. Assembly of the daughter centriole begins when a cartwheel (red) forms at a right angle to the mother centriole. In the second step, an inner tube forms and subsequently mediates the peripheral assembly of nine symmetrically arranged microtubules (light blue) forms around the cartwheel. Assembly of the paddlewheel and acquisition of the ability to duplicate again (centriole maturation: daughter to mother centriole transition) requires SAS-7. (E) Early steps of cartwheel assembly with the ninefold symmetry dictated by the structure of SAS-6. SAS-6 contains an N-terminal globular domain, a long coiled-coil, and an unstructured C-terminal region. *C. elegans* SAS-6 alone forms an antiparallel tetramer, whereas binding of SAS-5 (green) disrupts the tetrameric association of SAS-6. The N-terminal globular domains of SAS-6 form the hub of the cartwheel with the coiled-coil dimerized spokes projecting outward.

array of stabilized microtubules. Depending on the species and tissue, the microtubule blades that make up the centriole outer wall can contain singlet, doublet, or triplet microtubules [reviewed in Carvalho-Santos *et al.* (2011), Bornens (2012), Gönczy (2012), Fırat-Karalar and Stearns (2014), Conduit *et al.* (2015), Fu *et al.* (2015)]. During interphase, centrioles are surrounded by a thin highly ordered layer of PCM. Upon entry to mitosis, centrosomes undergo a mitotic maturation that prepares them for spindle assembly. During this process, the amount of PCM and its microtubule nucleation capacity increases 5- to 10-fold. In addition to being larger, the mitotic PCM matrix also appears less organized than its interphase counterpart [reviewed in Mennella *et al.* (2014), Woodruff *et al.* (2014)].

C. elegans centrioles are relatively small and simple compared to those in other model systems, being roughly 200 nm long and 100 nm wide (Figure 2A). Rather than doublets or triplets, centrioles in the *C. elegans* embryo have singlet microtubules and lack the appendages that decorate the distal ends of mature mother centrioles in vertebrate cells. *C. elegans* centrioles are similar in size and structure to those in the early *Drosophila* embryo (Moritz *et al.* 1995), which has a similarly short cell-cycle time, and to the early intermediates observed during centriole assembly in mammalian cultured cells (Vorobjev and Nadezhdina 1987), suggesting that they represent a minimal form of the centriole that can assemble quickly to provide centrosome function in a rapidly developing embryo. Nevertheless, centrioles in later-stage embryos appear to be even smaller, roughly 100 nm in both length and width (Feldman and Priess 2012).

Centriole disengagement and the initiation of centriole duplication

Centriole duplication begins after the two centrioles at each spindle pole disengage during anaphase, a step that licenses their subsequent duplication (Fırat-Karalar and Stearns 2014; Lu and Roy 2014). Intriguingly, chromatin factors required for the cohesion of duplicated chromosomes during meiosis and mitosis also regulate centriole disengagement, although their requirements vary among species and at different developmental times. In *C. elegans*, these cohesion factor requirements are most apparent during spermatogenesis, when the two centrioles at each spindle pole remain associated throughout sperm maturation after the completion of meiosis II (Schvarzstein *et al.* 2013). Before disengagement, HORMA domain proteins that initially form axial elements to promote homologous chromosome pairing and cohesion, and the meiosis-specific cohesin REC-8, prevent centriole disengagement, until cell-cycle regulation leads to activation of the cysteine protease SEP-1/separase, cleavage of REC-8, and centriole disengagement. Loss of the HORMA domain proteins or REC-8 leads to premature disengagement and overduplication during spermatogenesis (Schvarzstein *et al.* 2013).

After fertilization of the oocyte and the completion of meiosis I and II, the two centrioles contributed by the sperm normally disengage and separate, and then each assembles a daughter centriole. Analysis of separase-depleted embryos revealed that the centrioles disengage but fail to separate further or duplicate. The defects in centriole separation and duplication resulting from lack of separase could both be rescued by perturbations that enhance PCM or microtubule assembly (Cabral *et al.* 2013). These results suggest that at the meiosis-to-mitosis transition physical proximity of centrioles, rather than engagement *per se*, is sufficient to suppress duplication; that separase promotes separation of the sperm centrioles, rather than their disengagement; and that enhanced microtubule-dependent forces acting on centrosomes can substitute for the role of separase in promoting centriole separation.

In contrast to their critical role in controlling centriole cohesion and separation during the meiosis to mitosis transition, cohesion and separase do not seem to control centriole dynamics during subsequent mitotic divisions. However, premature disruption of the PCM in these later divisions, by upshifting a fast-acting TS allele of the PCM component SPD-5, resulted in premature centriole separation and overduplication (Cabral *et al.* 2013), suggesting that the mother and daughter centrioles may be held together by the PCM matrix that forms around the mother. Disassembly of this matrix would then release the daughter and allow the two centrioles to separate and duplicate.

The centriole assembly pathway

After disengagement, a daughter centriole grows out from a single spot near the proximal end of each mother centriole, initially forming an electron-dense central tube that subsequently becomes decorated with a ninefold symmetric array of singlet microtubules (the outer centriole wall) (Pelletier *et al.* 2006) (Figure 2C). The core components required for centriole assembly were discovered in *C. elegans* and are widely conserved. Indeed, identification of the centriole assembly pathway is a premier *C. elegans* contribution to our understanding of fundamental biological processes.

Six *C. elegans* centriole assembly genes have been identified through chemical mutagenesis and genome-wide RNAi knockdown screens: *sas-4*, -5, *-6*, and *-7*, *spd-2*, and *zyg-1* (*sas* for spindle assembly abnormal, *spd* for spindle defective, and *zyg* for zygote-defective; Figure 2D) (O'Connell *et al.* 2001; Kirkham *et al.* 2003; Leidel and Gönczy 2003; Dammermann *et al.* 2004; Delattre *et al.* 2004; Kemp *et al.* 2004; Pelletier *et al.* 2004; Leidel *et al.* 2005; Sugioka *et al.* 2017). Four of these genes encode the conserved coiled-coil proteins SAS-4/CPAP, SAS-5/STIL, SAS-6/SAS6, and SPD-2/Cep192, while *zyg-1* encodes the *C. elegans* ortholog of mammalian Plk4 (Table 1). When any one of these genes is reduced in function in the oocyte, the sperm still brings a pair of centrioles into the egg during fertilization to support mitotic spindle assembly. However, the mutant oocyte cytoplasm fails to support duplication of the sperm-derived centrioles, and the two daughters each inherit a single centriole and form monopolar spindles (Figure 3A). Thus, inhibition of the four genes that are specifically required for centriole assembly (*zyg-1*, *sas-4*, *sas- 5*, and *sas-6*) leads to a signature phenotype in which the first division is normal and monopolar spindles are observed in both daughters at the second division (O'Connell *et al.* 2001; Kirkham *et al.* 2003; Leidel and Gönczy 2003; Delattre *et al.* 2004; Dammermann *et al.* 2004). In contrast, SPD-2 is a bifunctional protein required for centriole duplication and PCM assembly, and its inhibition results in a more severe defect in which centrosome formation and spindle assembly are compromised during the first division, in addition to a failure in centriole duplication (Kemp *et al.* 2004; Pelletier *et al.* 2004). SAS-7 is uniquely required for daughter centrioles to acquire the ability to reproduce (Sugioka *et al.* 2017). Thus, in *sas-7* mutant oocytes fertilized by wild-type

Table 1 Centrosomes and spindle assembly proteins

C. elegans protein	*C. elegans* gene	Vertebrate ortholog	Brief description of localization and function	TS alleles (* indicates fast acting)
REC-8	*rec-8*(W02A2.6)	Rec8	Meiosis specific cohesin: prevents centriole disengagement during spermatogenesis	
SEP-1/Separase	*sep-1*(Y47G6A.12)	Separase	Cysteine protease: cleaves REC-8 and promotes centriole separation after fertilization	
SAS-1	*sas-1*(Y111B2A.24)	C2CD3, RPH3A, DOC2A	Centriole protein required for centriole stability	
SAS-4	*sas-4*(F10E9.8)	CPAP	Centriole protein required for centriole assembly	
SAS-5	*sas-5*(F35B12.5)	Not identified	Centriole protein required for centriole assembly	
SAS-6	*sas-6*(Y45F10D.9)	SAS-6	Centriole protein required for centriole assembly	*or1167*
SAS-7	*sas-7*(T07C4.10)	Not identified	Centriole protein required for centriole assembly	*or452*
ZYG-1	*zyg-1*(F59E12.2)	Polo-like kinase 4 (Plk4)	Centriole protein required for centriole assembly	*it25*, *or278*, *or297**, *or409**, *or1018*
SPD-2	*spd-2*(F32H2.3)	Cep192	Bifunctional protein required for new centriole formation and for the assembly of the PCM	*or655*, *or493*, *or454*, *or183*, *or188*, *or293**, *or1089*
SPD-5	*spd-5*(F56A3.4)	Not identified	PCM component required for PCM assembly	*or213**
PLK-1	*plk-1*(C14B9.4)	Polo-like kinase 1 (Plk1)	Localizes to the centrosomes regulates PCM assembly; binds SPD-2	*or683*
AIR-1	*air-1*(K07C11.2)	Aurora A kinase	PCM component required for PCM assembly	
TBG-1	*tbg-1*(F58A4.8)	γ-tubulin	Provides caps and anchors microtubule ends	
TBA-1	*tba-1(F26E4.8)*	α-tubulin	*tba-1* encodes one of the nine *C. elegans* α-tubulins; acts redundantly with TBA-2 in the early embryo	*or346*, *or594**
TBB-2	*tbb*-2(C36E8.5)	β-tubulin	*tbb-2* encodes a *C. elegans* β-tubulin; acts redundantly with TBB-1 in the early embryo	*or600*
ZYG-9	*zyg-9*(F22B5.7)	XMAP-215	PCM component	*or634*, *or628*, *or593*, *or635*, *or623**
TAC-1	*tac-1*(Y54E2A.3)	TACC-1, 2, 3	PCM component	*or369*, *or402*, *or455*
SZY-2	*szy-2*(Y32H12A.4)	Phosphatase 1 inhibitor 2 - I2	Localizes to centrosomes; negatively regulates centrosome size	
SZY-20	*szy-20*(C18E9.3)	Not identified	Localizes to centrosomes; negatively regulates centrosome size	
SDS-22	*sds-22*(T09A5.9)	PPP1R7	Localizes to centrosomes; negatively regulates centrosome size	
GSP-1	*gsp-1*(F29F11.6)	PPP1CB (protein phosphatase 1 catalytic subunit β)	Downregulates ZYG-1 levels	
GSP-2	*gsp-2*(F56C9.1)	PPP1CA (protein phosphatase 1 catalytic subunit α)	Downregulates ZYG-1 levels	
FZR-1	*fzr-1*(ZK1307.6)	Cdh1	Substrate recognition subunit of the APC/C E3-ligase	
LIN-23	*lin-23*(K10B2.1)	βTrcp	F-box protein: substrate recognition subunit of Cullin 1-RING E3-ligases (CRL1)	
SEL-10	*sel-10*(F55B12.3)	Fbw7	F-box protein: substrate recognition subunit of Cullin 1-RING E3-ligases (CRL1)	
CYE-1	*cye-1*(C37A2.4)	Cyclin E	Promotes PCM assembly during embryo polarization	
CDK-2	*cdk-2*(K03E5.3)	Cdk2	Promotes PCM assembly during embryo polarization	
KLP-7	*klp-7*(K11D9.1)	MCAK	Kinesin-13 microtubule depolymerase; localizes to the kinetochores, the centrosomes.	*or1092*, *or1292*
TPXL-1	*tpxl-1*(Y39G10AR.12)	TPX2	Targets Aurora A to microtubules, activates Aurora A; colocalizes with Aurora A at the centrosomes	
RSA-1	*rsa-1*(C25A1.9)	Protein phosphatase 2A regulatory subunit	Localizes to centrosomes, required for PP2A localization to centrosomes; binds RSA-1	*or598**
LET-92	*let-92*(F38H4.9)	Protein phosphatase 2A catalytic subunit	Localizes to centrosomes	
PAA-1	*paa-1*(F48E8.5)	Protein phosphatase 2A structural subunit	Localizes to centrosomes	
RSA-2	*rsa-2*(Y48A6B.11)	Not identified	Localizes to centrosomes; binds SPD-5 and RSA-1, targets PP2A to centrosomes	

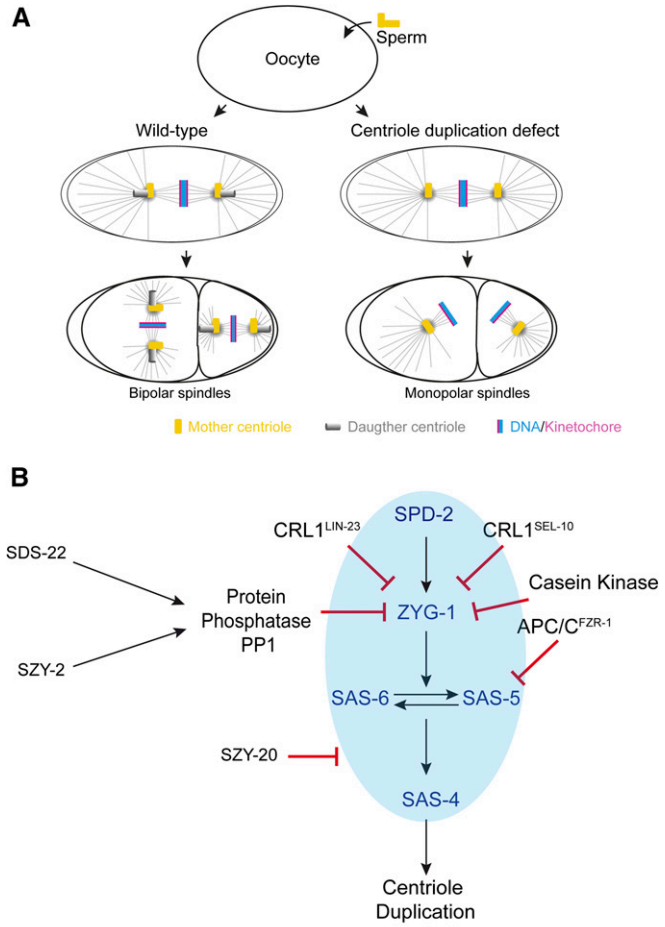

Figure 3 Pathways regulating *C. elegans* centriole duplication and assembly. (A) Phenotypes observed when a sperm cell containing a wild-type pair of centrioles fertilizes a wild-type oocyte (left column) or an oocyte lacking a component essential for daughter centriole formation (*e.g.*, *zyg-1* or *sas-4* mutants; right column). Defects in centriole duplication lead to the assembly of monopolar spindles during mitosis in two-cell stage embryos. (B) Different inputs involving E3-ligases, the cullin 1-RING E3-ligases $CRL1^{LIN-23}$ and $CRL1^{SEL-10}$, and the APC/C^{FZR-1} that regulate ZYG-1 and SAS-5 protein levels to limit centriole duplication. Red blunt arrows represent negative regulation. SZY-20 also inhibits the pathway controlling centriole duplication (blue oval), but the underlying mechanism is not known.

sperm, new daughter centrioles can recruit mitotic PCM and assemble bipolar spindles during the first and second mitotic divisions. However, the new daughter centrioles cannot reproduce, resulting in cells with monopolar spindles during the third cell division.

Investigation of these six genes has identified a conserved centriole assembly pathway (Figure 2D) (Hodges *et al.* 2010; Carvalho-Santos *et al.* 2011). The order in which they act has been determined by taking advantage of the initial contribution of centrioles to the one-cell stage zygote by the sperm, with oocytes lacking centrioles due to their earlier elimination during oogenesis (Albertson and Thomson 1993; Mikeladze-Dvali *et al.* 2012). Maternally expressed centriolar proteins present in the oocyte are required to assemble the new daughter centrioles adjacent to each of the sperm-provided parent centrioles. A sequence of centriolar protein assembly has been established using immunofluorescence in fixed embryos to detect native sperm-donated centriolar proteins and maternal centriolar proteins, marked by translational fusions to green fluorescent protein (GFP) (Dammer mann *et al.* 2004; Delattre *et al.* 2006; Pelletier *et al.* 2006).

In brief, such studies have shown that SPD-2 is recruited to the sperm centrioles early in meiosis I and is the centriolar receptor for ZYG-1/Plk4, the master regulatory kinase that controls centriole assembly (Delattre *et al.* 2006; Pelletier *et al.* 2006). ZYG-1/Plk4 is in turn required for the recruitment of SAS-5 and SAS-6, which, together with ZYG-1/Plk4, direct formation of the first intermediate in centriole assembly, called the central tube or cartwheel (Figure 2E) (Dammermann *et al.* 2004; Delattre *et al.* 2004; Leidel *et al.* 2005; Delattre *et al.* 2006; Pelletier *et al.* 2006)

Downstream of cartwheel assembly, SAS-4 controls formation of the microtubule-containing centriole outer wall (Figure 2D) (Kirkham *et al.* 2003; Leidel and Gönczy 2003; Pelletier *et al.* 2006). Live imaging of SAS-4 recruitment following fertilization indicates that it is recruited to daughter centrioles at the same time as SAS-6. Based on recovery after photobleaching, centriolar SAS-4 initially exists in a form that can rapidly exchange with SAS-4 in the cytoplasm, and subsequently converts to a more stable form that does not undergo rapid exchange. The conversion of SAS-4 to a stably associated form requires γ-tubulin and microtubule assembly (Dammermann *et al.* 2008). These results suggest that SAS-4 is stably incorporated into the centriole outer wall as the centriolar microtubules are formed. After assembly of the microtubule-containing outer wall, the coiled-coil protein SAS-7 directs assembly of an additional ninefold symmetric structure, termed the paddlewheel, that runs along the length of the centriolar microtubules (Figure 2D) (Sugioka *et al.* 2017). The paddlewheel forms concurrent with centriole maturation, the acquisition by the daughter centriole of the ability to reproduce, and SAS-7 is the first *C. elegans* protein known to be required for centriole maturation. Consistent with a role in maturation, SAS-7 binds to SPD-2 in yeast two-hybrid assays (Boxem *et al.* 2008; Sugioka *et al.* 2017), localizes to daughter centrioles independently of SPD-2, and is at least partially required for SPD-2 to localize to centrioles (Sugioka *et al.* 2017). Thus, in addition to being required for addition of the paddlewheel to the outer wall that completes formation of the daughter centriole, SAS-7 is also the earliest acting protein in centriole assembly through its role in recruiting SPD-2. SAS-7 shows some weak homology to the centriole-localized Chibby family of proteins, but thus far these potential homologs have not been found to have roles in centriole duplication.

Finally, SAS-1 (a C2 domain protein that binds to and stabilizes microtubules *in vitro*) is a centriole-localized protein that appears to promote normal centriole structure and stability, but may not be required for centriole duplication

(Table 1) (von Tobel *et al.* 2014). Centriole proteins themselves in *C. elegans* are remarkably stable: when introduced by fertilization into wild-type hermaphrodites expressing unmarked centriolar proteins, GFP fusions to some sperm centriole proteins persist throughout embryogenesis (Balestra *et al.* 2015).

Centriole assembly: a higher resolution view

From a structural perspective, the best-understood centriole component is SAS-6 [reviewed in Gönczy (2012), Hirono (2014), Dong (2015)]. This widely conserved protein includes an unstructured C-terminal region, a long central coiled-coil domain that mediates homodimerization, and a globular N-terminal domain (Figure 2E). Interactions between the globular head domains of SAS-6 dimers oligomerizes them to form a remarkable ring-shaped structure called the cartwheel, with ninefold radial symmetry that beautifully explains the ninefold symmetric organization of the microtubules that assemble along the length of each centriole (Figure 2E) (Kitagawa *et al.* 2011b; van Breugel *et al.* 2011). The N-terminal globular domains form the hub of this cartwheel, with the coiled-coil dimerized spokes projecting outward (Figure 2E). In other organisms, the SAS-6 cartwheels appear to be stacked upon each other with aligned spokes, forming an electron-dense central tube and spoke structure visible in electron micrographs (Figure 2). In *C. elegans*, it has been suggested that SAS-6 may instead form a spiral in which the spokes are not fully aligned (Hilbert *et al.* 2013), which could explain the lack of prominent electron-dense spokes in electron micrographs (Figure 2) (Pelletier *et al.* 2006; Sugioka *et al.* 2017). How the termini of the coiled-coil spokes promote centriole microtubule assembly is not known, but the process requires SAS-4 (Pelletier *et al.* 2006), which resides at the centriole perimeter near the spoke termini (Kirkham *et al.* 2003).

Mechanisms that promote assembly of the SAS-6–containing cartwheel have begun to emerge. Across species, ZYG-1/Plk4 forms a homodimer through the antiparallel association of its cryptic polo box domain (Park *et al.* 2014; Shimanovskaya *et al.* 2014). Dimerization of the cryptic polo box domain of ZYG-1/Plk4 generates a basic patch that interacts with an acidic region at the N-terminus of SPD-2, to recruit Plk4/ZYG-1 to the mother centriole (Shimanovskaya *et al.* 2014). The recruitment of SAS-6 to the assembly site on the mother centriole requires ZYG-1 and SAS-5, which interact with adjacent regions on the SAS-6 coiled-coil (Qiao *et al.* 2012; Lettman *et al.* 2013). The kinase activity of ZYG-1/Plk4 is not required to recruit SAS-6 to the vicinity of the mother centriole, but is required for its subsequent oligomerization to form the cartwheel (Lettman *et al.* 2013). The critical function of the kinase activity of ZYG-1/Plk4 in promoting SAS-6 oligomerization is not known, although the phosphorylated target is unlikely to be SAS-6 (Kitagawa *et al.* 2009; Lettman *et al.* 2013). SAS-5 forms homo-oligomers through two distinct domains, and mutations that disrupt oligomerization prevent centriole duplication (Qiao *et al.* 2012; Rogala *et al.* 2015), although the function of SAS-5 oligomerization in centriole assembly remains unclear.

The PCM also has a role in centriole assembly. While not absolutely required for centriole duplication, RNAi knockdown of the coiled-coil PCM scaffolding protein SPD-5 prevents centriole duplication in roughly half of the mutant embryos based on both light microscopy (Dammermann *et al.* 2008) and electron tomography studies (O'Toole *et al.* 2012). This requirement reflects a role for the SPD-5 dependent accumulation of PCM-associated γ-tubulin in centriole duplication, as γ-tubulin knockdown also partially compromises centriole duplication but does not affect SPD-5 accumulation. As noted earlier, at least one role for γ-tubulin appears to involve conversion of the initially exchangeable centriolar SAS-4 into a form that cannot rapidly exchange with cytoplasmic pools, likely by promoting assembly of the microtubules that make up the outer centriole wall (Dammermann *et al.* 2008).

Further evidence for a link between PCM assembly and centriole duplication has come from the identification of SZY-20, a conserved protein with RNA binding domains that negatively regulates centrosome maturation (Song *et al.* 2008). The *szy-20* gene was identified in a mutagenesis screen for suppressors of a conditional *zyg-1* allele (Kemp *et al.* 2007), and reducing SZY-20 function can restore centriole duplication in both *zyg-1* and *spd-2* mutants. Normally, SZY-20 limits the accumulation of ZYG-1, SPD-2, and other PCM components to centrosomes, and loss of SZY-20 results in abnormally large centrosomes and ensuing defects in spindle position and function that can be suppressed by reducing ZYG-1 levels. Thus, reducing SZY-20 function might restore centriole duplication in *zyg-1* and *spd-2* mutants by increasing the accumulation of partially functional ZYG-1 and SPD-2 mutant proteins, or alternatively by increasing the PCM levels of γ-tubulin (Dammermann *et al.* 2004; Song *et al.* 2008).

In addition to limiting centriole assembly such that each mother produces only a single daughter, centriole length also is tightly controlled to produce a structure that is about 200 nanometers long. How centriole length is determined remains poorly understood, but some of the genes required for centriole duplication also are required for daughter centrioles to achieve their normal length. Intriguingly, the coiled-coil protein SAS-7, which acts at both the earliest and last steps in centriole duplication, is required for daughter centrioles to grow to their full length (Sugioka *et al.* 2017). Partial depletion of SAS-4 leads to the assembly of centrioles with less microtubule-containing outer wall that recruit PCM in proportion to the amount of SAS-4 that they contain (Kirkham *et al.* 2003). Partial depletion of ZYG-1 or SAS-5, which control assembly of the cartwheel, can also lead to the production of short centrioles (Delattre *et al.* 2004). Knockdown of the PCM components SPD-5 or γ-tubulin, which both result in a partially penetrant failure in centriole duplication, also result in the production of daughter centrioles with abnormally low levels of SAS-4 incorporation (Dammermann *et al.* 2004). Together these results suggest that the amount of

SAS-4–containing outer wall that forms may depend on the size of the cartwheel and its ability to nucleate outer wall microtubules.

Intriguingly, electron tomography has shown that in the absence of either γ-tubulin or SPD-5, both of which result in the production of daughter centrioles with abnormally low levels of SAS-4 (Dammermann *et al.* 2004), centriolar microtubules often extend well beyond the ends of both the mother and daughter central tubes (O'Toole *et al.* 2012). It is surprising that lower levels of SAS-4, which is required for centriolar microtubule assembly, and of γ-tubulin, which can nucleate microtubule assembly, result in abnormally long centriolar microtubules. However, it is possible that reducing the number of PCM-associated microtubule polymers might increase local tubulin levels and thereby promote centriolar microtubule growth. Alternatively, PCM disruption in these mutants might interfere with factors that normally limit centriolar microtubule length.

Limiting centriole duplication by controlling the levels of centriole components

How does a mother produce only a single daughter? Studies in multiple systems indicate that tight regulation of Plk4 levels is critical (Nigg and Stearns 2011). Indeed, Plk4 is a suicide kinase regulated by autophosphorylation on an internal phosphodegron; disruption of the phophodegron increases Plk4 levels and produces centriolar rosettes with multiple daughters per mother centriole (Guderian *et al.* 2010; Holland *et al.* 2010; Cunha-Ferreira *et al.* 2013; Klebba *et al.* 2013). Furthermore, superresolution microscopy has shown that Plk4 initially forms a ring around one end of the mother centriole that subsequently resolves into a single spot to establish the site of daughter centriole assembly (Mennella *et al.* 2014). Presumably, tight regulation of Plk4 levels is important for resolving its localization to a single spot.

While genetic studies in *C. elegans* have focused on positive regulation of centriole duplication, a screen for suppressors of a TS *zyg-1* mutant (Kemp *et al.* 2007) has identified negative regulators of centriole duplication that provide insight into how ZYG-1/Plk4 levels are regulated (Figure 3B). Two suppressors of TS *zyg-1* embryonic lethality and centriole duplication, *szy-2/I-2* and *sds-22*, reduce the function of the protein phosphatase PP1 (Peel *et al.* 2017). Reducing PP1 function, or its positive regulators SZY-2/I-2 and SDS-22, increases early embryo ZYG-1 levels and results in the production of extra centrioles, and ZYG-1 overexpression in larval neuroectodermal stem cell lineages also results in centriole amplification (Wolf *et al.* 2018). Live-cell and superresolution imaging suggest that the extra centrioles in early embryos result from the simultaneous assembly of two or more daughters from one mother centriole (Peel *et al.* 2017). Thus, increased ZYG-1 levels may interfere with resolution of the ZYG-1/Plk4 ring into a single focus to produce only one daughter centriole, although whether ZYG-1 initially forms a ring at one end of the mother centriole has yet to be determined.

Additional inputs serve to further limit ZYG-1 levels during centriole duplication (Figure 3B). In addition to PP1 negatively regulating ZYG-1 levels, ubiquitin-dependent proteasome degradation, mediated by the Cullin 1 family member CUL-1 as an E3 ligase scaffold and two F-box substrate adaptor proteins LIN-23/βTrcp and SEL-10/Fbw7/Cdc4, also negatively regulates ZYG-1 levels, although loss of this regulation has not been shown to result in centriole overduplication (Peel *et al.* 2012). More recently, Casein kinase II also has been shown to negatively regulate ZYG-1 levels (Medley *et al.* 2017b). While it remains unclear how these different negative regulators cooperate, precise regulation of ZYG-1 levels appears to be critical for limiting centriole duplication to produce a single daughter.

Other factors also influence centriole assembly by regulating the levels of centriole components. The phosphatase PP2A influences the SAS-5–SAS-6 complex by inhibiting both ZYG-1 and SAS-5 degradation (Kitagawa *et al.* 2011a; Song *et al.* 2011), while the transcriptional regulator E2F/DP1 negatively regulates SAS-6 expression and positively regulates ZYG-1 and other centriole duplication factors. Reducing E2F/DP1 can rescue reduced ZYG-1 function through the resulting increased SAS-6 levels (Miller *et al.* 2016). Finally, negative regulation of SAS-5 levels also is important for the control of centriole duplication. The same screen that led to the identification of a role for PP1 in limiting ZYG-1 levels also recovered two loss-of-function alleles of the FZR-1/Cdh1 subunit of the anaphase promoting complex/cyclosome (APC/C) E3 ligase as suppressors of a TS *zyg-1* centriole duplication defect (Medley *et al.* 2017a). Further investigation showed that FZR-1/Cdh1 and other APC/C subunits are required to limit SAS-5 levels (Figure 3B). The higher SAS-5 levels are at least partially responsible for restoring centriole duplication in the *zyg-1* mutant background, although reducing APC/C function was not found to result in overduplication.

PCM assembly dynamics and structure

Centrioles recruit PCM to form centrosomes that can nucleate and anchor microtubules (Figure 4). Studies of PCM assembly in *C. elegans* first identified two coiled-coil proteins required for this process, SPD-2/Cep192 and SPD-5 (O'Connell *et al.* 2000; Hamill *et al.* 2002; Kemp *et al.* 2004). PCM assembly occurs in three different phases. First, after they enter the embryo, the sperm-donated centrioles acquire a shell of PCM as the maternal pronucleus completes its meiotic divisions (meiotic PCM assembly). The PCM component SPD-2 is detected in association with centrioles in mature sperm, but is not detectable shortly after fertilization following RNAi knockdown of maternal SPD-2, suggesting that this pool of SPD-2 is not stably associated (Pelletier *et al.* 2004). A more recent study indicates that SPD-2 is present both at the centrioles and in a shell that surrounds the sperm DNA and centrioles after fertilization (McNally *et al.* 2012). While SPD-5 has not been detected postfertilization at the sperm pronucleus-associated centrosome until after SPD-2

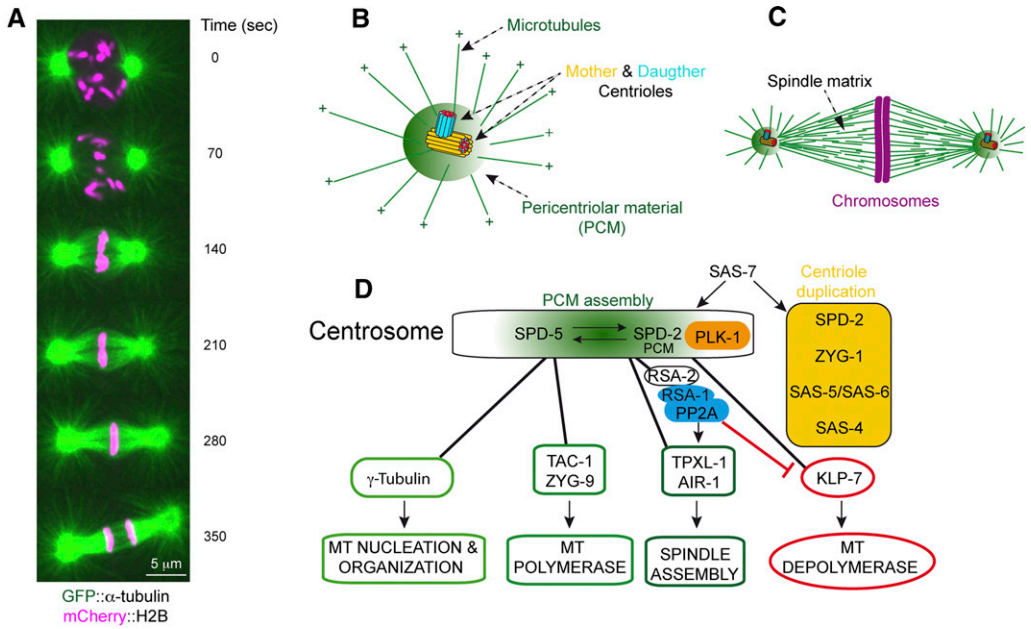

Figure 4 Centrosomes and mitotic spindle assembly in the one-cell *C. elegans* embryo. (A) Frames from *in vivo* live imaging of mitotic embryos expressing GFP-tagged β-tubulin (green) and mCherry-tagged H2B (magenta) to mark microtubules and chromosomes, respectively, from nuclear envelope breakdown to anaphase. (B) Schematic of the *C. elegans* centrosome containing a pair of centrioles surrounded by pericentriolar material (green) and microtubules anchored at their minus ends. (C) Schematic of the mitotic spindle in the one-cell *C. elegans* embryo. (D) Centrosome localized factors regulating centriole duplication (yellow) and PCM assembly (green).

is detectable (Pelletier *et al.* 2004), and γ-tubulin is detectable soon after fertilization (Hannak *et al.* 2001), the order of appearance of these different PCM components and their limits of detection using available reagents remain unclear.

The amount of PCM surrounding the centrioles subsequently increases coincident with the onset of polarity establishment via a process that depends on Cyclin E/CYE-1 and its partner CDK-2 (Cowan and Hyman 2006). We refer to this process as interphase PCM assembly, to distinguish it from the mitotic maturation of the PCM described below. Interphase PCM assembly is required for the proper temporal specification of the anterior–posterior axis of zygote polarity, which depends on signal(s) from the maturing centrosome (O'Connell *et al.* 2000; Wallenfang and Seydoux 2000; Cowan and Hyman 2006). In the absence of Cyclin E/CDK-2, PCM assembly is delayed but ultimately recovers and reaches wild-type levels during mitosis (Cowan and Hyman 2006). Assembly of the interphase PCM also has recently been shown to require SAS-7, which interacts with SPD-2 and is required for assembly of the paddlewheel that runs along the length of centriolar microtubules (Sugioka *et al.* 2017), raising the possibility that interphase PCM assembly involves recruitment of SPD-2 to the paddlewheel.

As the embryo enters mitosis, the amount of PCM surrounding the centrioles dramatically increases. During the first cell division in the *C. elegans* embryo, both the volume of the centrosome and the amount of γ-tubulin increase 5- to 10-fold during this mitotic PCM assembly process (Hannak *et al.* 2001; Decker *et al.* 2011). The amount of PCM that the centrosomes acquire during mitotic maturation depends on the size of the cell and presumably the amount of maternally loaded centrosome components that it contains. This proportionality is thought to contribute to the scaling process that reduces the size of the spindles as cell size decreases during development (Greenan *et al.* 2010; Decker *et al.* 2011). Interestingly, SAS-7 is required for the recruitment of interphase PCM and polarity establishment but may not be required for assembly of the mitotic PCM, suggesting that these two processes are distinct (Sugioka *et al.* 2017). However, complete loss of *sas-7* results in adult sterility due to failed germline proliferation, and the reduction of SAS-7 function in mutant zygotes is likely incomplete. Thus it remains possible that a complete loss of SAS-7 might result in more severe mitotic PCM assembly defects.

SPD-2 and SPD-5 are required for all PCM assembly in the embryo including mitotic PCM maturation (O'Connell *et al.* 2000; Hamill *et al.* 2002; Kemp *et al.* 2004), which is controlled by the mitotic kinases PLK-1 and Aurora A (Hannak *et al.* 2001; Woodruff *et al.* 2015). SPD-2 and SPD-5 have each been shown to exist primarily as monomers within the cytoplasm, and thus any interactions between them appear to occur only within the PCM (Wueseke *et al.* 2014). The polo-like kinase PLK-1 docks onto SPD-2 via an interaction that is essential for mitotic PCM assembly (Decker *et al.* 2011), and like the interaction between SPD-2 and SPD-5, their interaction is thought to occur only on the PCM and not in the cytoplasm (Wueseke *et al.* 2014). These studies have established that in *C. elegans*, SPD-2, SPD-5, PLK-1, and Aurora A/AIR-1 are key players required for assembly of the PCM that serves as a docking site for other centrosomal proteins, including the microtubule nucleating and anchoring protein γ-tubulin and the microtubule polymerase ZYG-9/XMAP215 (O'Connell *et al.* 2001; Hamill *et al.* 2002; Brouhard *et al.* 2008). How SPD-2 and SPD-5 interact during the mitotic maturation of the PCM and how they recruit other PCM components remain important areas for further investigation.

Superresolution light microscopy studies of mammalian centrosomes have revealed a substantial degree of organization within the interphase PCM, with both ninefold symmetry and an ordered molecular layering. In contrast, the mitotic

PCM is much less ordered (Mennella *et al.* 2014; Woodruff *et al.* 2014). In contrast to prior work examining PCM incorporation of centrosomin in *Drosophila* embryos (Conduit *et al.* 2010), where it was suggested that new PCM is added near the centriole and then moves outward, Fluorescence recovery after photobleaching (FRAP) experiments examining the *C. elegans* PCM matrix component SPD-5 suggest it is incorporated uniformly throughout the PCM during mitotic maturation (Laos *et al.* 2015). Nevertheless, light microscopy and electron tomography have suggested that the *C. elegans* PCM at metaphase may also have substantial internal structure, with microtubule density forming a ring around a core of γ-tubulin density and microtubule ends embedded at this interface (O'Toole *et al.* 2012). Knockdown of γ-tubulin results in loss of this internal structure and a more random distribution of microtubule ends throughout the PCM. Moreover, most of the microtubule ends within the PCM no longer appear capped and blunt, but frayed, suggesting that centrosomal microtubules in wild-type embryos are oriented with minus ends capped by γ-tubulin complexes anchored within the PCM at the γ-tubulin/microtubule interface. Knockdown of SPD-5 also results in a more random distribution of microtubule ends within the PCM and a loss of blunt ends, perhaps due not only to the loss of γ-tubulin, but also to a disorganization of other factors that normally regulate microtubule nucleation and organization. Superresolution imaging of multiple PCM components, together with electron tomography studies, both during polarity establishment and mitosis, promise to further improve our understanding of PCM organization in *C. elegans*.

In vitro reconstitution of PCM assembly

Reconstitution of PCM assembly *in vitro* has recently shed new light on the mitotic maturation of the centrosomes and highlights the central roles played by SPD-2, SPD-5, and the polo-like kinase PLK-1. First, purified SPD-5 was shown to assemble into large scaffolds *in vitro*, with both SPD-2 and PLK-1 accelerating this process (Woodruff *et al.* 2015). Mutant SPD-5 protein that cannot be phosphorylated by PLK-1 can still form scaffolds, but at a reduced rate (Wueseke *et al.* 2016). Most recently, SPD-5 was shown to form liquid droplets, in a phase separation process, when the crowding reagent glycol was added to the *in vitro* assays (Woodruff *et al.* 2017). Addition of PLK-1 and the tubulin dimer-binding TOG domain protein ZYG-9/XMAP215 to these assembly reactions led to the assembly of liquid droplets that concentrate tubulin dimers and promote microtubule nucleation in the absence of γ-tubulin. Moreover, computational modeling indicates that phase separation can account for the scaling of cell size and centrosome size, and for PCM defects in centriole-defective mutants (Zwicker *et al.* 2014). Thus phase separation may play a role in PCM assembly, with locally increased tubulin dimer concentration promoting microtubule nucleation and assembly. These results, and electron tomography analysis (O'Toole *et al.* 2012), raise the possibility that microtubule nucleation may be, at least in part, γ-tubulin–independent, and that γ-tubulin ring complexes may play more of a capping and anchoring role, in addition to or instead of a nucleation role. How AIR-1 might interface with such a phase separation process is not clear, and it remains to be shown that PCM *in vivo* exhibits liquid droplet-like properties. Furthermore, recent studies in *Xenopus* extracts and in fission yeast indicate that XMAP215/ZYG-9 family members and γ-tubulin cooperate to promote microtubule nucleation (Flor-Parra *et al.* 2018; Thawani *et al.* 2018). Future work on phase separation, both *in vivo* and *in vitro* and with *C. elegans* and mammalian proteins, may provide further insight into PCM assembly and function.

The past 20 years have witnessed remarkable advances in our understanding of centriole duplication and centrosome maturation, with studies in *C. elegans* being of particular importance for identifying a widely conserved pathway of centriole assembly. Future research promises to further advance our understanding of the initiation of centriole duplication, the link between central tube formation and centriolar microtubule assembly, centriole length control, and the role of the PCM in nucleating and organizing mitotic spindle microtubules.

Kinetochore Assembly, Function, and Regulation

Mitosis equally partitions a replicated genome between two daughter cells. Essential to this process is a large, multi-subunit protein complex called the kinetochore that connects chromosomes to spindle microtubules (Cheeseman 2014; Musacchio and Desai 2017). The kinetochore is composed of numerous polypeptides (Table 2) that assemble on chromosomes in a cell-cycle–dependent manner and coordinate multiple functions. The most apparent functions are to mediate microtubule/chromosome attachment and to generate force and tension required for chromosome movement. Kinetochores also must sense microtubule attachments, correct any errors, and signal the cell-cycle machinery to delay anaphase onset until all chromosomes achieve proper bipolar attachments.

The early *C. elegans* embryo offers relatively simple functional assays for investigating kinetochore assembly and function (Figure 5) (Oegema *et al.* 2001; Gassmann *et al.* 2007). Cortical pulling forces on astral microtubules asymmetrically position the one-cell stage mitotic spindle before cytokinesis (Grill *et al.* 2001), with sister chromatid cohesion and stable kinetochore attachments resisting these forces. In the absence of kinetochores, no forces counteract the astral pulling forces (Oegema *et al.* 2001), resulting in a failure to align chromosomes at the metaphase plate, an absence of chromosome segregation, and premature and excessive pole separation (the "Kinetochore-null" or KNL phenotype; see Figure 5A). As chromosome and spindle pole dynamics are highly reproducible from embryo to embryo, one can detect and quantify even subtle defects after RNAi knockdown of kinetochore components and regulators (Figure 5B) (Cheerambathur *et al.* 2017).

Table 2 Kinetochore proteins

C. elegans protein	*C. elegans* gene	Vertebrate ortholog	Brief description of localization and function	TS alleles
HCP-3/CENP-A	*hcp-3*;*cenp-a* (F58A4.3)	CENP-A	Histone H3-variant CeCENP-A epigenetically marks functional centromere, required for localization of all kinetochore components except KNL-2	
HCP-4/CENP-C	*hcp-4*;*cenp-c* (T03F1.9)	CENP-C	Kinetochore localization (prophase to telophase); required for localization of all kinetochore components except CENP-A and KNL-2	
LIN-53	*lin-53*(K07A1.12)	RbAp46/48	Histone chaperone, localizes to the centromere in metaphase and disappears in anaphase, required for CENP-A localization to the centromere	
KNL-2	*knl-2*(K06A5.4)	Mis18BP1	Kinetochore localization (prophase to telophase): required for CENP-A localization at kinetochore	
KNL-1	*knl-1*(C02F5.1)	AF15q14	Scaffolding protein at the kinetochore localization (prophase to telophase)	
KNL-3	*knl-3*(T10B5.6)	Not identified	Mis 12 complex, kinetochore localization (prophase to telophase)	
MIS-12	*mis-12*(Y47G6A.24)	Mis12	Mis 12 complex, kinetochore localization (prophase to telophase)	
KBP-1	*kbp-1*(R13F6.1)	Not identified	Mis 12 complex, kinetochore localization (prophase to telophase)	
KBP-2	*kbp-2*(F26F4.13)	Not identified	Mis 12 complex, kinetochore localization (prophase to telophase)	
NDC-80	ndc-80(W01B6.9.1)	Ndc80/ HEC	Ndc80 complex, kinetochore localization (prophase to telophase)	
HIM-10	*him-10*(R12B2.4)	Nuf2	Ndc80 complex, kinetochore localization (prophase to telophase)	
KBP-3	*kbp-3*(F26H11.1)	Spc25	Ndc80 complex, kinetochore localization (prophase to telophase)	
KBP-4	*kbp-4*(Y92C3B.1)	Spc24	Ndc80 complex, kinetochore localization (prophase to telophase)	
KBP-5	*kbp-5*(C34B2.2)	Not identified	Kinetochore localization (prophase to telophase)	
HCP-1, HCP-2	*hcp-1*(ZK1055.1) *hcp-2*(T06E4.1)	CENP-F	Functionally redundant proteins that localize to the mitotic spindle and to kinetochores between late prometaphase and early anaphase.	
CLS-2	*cls-2*(R107.6)	CLASP	Microtubule-associated protein that localizes to spindle poles, the miotic spindle and kinetochores, required for central spindle assembly	
CZW-1	*czw-1*(F20D12.4)	Zw10	RZZ complex, requires the other RZZ subunits for kinetochore localization	
ROD-1	*rod-1*(F55G1.4)	Rod	RZZ complex, requires the other RZZ subunits for kinetochore localization	
ZWILCH	*zwl-1*(Y39G10AR.2)	Zwilch	RZZ complex, requires the other RZZ subunits for kinetochore localization	
NUD-2	*nud-2*(R11A5.2)	NudE/NudL	Dynein receptor at the kinetochore	
SPDL-1	*spdl-1*(C06A8.5)	Spindly	Acts downstream of RZZ; kinetochore-specific adaptor for dynein	
BUB-1	*bub-1*(R06C7.8)	Bub1	Mitotic checkpoint serine/threonine kinase, kinetochore localization (prophase to telophase)	
BUB-3	*bub-3*(Y54G9A.6)	Bub3	Mitotic checkpoint pathway	
MDF-1/MAD-1	*mdf-1*(C50F4.11)	Mad1	Mitotic checkpoint pathway, localizes to unattached kinetochores	
MDF-2/MAD-2	*mdf-2*(Y69A2AR. 30)	Mad2	Mitotic checkpoint pathway, protein with similar functions as MAD-1, localizes to unattached kinetochores	
SAN-1/MAD-3	*san-1*(ZC328.4)	BubR1	Mitotic checkpoint pathway, localizes to kinetochores after nuclear envelope breakdown	
FZY-1/CDC-20	*cdc-20*(zk177.6)	Cdc20	Substrate recognition subunit of the APC/C E3-ligase and part of the Mitotic checkpoint complex (MCC)	
PLK-1	*plk-1*(C14B9.4)	Polo-like kinase 1 (Plk1)	Localizes to the kinetochores: regulates the mitotic checkpoint pathway; phosphorylates KNL-1 to promote BUB-1 recruitment to KNL-1	*or683*

C. elegans chromosomes are unusual in being holocentric, with diffuse kinetochores that form along their entire length, but their kinetochore structure and molecular composition are similar to those in other metazoans (Maddox *et al.* 2004; Musacchio and Desai 2017). The first *C. elegans* kinetochore proteins were identified by homology and their uniform localization along chromosomes, leading to the term *hcp* for *holocentric* proteins (Moore *et al.* 1999; Moore and Roth 2001; Kitagawa 2009). Later, essential kinetochore proteins were identified through RNAi-based genome-wide screens for the KNL phenotype (Desai *et al.* 2003; P. S. Maddox *et al.* 2007; Gassmann *et al.* 2008), and by tandem mass spectrometry interrogation of affinity purified KNL complexes (Cheeseman *et al.* 2004). Immunofluorescence combined with RNAi-mediated depletion revealed a hierarchy of kinetochore protein assembly (Cheeseman *et al.* 2004; Oegema and Hyman 2006). Subsequent *in vitro* reconstitution using purified components identified various subcomplexes and microtubule-binding activities within the kinetochore, and regulatory mechanisms controlling

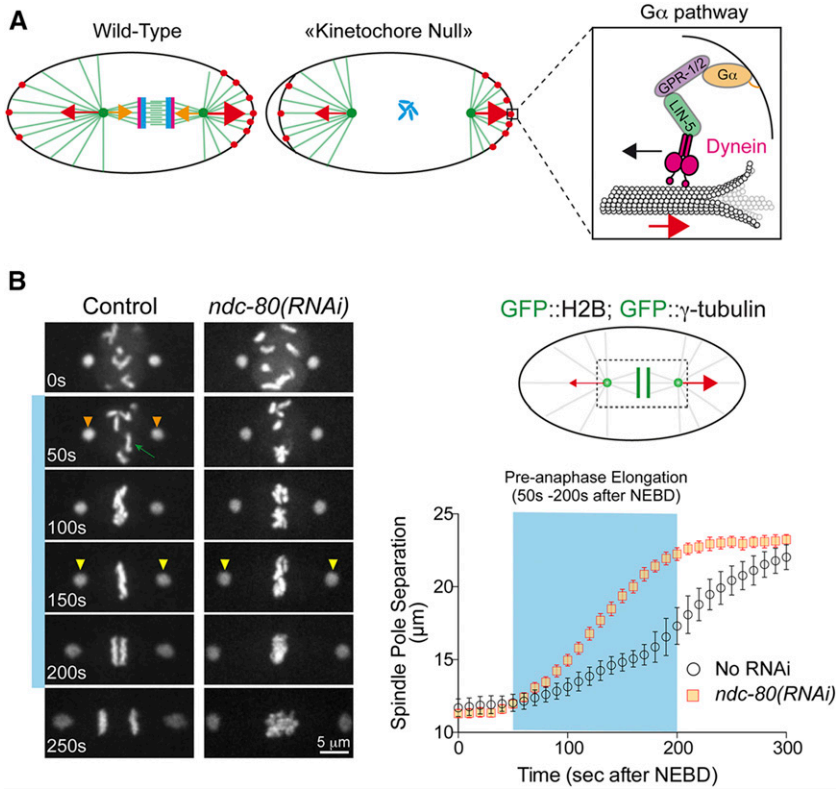

Figure 5 Functional analysis of kinetochore assembly and function in one-cell *C. elegans* embryos. (A) Schematics illustrating the kinetochore null (KNL) phenotype, characterized by a failure to assemble kinetochores competent for spindle microtubule attachment. Consequently, DNA segregation is severely defective and spindle poles separate prematurely and excessively in response to the astral pulling forces mediated by the Gα pathway, as schematized in the inset. This pathway, which comprises a complex of Gα, GPR-1/2, and LIN-5, anchors dynein to the cell cortex to generate pulling forces when dynein walks toward microtubule minus ends anchored at the spindle poles. These force generators are enriched posteriorly in response to cell polarity factors such that the spindle becomes posteriorly displaced and the division is asymmetric. (B) Spindle pole tracking detects defective kinetochore-microtubule attachments. Frames from *in vivo* live imaging videomicrographs of control and ndc-80(RNAi) one-cell embryos expressing GFP::H2b and GFP::γ-tubulin to label chromosomes (green arrow) and spindle poles (orange arrowheads), respectively, from NEBD to anaphase. Yellow arrowheads highlight premature spindle pole separation in ndc-80-(RNAi) embryos as compared to wild-type. Bar, 5 µm. Modified from Cheerambathur *et al.* (2017).

kinetochore-microtubule attachments (Cheeseman *et al.* 2006). Kinetochore proteins coordinate multiple functions, and the generation and analysis of "separation-of-function" mutants via genome editing has led to an impressive understanding of kinetochore protein functions.

Molecular architecture of the C. elegans kinetochore

Inner kinetochore proteins: connecting with chromosomal DNA: The kinetochore assembles on a specific chromosomal region called the centromere (Figure 5), which includes specialized nucleosomes containing the histone H3 variant HCP-3/CENP-A (Buchwitz *et al.* 1999) that epigenetically marks functional centromeres (Musacchio and Desai 2017). The histone chaperone LIN-53/RbAp46/48 is required for HCP-3/CENP-A localization to holocentric *C. elegans* centromeres and for accurate chromosome segregation, suggesting that LIN-53/RbAp46/48 mediates HCP-3/CENP-A deposition (Lee *et al.* 2016). To date, one other factor modulates HCP-3/CENP-A deposition at holocentromeres: the myb/SANT-domain protein KNL-2 (P. S. Maddox *et al.* 2007). Downstream of KNL-2 and HCP-3/CENP-A is HCP-4/CENP-C (Moore and Roth 2001; Oegema *et al.* 2001). These three inner kinetochore proteins connect chromosomal DNA to the outer kinetochore in *C. elegans* (Figure 6). In humans, the inner kinetochores are built by additional CENP subunits, which form the constitutive centromere-associated network (CCAN). With the notable exception of HCP-4/CENP-C, CCAN subunits have not been found to date in some species, including *C. elegans* and *Drosophila* [for review see Musacchio and Desai (2017), van Hooff *et al.* (2017)].

Outer kinetochore proteins: connecting with microtubules: RNAi screens for KNL phenotypes initially identified KNL-1 and KNL-3, in addition to the inner kinetochore proteins HCP-3/CENP-A, HCP-4/CENP-C, and KNL-2 (Desai *et al.* 2003; P. S. Maddox *et al.* 2007) (Figure 6A). Subsequent mass spectrometry analysis of KNL-1– and KNL-3–interacting proteins identified a network of 10 proteins: KNL-1, MIS-12, KNL-3, KBP-1, KBP-2, NDC-80, HIM-10/Nuf2, KBP-3/Spc24, KBP-4, and KBP-5 (Cheeseman *et al.* 2004). Analysis of their assembly hierarchy revealed that KNL-1, which acts downstream of KNL-3, is required for the localization of all known outer kinetochore proteins (Cheeseman *et al.* 2006) (Figure 6A). Biochemical reconstitution and characterization of this network from purified components identified three interacting and conserved units: KNL-1 and the Mis12 and Ndc80 complexes (together known as KMN), bearing distinct microtubule-binding activities in the Ndc80 complex and KNL-1 (Cheeseman *et al.* 2006). The Ndc80 complex forms load-bearing attachments to dynamic microtubules (Cheeseman *et al.* 2006), while the KNL-1 microtubule-binding domain is required for checkpoint signaling, but dispensable for chromosome segregation (Espeut *et al.* 2012).

The Ndc80 complex is a 50–60 nm long heterotetrameric and evolutionarily conserved complex consisting of two

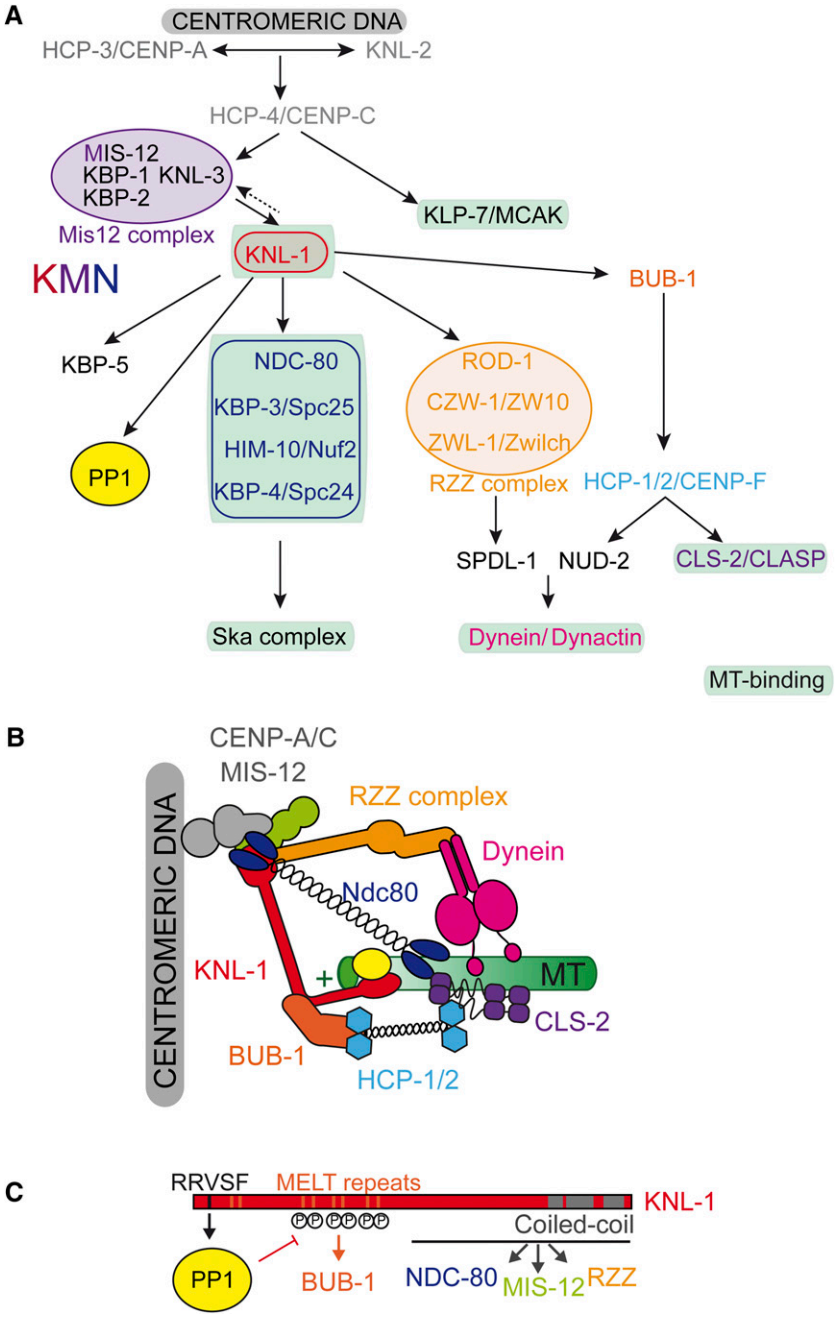

Figure 6 *C. elegans* kinetochore assembly: building spindle microtubule attachment sites. (A) Schematic of *C. elegans* kinetochore assembly. Proteins directly interacting with microtubules are highlighted in light green. Arrows indicate dependencies. (B) Schematics of kinetochore proteins and activities that interact with microtubules. (C) Domain organization of the scaffolding protein KNL-1 (red), a large multidomain and multifunctional scaffold protein required for kinetochore targeting of several other outer-domain kinetochore proteins, including, the SAC proteins BUB-1 and BUB-3, NDC-80, MIS-12, and the RZZ complex. KNL-1 also contains a docking site (RRVSF) for the PP1 phosphatase, which dephosphorylates KNL-1 on the MELT repeats and thereby eliminates the interaction between BUB-1 and KNL-1.

heterodimers—NDC-80 and HIM-10/Nuf2, and KBP-3/Spc25 and KBP-4/Spc24—joined by overlapping α-helical coiled-coiled domains located at the C and N termini of the NDC-80-HIM-10/Nuf2 and KBP-3/Spc25-KBP-4/Spc24 heterodimers, respectively (Figure 6B) (Cheeseman *et al.* 2006; DeLuca *et al.* 2006; Wei *et al.* 2007; Ciferri *et al.* 2008). Therefore MT-binding, mediated by the N-terminal globular domain of NDC-80 and HIM-10 subunits, and kinetochore-targeting, mediated by the C-terminal regions of KBP-3 and KBP-4 subunits, are located at opposite ends of the complex. The microtubule-binding region of the Ndc80 complex consists of a basic, unstructured N-terminal tail of the NDC-80 subunit and a pair of calponin-homology (CH) domains in the NDC-80 and HIM-10 subunits (Cheeseman *et al.* 2006). The microtubule-binding region is subject to extensive phosphorylation by Aurora kinases that regulate kinetochore-microtubule attachments (Cheerambathur *et al.* 2017) (see below).

Beyond these subunits, a number of additional proteins, notably factors linking kinetochores to microtubules (HCP-1/2/CENP-F, CLS-2/CLASP, Dynein, and SKA complex) dynamically associate with kinetochores during chromosome congression and segregation to regulate microtubule attachment and coordinate attachment with anaphase onset (Figure 6). For instance, downstream of KNL-1, the checkpoint kinase BUB-1 lies at the top of a hierarchy that comprises the CENP-F-like proteins HCP-1/2 and the microtubule-binding

protein CLS-2/CLASP (Figure 6A). In turn, CLS-2/CLASP controls kinetochore-bound microtubule dynamics to promote chromosome biorientation (Cheeseman *et al.* 2005).

From lateral to end-on microtubule attachment: cross-talk between RZZ-Spindly and the Ndc80 complex

Chromosomes are initially captured along microtubule sides (lateral attachments) and then tethered to microtubule ends, with end-on attachments that can resist the forces that pull on chromosomes during anaphase (Cheerambathur and Desai 2014). Furthermore, the two sister chromatids must attach to spindle microtubules emanating from opposite spindle poles to become bioriented. A lack of chromosome attachment, or incorrect ones such as merotelic attachments (a single sister kinetochore is simultaneously attached to microtubules from both spindle poles), must be corrected before anaphase onset. Proper orientation of sister kinetochores before stable microtubule attachment is of critical importance in *C. elegans*, with large holocentric kinetochores prone to merotely. The nonkinetochore motor protein KLP-19 discourages merotelic attachments by providing a polar ejection force that favors an orientation in which sister chromatid kinetochores face opposite spindle poles (Powers *et al.* 2004). The spindle assembly checkpoint (SAC) also promotes proper segregation by delaying sister chromatid separation until all chromosomes are bioriented and under tension (Musacchio and Salmon 2007) (discussed below).

In addition, regulatory mechanisms ensure that robust end-on microtubule attachments are achieved after transient, lateral kinetochore-microtubule attachments (Gassmann *et al.* 2008; Cheerambathur *et al.* 2013; Cheerambathur *et al.* 2017; Gama *et al.* 2017; Simões *et al.* 2018). *spdl-1*, the *C. elegans* ortholog of the conserved dynein adaptor Spindly, also results in a kinetochore null phenotype when inactivated. Removal of SPDL-1 is nearly equivalent to removal of NDC-80 (Gassmann *et al.* 2008). SPDL-1 interacts with ROD-1/rough deal, CZW-1/Zeste-white, and ZWL-1/Zwilch, which form the evolutionarily conserved RZZ complex, itself recruited to the kinetochore by KNL-1 (Figure 6A) (Gassmann *et al.* 2008). Paradoxically, although RZZ recruits SPDL-1 to kinetochores, *spdl-1* inactivation results in a much more severe phenotype than depletion of upstream RZZ complex subunits (Gassmann *et al.* 2008). The reason is that in the absence of SPDL-1, RZZ binds to and inhibits the Ndc80 complex, preventing end-on microtubule attachments (Cheerambathur *et al.* 2013). Codepleting RZZ relieves this inhibition and ameliorates the more severe defect resulting from *spdl-1* depletion alone.

SPDL-1 recruits dynein-dynactin to kinetochores (Griffis *et al.* 2007; Gassmann *et al.* 2008; Yamamoto *et al.* 2008; Chan *et al.* 2009), which contributes to initial lateral microtubule capture, accelerates stable end-on attachment by the Ndc80 complex, and relieves the inhibition of RZZ on Ndc80 by an as yet unknown mechanism (Cheerambathur *et al.* 2013). Cross-talk between Ndc80 and dynein-dynactin thus ensures accurate chromosome segregation. SPDL-1 recruits dynein-dynactin to kinetochores via two conserved motifs, the N-terminally located CC1 box and the spindly motif, through which SPDL-1 binds dynein light-intermediate chains and the dynactin pointed complex, respectively (Gama *et al.* 2017). Specific SPDL-1 point mutations, or deletion of the dynactin-pointed complex subunit DNC-6/p27, abrogate the interaction between SPDL-1 and the dynein-dynactin complex and phenocopy loss of *spdl-1* function, demonstrating the importance of dynein recruitment to kinetochores for chromosome segregation (Cheerambathur *et al.* 2013; Gama *et al.* 2017).

The recruitment of dynein at kinetochores also depends on the dynein cofactor NUD-2/NudE/NudL, which is recruited to kinetochores by HCP-1/2 (Figure 6A) (Simões *et al.* 2018). Loss of NUD-2 delays the formation of load-bearing microtubule attachments and causes chromatin bridges during anaphase. This phenotype is identical to that resulting from inactivation of RZZ complex subunits. However, loss of NUD-2 reduces dynein levels at kinetochores by only 50%, whereas RZZ depletion completely removes dynein from kinetochores, indicating that high levels of dynein at kinetochores are required for proper kinetochore function (Simões *et al.* 2018). To summarize, SPDL-1 and NUD-2 function as dynein receptors at unattached kinetochores to promote initial lateral microtubule capture and facilitate their maturation into end-on Ndc80 complex attachments.

Once proper end-on kinetochore-microtubule attachments are achieved, they must be stabilized to dampen chromosome motion before segregation. Dephosphorylation of the NDC-80 N-tail and/or tension on the attachments generated by GPR-1/2–dependent cortical pulling forces, promotes a microtubule-bound conformation of NDC-80 that is recognized by the conserved microtubule-binding SKA complex that stabilizes kinetochore-MT attachments and dampens chromosome motions (Cheerambathur *et al.* 2017).

Together, these sophisticated regulatory events ensure that chromosomes are bioriented and under tension before chromosome segregation begins. In addition, these steps are monitored by the SAC, which prevents anaphase onset until all sister chromatid pairs have attached to spindle microtubules emanating from opposite spindle poles (Musacchio and Salmon 2007).

Microtubule attachments and the SAC

The SAC: The spindle checkpoint is conserved from yeast to human, with subtle variations, and has been extensively studied in the early *C. elegans* embryo (Kitagawa and Rose 1999; Nystul *et al.* 2003; Encalada *et al.* 2005; Stein *et al.* 2007; Essex *et al.* 2009). In response to improper kinetochore-microtubule attachments, the SAC generates a diffusible "wait anaphase" inhibitor that delays sister chromatid separation and mitotic exit by preventing securin and Cyclin B proteosomal degradation (Barford 2011). This inhibitor, called the mitotic checkpoint complex (MCC), inhibits the APC/C(Cdc20) E3-ligase that polyubiquitinates securin and Cyclin B (Figure 7) (Liu and Zhang 2016; Lara-Gonzalez *et al.* 2017).

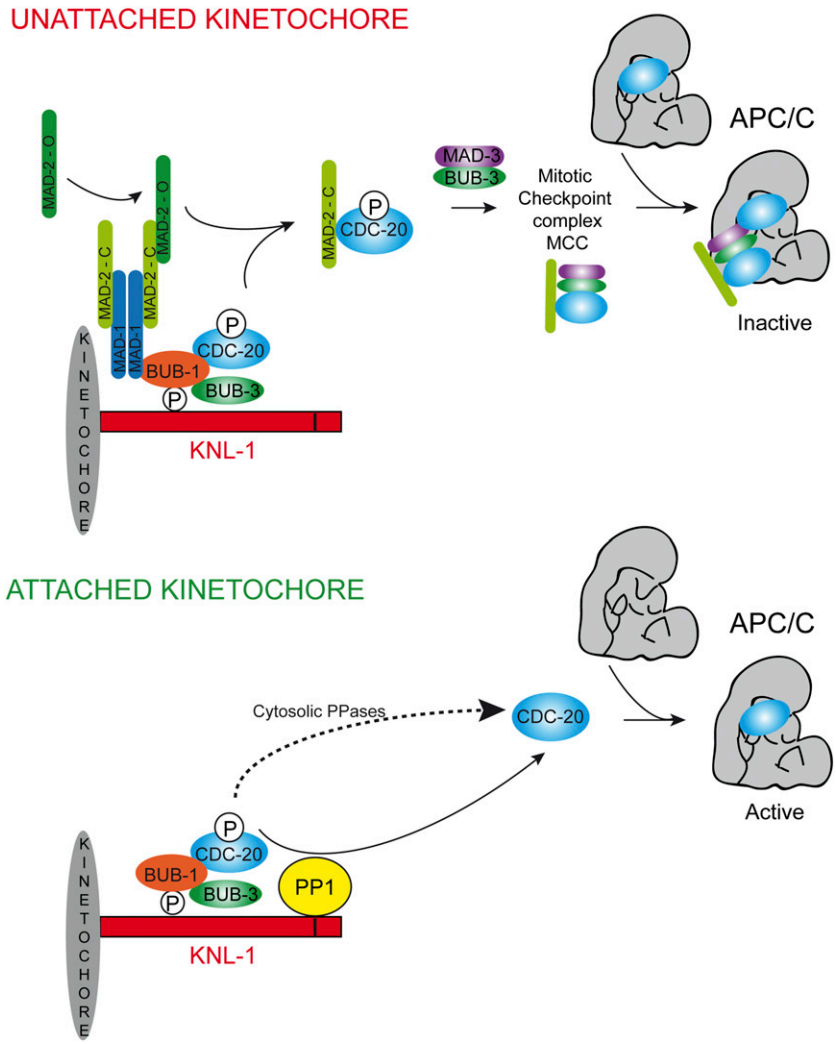

Figure 7 Kinetochores direct FZY-1/CDC-20 to the mitotic accelerator (APC/C) or brake (SAC) in response to microtubule attachment status: Schematics showing the two fates of CDC-20. CDC-20 is dynamically recruited to kinetochores where it interacts with BUB-1, via the ABBA motif. In the presence of unattached kinetochores (top panel) phosphorylated CDC-20 binds MAD-2 in the closed conformation and together with MAD-3 and BUB-3 assembles the mitotic checkpoint complex (MCC) that prevents APC/C activation. The MCC binds to a CDC-20 subunit physically associated with the APC/C and prevents it from binding substrates. It is known in mammalian cells that the MCC is continuously degraded as it binds the APC/C. When all kinetochores are properly attached to spindle microtubules and chromosomes are aligned at the metaphase plate (bottom panel), the generation of new MCC complexes is stopped. CDC-20 is dephosphorylated at the kinetochore by PP1 (and unknown cytosolic phosphatases) and then binds the APC/C after the previously bound MCC has been degraded [for review see Alfieri *et al.* (2017)].

The early *C. elegans* embryo mounts a checkpoint response that delays mitotic entry upon exposure to microtubule depolymerizing drugs such as nocodazole (Encalada *et al.* 2005), and robust genetic assays have been developed to monitor spindle checkpoint signaling (Essex *et al.* 2009). One assay uses two-cell stage embryos with monopolar spindles caused by inactivation of centriolar duplication proteins (Essex *et al.* 2009). Monopolar spindles, which contain unattached kinetochores and attached kinetochores that are not under tension, elicit a robust and quantifiable checkpoint-mediated cell-cycle delay that is suppressed by inactivation of checkpoint components (Essex *et al.* 2009). Another assay uses genetic interactions with a TS mutation in the essential APC/C subunit MAT-3/Apc8, *mat-3(or344ts)* (Rappleye *et al.* 2002; Kim *et al.* 2017). As the checkpoint prevents cell-cycle progression by inhibiting the APC/C, compromising the APC/C enhances a checkpoint defect (Bezler and Gönczy 2010; Kim *et al.* 2017).

Most SAC components, including MDF-1/MAD-1, MDF-2/MAD-2, BUB-1/Bub1, BUB-3/Bub3, and SAN-1/MAD-3/Mad3 (known as BubR1 in vertebrates and *Drosophila*), are conserved in *C. elegans* (Table 2), with the noticeable exception of the Mps1 kinase (Kitagawa and Rose 1999; Essex *et al.* 2009; Vleugel *et al.* 2012; Espeut *et al.* 2015). Depleting these proteins does not affect cell-cycle timing in basal conditions, except for BUB-1/BUB-3 (Kim *et al.* 2015), but all are required to delay cell-cycle progression by roughly 60% in the presence of monopolar spindles (Essex *et al.* 2009). In this section, for clarity and consistency with the cell-cycle field, we use the gene names *mad-1*, *mad-2*, and *mad-3* instead of *mdf-1*, *mdf-2*, and *san-1*.

Hierarchy of SAC assembly during checkpoint activation: How checkpoint proteins are recruited to kinetochores to generate the MCC is an important focus of SAC research. A key pathway component is the MAD-1–MAD-2 complex, which accumulates at unattached kinetochores (Kitagawa and Rose 1999; Essex *et al.*, 2009). Kinetochore recruitment of MAD-2 is observed only when the checkpoint is activated (Essex *et al.* 2009). The MAD-1–MAD-2 complex catalyzes a conformational transition of free cytoplasmic MAD-2 that promotes its association with FZY-1/CDC-20/Cdc20 (henceforth called CDC-20 for clarity and consistency with the cell-cycle field) to generate the MCC that inhibits the APC/C

(Figure 7). In vertebrates, the BubR1 subunit of the MCC binds to a Cdc20 subunit physically associated with the APC/C and prevents it from binding substrates (Izawa and Pines 2015; Alfieri *et al.* 2016); this inhibitory mechanism likely is conserved in *C. elegans* (Figure 7).

The mechanism by which the MAD-1–MAD-2 complex is recruited to kinetochores is emerging. MAD-1 physically interacts with the BUB-1 kinase (Moyle *et al.* 2014), which is at the top of the checkpoint protein kinetochore localization hierarchy from yeast to human (Musacchio and Salmon 2007). BUB-1 acts in a complex with its binding partner, the WD40-fold protein BUB-3 (Kim *et al.* 2015), to recruit MAD-1–MAD-2 to unattached kinetochores (Moyle *et al.* 2014). MAD-1 mutants defective in binding to BUB-1 do not localize to unattached kinetochores and are defective in checkpoint signaling (Moyle *et al.* 2014).

BUB-1 itself is recruited to kinetochores by KNL-1, an interaction regulated by phosphorylation (Figure 6B, bottom panel). In most organisms, the Mps1 kinase phosphorylates KNL-1 MELT repeats (Met-Glu-Leu-Thr) to create phospho-docking sites for Bub1/Bub3 and initiate checkpoint signaling. However, Mps1 is absent in *C. elegans* and instead, the polo-like kinase PLK-1 phosphorylates KNL-1 to direct BUB-1 recruitment to kinetochores (Espeut *et al.* 2015). How PLK-1 gets recruited to kinetochores in *C. elegans* is not understood (Cheerambathur and Desai 2014). Other kinetochore components are also required for the localization of MAD-1–MAD-2 at unattached kinetochores, in particular the RZZ complex, SPDL-1, and NDC-80 (Gassmann *et al.* 2008; Yamamoto *et al.* 2008; Essex *et al.* 2009), but the molecular mechanisms are unclear.

Checkpoint inactivation: Once all kinetochores are properly attached to microtubules and all chromosomes are bi-oriented, the SAC must be inactivated. The RZZ-spindly-Dynein pathway plays a prominent role in checkpoint inactivation by shedding MAD-1—MAD-2 from kinetochores to the spindle poles (Howell *et al.* 2001; Wojcik *et al.* 2001). The N-terminus of KNL-1 also senses microtubules attachment to kinetochores and recruits the PP1 phosphatase that contributes to checkpoint silencing (Espeut *et al.* 2012). In particular, PP1 dephosphorylates KNL-1 on the MELT repeats to disrupt the KNL-1—BUB-1 interaction (Espeut *et al.* 2012), with higher levels of BUB-1 at kinetochores in a KNL-1 PP1-binding mutant (Maton *et al.* 2015; Kim *et al.* 2017).

Kinetochores direct CDC-20 to the mitotic accelerator (APC/C) or brake (SAC)

While the spindle checkpoint pathway is conserved in *C. elegans* and delays mitosis in the presence of unattached kinetochores, its inhibition in unperturbed one-cell embryos does not lead to significant defects in chromosome segregation nor accelerated mitotic progression (Essex *et al.* 2009). Moreover, in embryos depleted of MAD-2, cyclin B degradation occurs with normal kinetics (Kim *et al.* 2017), indicating the existence of additional mechanisms restraining APC/C^{Cdc20} activity. A key factor that controls the timing of mitosis in one-cell embryos is BUB-1, which not only directs the SAC proteins to inhibit the APC/C in the presence of unattached kinetochores, but also promotes APC/C activation. BUB-1 thus plays a dual role, inhibiting and activating anaphase onset, with the balance between these potentially being modulated by microtubule attachment status (Figure 7). A role for BUB-1 in promoting anaphase onset emerged from the observation that inactivation of BUB-1, or its binding partner BUB-3, increased the time between NEBD and anaphase onset independently of the SAC, as this delay is still observed in BUB-1; MAD-2 or BUB-1; MAD-3 double depletions (Kim *et al.* 2015; Kim *et al.* 2017). A similar delay in anaphase onset is observed when BUB-1 recruitment to the kinetochores is prevented, indicating that BUB-1 is required at kinetochores for the normal timing of anaphase onset.

How might BUB-1 influence anaphase onset? In other systems, Bub1 and BubR1, which share a common Cdc20 interaction motif known as the ABBA motif (Di Fiore *et al.* 2015) or the Phe box (Diaz-Martinez *et al.* 2015), recruit Cdc20 via these motifs to kinetochores. However, the functional significance of Cdc20 recruitment to the kinetochore for its role in APC/C activation and anaphase onset has remained unclear. In *C. elegans*, BUB-1 similarly recruits CDC-20 to kinetochores through an ABBA motif (Kim *et al.* 2017). Mutation of the ABBA motif prevents CDC-20 recruitment to kinetochores and delays anaphase onset, demonstrating that CDC-20 recruitment to kinetochores is required for BUB-1 to promote anaphase onset. FRAP experiments showed that the residence time of CDC-20 at the kinetochores is only 1.2 sec, suggesting that it is dynamically fluxing through kinetochores (Figure 7).

How does BUB-1 influence CDC-20 activation? In human cells, the APC/C and Cdc20 are both regulated by Cdk1 phosphorylation. APC/C subunit phosphorylation enhances the affinity of Cdc20 for the APC/C (Fujimitsu *et al.* 2016; Qiao *et al.* 2016; Zhang *et al.* 2016), while Cdc20 phosphorylation inhibits its binding to the APC/C (Kramer *et al.* 2000; Labit *et al.* 2012; Hein and Nilsson 2016). Therefore, Cdc20 must be dephosphorylated to interact with, and activate the APC/C. *C. elegans* CDC-20 contains three evolutionarily conserved N-terminal Cdk1 sites (Kim *et al.* 2017). Remarkably, one-cell embryos expressing nonphosphorylatable alanine CDC-20 3A variants as the sole source of CDC-20 transit more rapidly through mitosis and enter anaphase before full chromosome congression (Kim *et al.* 2017). Of these three phosphorylation sites, Thr32 is the most important. Notably, the CDC-20 3A and T32A mutations bypass the anaphase-onset delay observed in the BUB-1 ABBA mutant, suggesting that BUB-1 promotes anaphase onset by promoting CDC-20 dephosphorylation. The PP1c phosphatase, which docks on KNL-1, likely fulfills this role at kinetochores. Accordingly, a PP1-binding mutant of KNL-1, which drastically impairs PP1 recruitment (Espeut *et al.* 2012) delays anaphase onset to an extent similar to that observed when CDC-20 recruitment to the kinetochores is prevented by mutating the ABBA motif of

BUB-1, and this delay is suppressed in the presence of CDC-20 nonphosphorylatable mutants (Kim *et al.* 2017). Overall, these data suggest that in embryos, CDC-20 is inhibited by Cdk-mediated phosphorylation and upon mitotic entry, CDC-20 recruitment to kinetochores through BUB-1 promotes its dephosphorylation by PP1c docked onto KNL-1 and its subsequent activation of the APC/C. Preventing CDC-20 recruitment to kinetochores delays anaphase onset but does not block it, possibly because CDC-20 is also dephosphorylated in the cytoplasm (Kim *et al.* 2017).

Strikingly, in the presence of unattached kinetochores, the BUB-1 ABBA motif mutant is also defective in checkpoint signaling. Thus, in situations where kinetochore-microtubule attachments are defective, CDC-20 recruited to the kinetochores by the BUB-1 ABBA motif also has the ability to delay anaphase onset (Kim *et al.* 2017). Therefore, CDC-20 dynamically fluxing through kinetochores via the ABBA motif of BUB-1 is either incorporated into the inhibitory checkpoint complex with MAD-2 (MCC) to inhibit the APC/C, or is dephosphorylated by PP1 to promote APC/C activation and anaphase onset (Figure 7). Whether and how microtubule attachment status influences the two fates of CDC-20 remains an open and important question. In principle, microtubule attachments trigger dynein-dependent MAD-1/2 removal from the kinetochores, and dephosphorylation of the MELT repeats of KNL-1 also promote BUB-1/BUB-3 removal. However, microtubule attachments remove MAD-1–MAD-2 but leave behind a substantial pool of BUB-1/BUB-3 (Kim *et al.* 2015), such that CDC-20 at attached kinetochores is more likely to be dephosphorylated (Figure 7). Microtubule attachments may also modulate PP1 activity and/or localization to the kinetochores. Further work is required to determine the relationship between microtubule attachment at kinetochores and alterations in the balance between the two fates of CDC-20 fluxing through kinetochores.

Tremendous progress has been made in understanding kinetochore assembly and function in *C. elegans*. However, despite considerable progress, important issues still need to be addressed. In particular, how do tension and mechanical forces stabilize kinetochore-microtubule attachments (Akiyoshi *et al.* 2010), and how do kinetochores sense and signal microtubule attachments to dephosphorylate NDC-80 and recruit the SKA complex to trigger anaphase onset? Interestingly, we have seen that dephosphorylation events trigger both stabilization of kinetochore-microtubule attachments and anaphase onset, further highlighting the importance of protein phosphatases in mitotic progression.

Mitotic Spindle Assembly and Chromosome Segregation

Mitotic spindle microtubules are organized and regulated by microtubule-associated proteins (MAPs), microtubule motors, and other associated proteins to generate three distinct sets of microtubules: astral microtubules emanating from the centrosomes and contacting the cortex, kinetochore microtubules (KMTs) that connect centrosomes to kinetochores, and central spindle microtubules that assemble between segregating sister chromatids after anaphase onset. KMTs mediate chromosome dynamics during mitosis. The central spindle, an array of antiparallel microtubules with their plus-ends overlapping at the center, coordinates chromosome segregation with furrow ingression during cytokinesis. Astral microtubules influence spindle position, contribute to chromosome segregation, and promote contractile ring assembly during cytokinesis.

Depending on the organism, different microtubule nucleation pathways support spindle assembly (Heald and Khodjakov 2015; Prosser and Pelletier 2017). In *Drosophila* and vertebrates, spindle assembly depends on three complementary mechanisms: (i) Ran-GTP produced in the vicinity of the chromatin binds to importin-β and thereby releases spindle assembly factors, (ii) centrosome-nucleated microtubules search and capture chromosomes, and (iii) Augmin-dependent recruitment of the microtubule nucleator γTuRC for branched microtubule nucleation. In the early *C. elegans* embryo, the Augmin pathway is absent and mitotic spindle assembly depends largely, if not exclusively on centrosomes. In this section, we review mitotic spindle assembly in *C. elegans* and discuss recent data highlighting the role of a subset of kinetochore proteins in the initial stages of central spindle assembly. Finally, we consider the mechanisms that mediate chromosome segregation, including a possible contribution from the central spindle via an inside-out pushing mechanism.

Centrosome-based spindle assembly in the early embryo

The one-cell stage mitotic spindle is large (14 μm from pole to pole), with prominent centrosomal spindle poles that nucleate microtubules (Figure 4A). Spindles fail to form in *C. elegans* embryos that lack functional centrosomes (Hamill *et al.* 2002; Kemp *et al.* 2004; Pelletier *et al.* 2004; Nahaboo *et al.* 2015), whereas half-spindles assemble in embryos that have one centrosome instead of two (O'Connell *et al.* 2001). Whether this strict requirement for centrosomes in spindle assembly is also true in later-stage embryos or larvae awaits further investigation. Fast-acting TS alleles of core centrosome components (*e.g.*, *spd-5* ts) can address this issue.

The centrosome-based mitotic spindle assembles rapidly. Early on, both cortical and sperm pronuclear envelope localized dynein promote centrosome separation in the one-cell zygote (De Simone *et al.* 2016). Subsequently, concomitant with nuclear envelope break down, centrosomal microtubules penetrate the nuclear space to capture chromosomes (Figure 4A), with further assembly also requiring functional kinetochores (Oegema *et al.* 2001). Spindle assembly is completed in the subsequent 2–3 min between nuclear envelope breakdown and anaphase onset, with astral microtubules attached to the cortex subject to pulling forces that position the spindle and promote chromosome segregation (Figure 4A) [for review see Kotak and Gönczy 2013, McNally 2013)].

Ultrastructural analysis of the mitotic spindle has provided important insights into spindle assembly and function

(O'Toole *et al.* 2003; Ozlü *et al.* 2005; Schlaitz *et al.* 2007; Redemann *et al.* 2017; Müller-Reichert *et al.* 2018). Tomographic reconstruction of metaphase and anaphase mitotic spindles in early embryos from electron micrographs combined with live-imaging confirmed that *C. elegans* mitotic spindles consist exclusively of microtubules radiating from the centrosomes (Figure 4, B and C) (O'Toole *et al.* 2003; Redemann *et al.* 2017). At metaphase, the mitotic spindle is composed of roughly 20,000 individual microtubules among which only 500 (2.5%) are KMTs, with only 6–50 KMTs attaching to each of the 12 chromosomes per pole-facing side (Redemann *et al.* 2017). These KMTs do not form bundles that resemble the kinetochore fibers observed in vertebrate and *Drosophila* cells, probably due to the holocentric nature of *C. elegans* chromosomes.

Do 6–50 KMTs directly connecting centrosomes to kinetochores provide a sufficiently strong mechanical connection between chromosomes and centrosomes? One possibility is that the number of microtubules directly connecting centrosomes to chromosomes might be underestimated, owing to the difficulty in tomography reconstructions and the density of microtubules emanating from the poles in all directions. Alternatively, one or two KMTs might be sufficient for chromosome segregation, as in budding yeast (Winey *et al.* 1995). Or, as supported by tomography reconstructions, chromosomes might be indirectly connected to centrosomes by anchoring into a spindle network, which raises questions about how KMTs form. Mathematical modeling of KMTs formation suggests that microtubules grow out from centrosomes and eventually either depolymerize or become KMTs upon attachment to a kinetochore. These KMTs might then selectively detach from the centrosomes and shrink from their minus ends. This model, supported by FRAP experiments, suggests that KMTs are short-lived relative to the timescale of mitosis (Redemann *et al.* 2017).

Because of the transient nature of KMTs, astral microtubules must rapidly find kinetochores. Given that chromosomes are holocentric, microtubules can bind at any point of the surface, and mitosis is semiclosed in the early embryo. The nuclear envelope breaks down in close proximity to the centrosomes, but remnants persist around the mitotic spindle until the metaphase-to-anaphase transition (Lee *et al.* 2000). The persistence of the nuclear envelope limits the space through which centrosome-based microtubules search and capture the chromosomes. Finally, a spindle matrix may concentrate free tubulin and other factors to favor assembly in a way that promotes kinetochore capture (Figure 4C) (Hayashi *et al.* 2012). Accordingly, during early spindle assembly, the density of EBP-2 foci, which decorate polymerizing microtubule plus-ends, is higher within the spindle (Srayko *et al.* 2005), suggesting that microtubules preferentially grow toward chromatin. The GTPase RAN-1, which is essential for nuclear import and spindle assembly (Askjaer *et al.* 2002; Bamba *et al.* 2002), contributes to this bias in microtubule outgrowth toward chromosomes, a function that is independent of kinetochores (Srayko *et al.* 2005). However, the mechanism by which RAN influences the direction of microtubule outgrowth toward chromatin is unclear (Srayko *et al.* 2005). Altogether, these traits likely contribute to the efficient capture of the chromosomes by astral microtubules, facilitating rapid spindle assembly.

Centrosome-localized factors regulating mitotic spindle assembly

Several centrosome-localized factors are required for mitotic spindle assembly (Figure 4D) (Sönnichsen *et al.* 2005; Srayko *et al.* 2005; Oegema and Hyman 2006; Müller-Reichert *et al.* 2010). Microtubule nucleation requires core centrosomal components, including the microtubule nucleator γ-tubulin and the AIR-1/Aurora A kinase, as discussed in the centrosome section. Other centrosome-localized factors further regulate microtubule dynamics (Srayko *et al.* 2005). Microtubule plus-end growth requires the evolutionarily conserved microtubule polymerase ZYG-9/XMAP215 and its activator TAC-1/TACC1 (Transforming and acidic coiled-coiled protein) (Srayko *et al.* 2005; Bellanger *et al.* 2007; Brouhard *et al.* 2008). The number of microtubules that grow out from centrosomes is controlled and limited by the microtubule depolymerizing kinesin KLP-7 (Srayko *et al.* 2005). Regulation of KMT length depends on the AIR-1/Aurora A kinase and its binding partner TPXL-1, the worm ortholog of the well-characterized microtubule-stabilizing protein TPX2 (Gruss *et al.* 2001; Schatz *et al.* 2003). In *tpxl-1*(*RNAi*) embryos, just after nuclear envelope breakdown, the assembling spindle collapses and centrosomes are pulled toward the chromatin (Ozlü *et al.* 2005); the rate of microtubule assembly is not affected (Srayko *et al.* 2005), but the microtubules are unstable. TPX-2 activates and targets AIR-1 to microtubules (Ozlü *et al.* 2005), which likely facilitates Aurora A-dependent phosphorylation of downstream substrates required for microtubule stability. For example, the *tpxl-1*(*RNAi*) phenotype might result at least in part from a defect in NDC-80 phosphorylation. As discussed in the kinetochore section, phosphorylation of the N-terminal NDC-80 tail prevents premature recruitment of the SKA complex to kinetochores such that microtubule attachments remain dynamic, allowing for tension-coupled polymerization. This phosphorylation is likely mediated by Aurora A (Cheerambathur *et al.* 2017), and preventing N-tail phosphorylation gives a short spindle phenotype that, although not as severe, is reminiscent of the *tpxl-1* phenotype.

The protein phosphatase PP2A complex, called regulator of spindle assembly (RSA), is also important for centrosome-based microtubule functions (Schlaitz *et al.* 2007). In particular, this complex is required for microtubule outgrowth from centrosomes and for microtubule stability during spindle assembly. This PP2A phosphatase complex is composed of RSA-1, (a regulatory subunit of PP2A), LET-92, (a catalytic subunit), and PAA-1, (a structural subunit). The complex localizes to the centrosomes via interaction with RSA-2, a coiled-coil–containing protein with no obvious homology in other organisms. RSA-2 recruits the PP2A complex to the centrosomes

by interacting with SPD-5 and RSA-1 (Figure 4D) (Schlaitz *et al.* 2007; Boxem *et al.* 2008). RSA influences spindle assembly both by inhibiting the microtubule depolymerase KLP-7 and by upregulating the microtubule-stabilizing protein TPXL-1. However, the phosphorylated substrates targeted by this phosphatase are not known. In summary, kinases and phosphatases are recruited to the centrosomes, where they coordinate the activities of different microtubule regulators to promote spindle assembly (Figure 4D).

Kinetochore proteins and central spindle assembly

After anaphase onset, antiparallel microtubules with overlapping plus-ends form the central spindle between segregating sister chromatids, which coordinates chromosome segregation with furrow ingression during cytokinesis. After anaphase onset, the central spindle bridges the two mitotic spindle halves and opposes cortical forces that pull on astral microtubules. When the mechanical integrity of the central spindle is compromised, the cortical pulling forces promote abnormally rapid and extensive sister chromatid separation during anaphase (Figure 8).

The central spindle comprises a network of interacting proteins that are essential for cytokinesis. At the top of this network are the microtubule-bundling factors SPD-1/PRC1 and centralspindlin, the latter being a 2:2 heterotetramer of the kinesin-6 motor ZEN-4/MKLP-1 and the nonmotor subunit CYK-4/MgcRacGAP/RACGAP1, with a GTPase-activating domain for Rho family GTPases (Mishima *et al.* 2002; White and Glotzer 2012). SPD-1/PRC1 and the centralspindlin complex physically interact at the central spindle and both are essential for its mechanical integrity (Lee *et al.* 2015; Maton *et al.* 2015; Nahaboo *et al.* 2015). Consistently, SPD-1/PRC1 or ZEN-4/MKLP-1 depletion, or specific CYK-4 mutations preventing its interaction with SPD-1, result in rupture of the central spindle such that two half-spindles, each connected to segregating sister chromatids, rapidly move apart (Figure 8C). Reducing the cortical pulling forces suppresses this phenotype and rescues central spindle integrity in *spd-1*, *zen-4*, and *cyk-4* mutant embryos (Figure 8, C and D) (Lee *et al.* 2015; Maton *et al.* 2015). These observations indicate that SPD-1/PRC1 and centralspindlin are required for mechanical integrity of the central spindle during its elongation, owing to their role in microtubule cross-linking, but are not absolutely required for central spindle assembly and function (Figure 8, C and D).

A subset of kinetochore proteins, including the multifaceted protein BUB-1, controls the initiation of central spindle assembly. More specifically, the BUB-1 kinetochore branch containing HCP-1/2/CENP-F and CLS-2/CLASP moves to the central spindle during anaphase and is required for its initial assembly. As mentioned earlier this pathway also is required for chromosome biorientation (Cheeseman *et al.* 2005). However, the defect in central spindle assembly in *hcp-1/2* or *cls-2(RNAi)* embryos is not an indirect consequence of defective chromosome biorientation because specific partial RNAi conditions that do not alter biorientation still cause a severe defect in central spindle mechanical integrity. Importantly, this defect is not rescued by dampening cortical pulling forces (Figure 8C), demonstrating an essential role for this central spindle assembly pathway.

How does the BUB-1 kinetochore branch promote central spindle assembly? CLS-2 is a microtubule polymerase that promotes *de novo* microtubule nucleation in the central spindle (Maton *et al.* 2015). CLS-2 recruitment to the central spindle after anaphase is accompanied by an accumulation of GFP::EBP-2 at polymerizing microtubule plus-ends (Srayko *et al.* 2005). This microtubule polymerization precedes the recruitment of SPD-1/PRC1 to the central spindle, indicating that SPD-1/PRC1 is not required for the initial stage of central spindle assembly. How kinetochore proteins, initially localized on the poleward face of the chromosomes, translocate to the central spindle during anaphase is unclear. As mentioned, BUB-1 is recruited to the kinetochores by the MELT repeats of KNL-1 phosphorylated by PLK-1. Upon dephosphorylation of the KNL-1 MELT repeats by the PP1 phosphatase, BUB-1 is released from KNL-1. Preventing KNL-1 dephosphorylation leads to the hyper-recruitment of BUB-1 to the kinetochores, which results in the assembly of a stronger central spindle, capable of resisting stronger astral pulling forces. This work echoes several observations in other systems where CLASP has been implicated in central spindle integrity (Inoue *et al.* 2004; Liu *et al.* 2009).

Chromosome segregation

Chromosome segregation typically involves both anaphase A and anaphase B chromosome movements. During anaphase A, the chromosomes move toward the spindle poles, whereas during anaphase B, the poles move away from each other, dragging sister chromatids with them. In *C. elegans*, the majority of chromosome movement is due to anaphase B via cortical pulling forces on astral microtubules that drive chromosome segregation (Oegema *et al.* 2001). Other factors influence the strength of the pulling forces, including KLP-7, as mentioned earlier, and EFA-6, which promotes microtubule catastrophe at the cell cortex. Cortical microtubules are more stable in *efa-6* mutant embryos, leading to an increase in astral pulling forces (O'Rourke *et al.* 2010; Lee *et al.* 2015).

Reducing cortical pulling forces by inactivating dynein regulators does not fully prevent chromatid separation in the early embryo (Colombo *et al.* 2003; Gotta *et al.* 2003), suggesting that some segregation can occur in absence of astral pulling forces on the centrosomes. Consistently, laser ablation of both centrosomes during anaphase does not prevent sister chromatid separation. RNAi-mediated depletion experiments in embryos with laser-ablated centrosomes provided evidence that CLS-2 and the RAN pathway contribute to sister chromatid separation in anaphase. A lack of RanGTP reduces the extent of chromatid segregation, whereas an excess of RAN GTP leads to increased chromatid separation in the absence of centrosomes (Nahaboo *et al.* 2015). These observations suggest that continuous microtubule polymerization in the central spindle provides an inside-out pushing

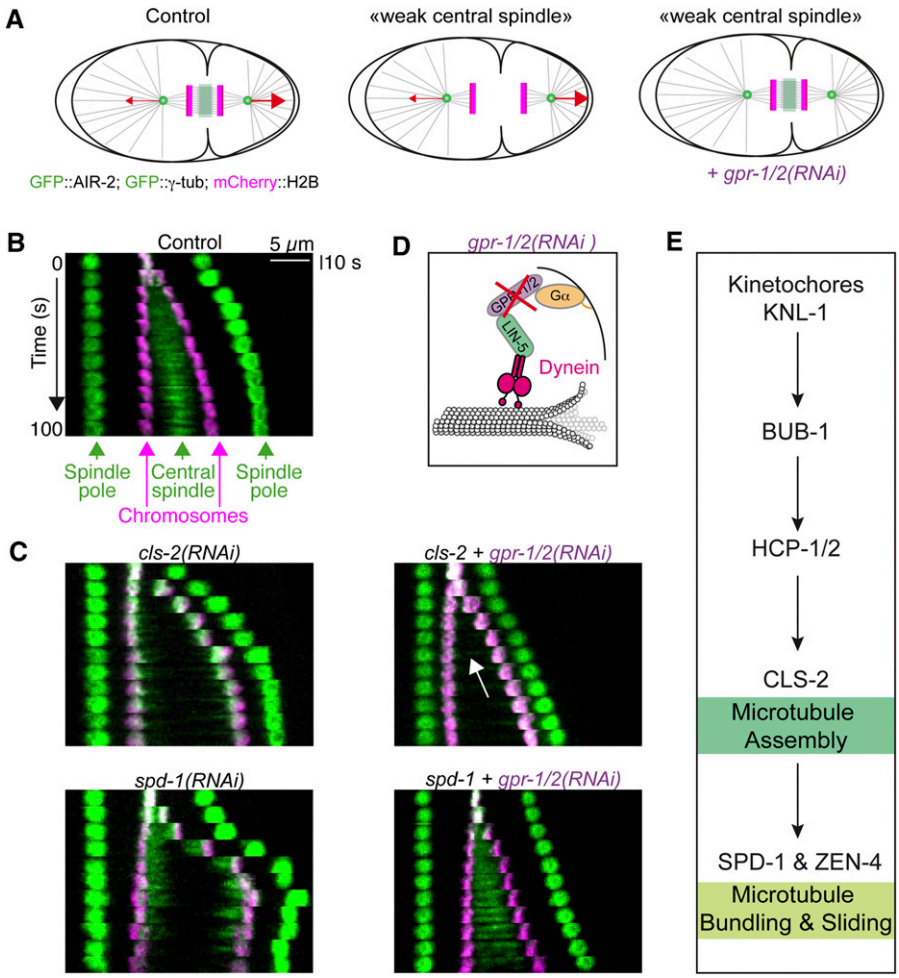

Figure 8 A subset of kinetochore components drive central spindle assembly. (A) Schematics of the assay used to assess the mechanical integrity of the central spindle. When the mechanical integrity of the central spindle is compromised ("weak central spindle"), spindle poles separate prematurely and excessively in response to the astral pulling forces (red arrows) as compared to control embryos. Downregulation of the astral pulling forces via *gpr-1/2* RNAi suppresses this phenotype. (B and C) Kymographs assessing rates of chromosome segregation due to central spindle and cortical pulling force defects in embryos of the indicated genotypes. Note that *gpr-1/2* inactivation rescues the excessive and premature spindle pole separation phenotype observed in *cls-2(RNAi)* and *spd-1(RNAi)*. However, in contrast to *spd-1+grp-1/2(RNAi)* embryos, the central spindle fails to assemble in *cls-2 + grp-1/2(RNAi)* embryos in these conditions, indicated by the absence of the central spindle marker GFP::AIR-2 (white arrow). (D) Schematic of the Gα pathway. *gpr-1/2* inactivation (red cross) suppresses the astral pulling forces. (E) A subset of kinetochore components acting downstream of KNL-1 regulate central spindle assembly.

force that contributes to sister chromatid segregation during anaphase. Such a mechanism is reminiscent of chromosome segregation during oocyte meiosis (Dumont *et al.* 2010; Laband *et al.* 2017). Ultrastructural analysis of the central spindle will be required to fully decipher how polymerizing microtubules between the sister chromatids contribute to chromosome segregation.

Cytokinesis: The Last Chapter in Cell Division

Mitotic cell division ends with cytokinesis, the partitioning of a dividing cell into two fully separated daughter cells (Green *et al.* 2012; White and Glotzer 2012; Glotzer 2017). This partitioning requires the actomyosin-based contractile ring, a differentiated region of cortex enriched in actin and myosin II that assembles around the cell equator beneath the plasma membrane (Figure 9 and Figure 10). Constriction of the contractile ring pulls the adjacent cortex and membrane inward to generate a cleavage furrow that ingresses toward the cell center with the contractile ring at its leading edge. The assembly and positioning of the cleavage furrow is specified by the mitotic spindle, ensuring that cytokinesis properly partitions the duplicated genome (Figure 8 and Figure 9). Constriction of the contractile ring changes the shape of the cell to generate two adjacent daughter cells connected by an intercellular bridge. This channel is closed by a separate process called abscission that remodels the membrane to generate two separate membrane-bound daughters. Experimental manipulations of wild-type and mutant embryos, fixed- and live-cell fluorescent image analysis, and electron tomography have identified requirements for contractile ring assembly, the positioning and rate of furrowing during cytokinesis, and the final step of abscission.

Contractile ring components

The contractile ring comprises a set of filamentous proteins that provide the structural framework for cytokinesis (Figure 10 and Table 3): (i) polarized actin polymers called microfilaments (Strome 1986), (ii) short bipolar filaments of the nonmuscle myosin NMY-2 (Munro *et al.* 2004), and (iii) the nonpolar filamentous septins UNC-59 and UNC-69 (Nguyen *et al.* 2000). The contractile ring also contains the scaffolding protein anillin/ANI-1 that binds and cross-links these filamentous ring components (Maddox *et al.* 2005).

Cortical microfilaments include long linear and short branched actin filaments that depend respectively on formin proteins and the Arp2/3 complex for their assembly. In the early *C. elegans* embryo, linear cortical microfilaments

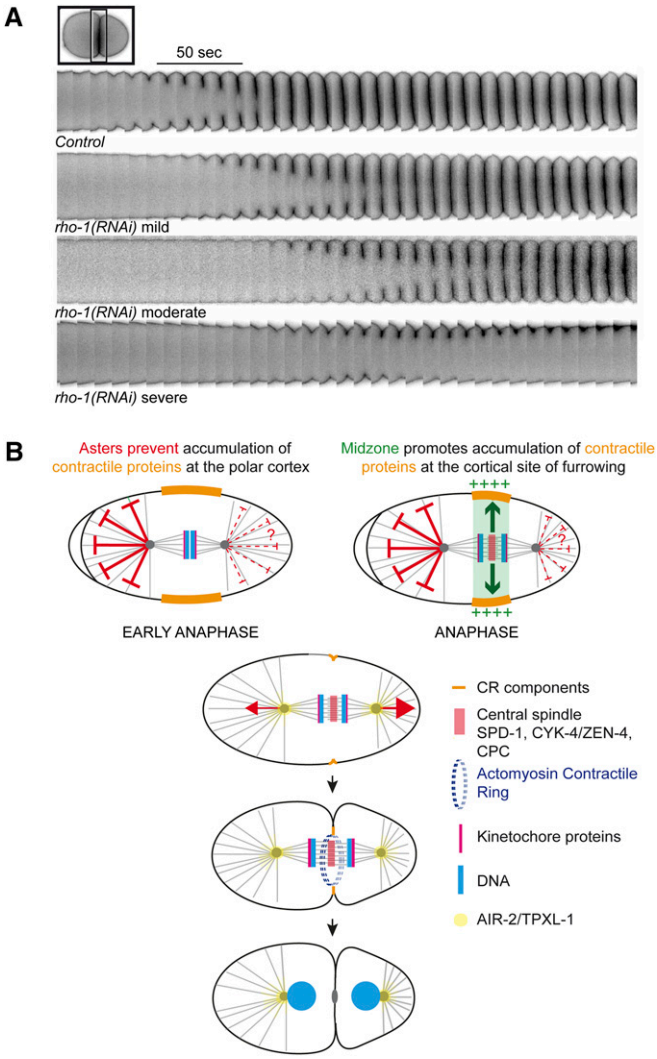

Figure 9 Contractile ring components and assembly at the cell equator during mitosis. (A) Kymographs of the equatorial region of one-cell stage embryos expressing GFP::PH to mark the plasma membrane and show furrow ingression during cytokinesis in wild type (Control) and upon mild, moderate, or severe depletion of *rho-1*/RhoA by RNA interference. Modified from Loria *et al.* (2012). (B) Schematics illustrating the role of astral microtubules and the midzone in furrow positioning. During anaphase, astral microtubules clear contractile ring proteins (orange) from the polar cortex at the anterior pole. Data supporting a role for astral microtubules in clearing contractile ring proteins at the posterior poles are less compelling. During anaphase, the spindle midzone, through centralspindlin, promotes the cortical accumulation of contractile ring proteins at the site of furrowing. In response to polar and centralspindlin signaling, the contractile ring assembles and ingresses during cytokinesis.

require the actin monomer binding protein PFN-1/profilin and the formin CYK-1 (Swan *et al.* 1998; Severson *et al.* 2002; Davies *et al.* 2014; Ding *et al.* 2017). CYK-1 also is enriched in the contractile ring (Swan *et al.* 1998), and strong reduction of CYK-1 function results in the loss of long linear cortical actin and of furrow ingression (Severson *et al.* 2002; Davies *et al.* 2014), but not of widely distributed punctate cortical actin foci that presumably represent branched Arp2/3-nucleated actin (Davies *et al.* 2014; Ding *et al.* 2017). Profilin in fission yeast has been shown to inhibit the Arp2/3-dependent assembly of branched microfilaments while promoting formin-dependent linear microfilament assembly (Suarez *et al.* 2015). Whether only linear and not branched microfilaments in *C. elegans* require PFN-1/profilin has not been addressed.

Other contractile ring components and regulators also localize to the assembling and ingressing cleavage furrow. The myosin motor NMY-2 exhibits a dynamic cortical localization pattern that undergoes extensive reorganization as the cell-cycle progresses (Munro *et al.* 2004), ultimately enriching in the cleavage furrow just before and during ingression (Nguyen *et al.* 2000; Maddox *et al.* 2005). Cortical NMY-2 localization depends on PFN-1 (Severson *et al.* 2002), although its dependence on actin filaments has not been reported. The widely conserved actin cross-linking protein PLST-1/Plastin colocalizes with cortical microfilaments in the early *C. elegans* embryo and contributes to cortical stiffness and cortical actomyosin dynamics (Ding *et al.* 2017). While cytokinesis fails in only about 15% of *plst-1* null mutant embryos, the initiation of furrow ingression is delayed and the initial rate of ingression is decreased in mutant embryos that complete cytokinesis.

The small GTPase RHO-1/RhoA is the central signaling molecule that controls contractile ring assembly. Signaling by the anaphase spindle activates RhoA at the cell equator, and active RhoA is in turn required for recruitment of all of the other components of the contractile ring (Jantsch-Plunger *et al.* 2000; Dechant and Glotzer 2003; Motegi *et al.* 2006; Loria *et al.* 2012). RhoA is activated by the guanine nucleotide exchange factor (GEF) ECT-2 (Somers and Saint 2003; Yüce *et al.* 2005; Burkard *et al.* 2009; Wolfe *et al.* 2009; Loria *et al.* 2012; Zhang and Glotzer 2015; Basant and Glotzer 2017), and is inactivated by the RhoA GAP RGA-3/4 (Schmutz *et al.* 2007; Schonegg *et al.* 2007). Active RHO-1/RhoA localizes to the furrow, while ECT-2 appears uniformly distributed over the cortex but is presumably activated only at the furrow (Motegi *et al.* 2006).

The septins UNC-59 and UNC-61 coassemble to form nonpolar membrane–associated filaments (John *et al.* 2007) that localize to cleavage furrows in early embryonic cells and are required for cytokinesis postembryonically. In early embryos, the septins are not essential for cytokinesis, but increase its fidelity, as septin knockdown results in low-level cytokinesis failure (Nguyen *et al.* 2000; Maddox *et al.* 2005; A. S. Maddox *et al.* 2007). The contractile ring component anillin/ANI-1 is required to recruit the septins but not NMY-2 or actin to localize at the cleavage furrow. Consistent with its role in targeting the septins, anillin inhibition also results in low-level cytokinesis failure (Nguyen *et al.* 2000; Maddox *et al.* 2005; A. S. Maddox *et al.* 2007; Tse *et al.* 2011). More recently, another contractile ring component, the BTB domain protein CYK-7, has been shown to be required for cytokinesis (Green *et al.* 2011), but how it contributes to contractile ring assembly or function has not been reported.

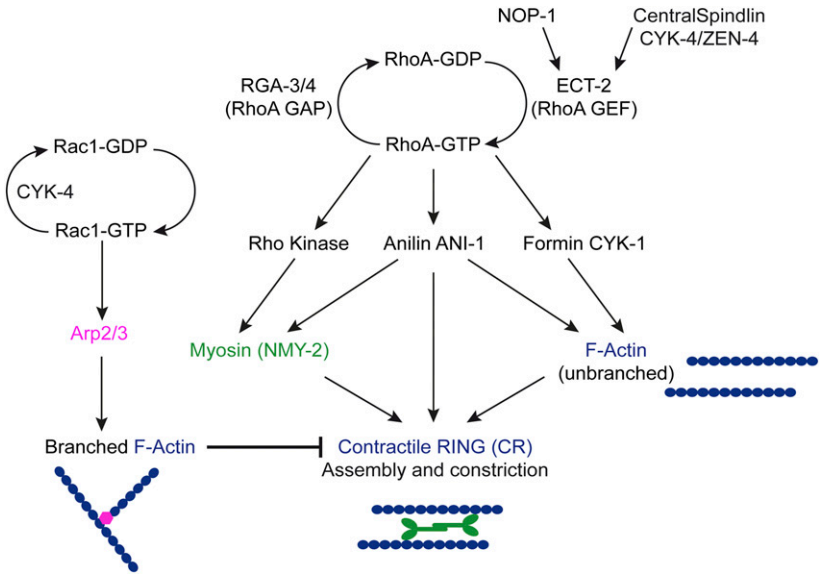

Figure 10 Signaling pathways that control cortical activity during cytokinesis. Summary of the genetic pathways that influence contractile ring assembly and ingression during cytokinesis in the early *C. elegans* embryo.

Contractile ring assembly and dynamics

Contractile ring assembly initiates during anaphase with the recruitment of contractile ring proteins to an ~10-μm-wide equatorial band that encircles the cell equator. Recruitment of contractile ring components causes the cortex within the ring to compress (Lewellyn *et al.* 2011; Reymann *et al.* 2016; Khaliullin *et al.* 2018), concentrating contractile ring components within the ring, aligning formin-nucleated actin filaments around the ring, and generating long-range cortical flows toward the ring (Reymann *et al.* 2016; Ding *et al.* 2017; Khaliullin *et al.* 2018). As the ring constricts, it pulls the adjacent cortex behind it to generate the division plane. Because of the geometry change that accompanies constriction, the surface area of the cell increases ~1.4-fold as the ring closes. Generation of a three-dimensional map of cortical flow during cytokinesis suggest that this additional surface is gained at the cell poles through a process that includes expansion of the polar cell cortex. Laser-cutting experiments combined with the flow map indicate that, in response to tension generated by the constricting ring, the cortex at the cell poles expands to generate the additional cortical surface area required to cover the daughter cells. During this process, myosin foci at the poles are inferred to move more rapidly, in contrast to more centrally located foci that were found to move at a constant rate, flowing toward the ring without expanding when pulled by the ring. These results suggest that the mechanical properties of the polar cortex are distinct from those of the intervening cortex, although the molecular mechanism underlying polar cortical expansion remains unknown (Khaliullin *et al.* 2018).

During each of the first three divisions of the *C. elegans* embryo, there is a substantial period following the onset of ring constriction when the overall rate of ring closure remains approximately constant despite the fact that the ring is progressively decreasing in size (Zumdieck *et al.* 2007; Carvalho *et al.* 2009; Bourdages *et al.* 2014). Thus the per-unit-length rate of closure increases as the circumference decreases. A recent study has shown that per unit length, the rate of ring closure, the amount of ring components, and the rate of cortical compression all increase with parallel exponential kinetics as the ring closes. The exponential kinetics suggest control by positive feedback. Mathematical modeling indicates that a positive feedback loop can explain the observed contractile ring dynamics: ring myosin compresses the cortex within the ring, which pulls additional cortex loaded with myosin into the ring, thereby increasing the amount of ring myosin (Khaliullin *et al.* 2018). Thus, it has been proposed that positive feedback between ring myosin and compression-driven cortical flow drives an exponential increase in the per-unit-length constriction rate that balances the reduction in ring size during constriction to allow the ring to maintain a high overall constriction rate. This proposed feedback-based mechanism for contractile ring component accumulation may render ring constriction robust to mechanical challenges including internal obstructions and cell-cell contacts. During the first three embryonic divisions, this approximately constant overall constriction rate is maintained until the ring comes into contact with the spindle midzone at a circumference of ~20 μm. After this transition point, the constriction rate progressively slows, likely due to the mechanical influence of the midzone as well as the signaling-based mechanisms that prepare the intercellular bridge for abscission (Carvalho *et al.* 2009).

Another recent study suggests that contractile ring constriction may also drive rotation of cell division axes during both *C. elegans* and mouse embryogenesis. In response to adhesive cell contact, cortical flow near the site of contact is inhibited. As the entire cell surface and cortex are an integrated mechanical unit that moves in a coordinated fashion during cytokinesis (Hird and White 1993; Reymann *et al.*

Table 3 Cytoskeletal proteins required for cytokinesis

C. elegans protein	*C. elegans* gene	Vertebrate ortholog	Brief description of localization and function	TS alleles (* indicates fast acting)
NMY-2	*nmy-2*(F20G4.3)	nonmuscle myosin II	Localizes to the contractile ring during cytokinesis as well as the other cortical contractile structure	*ne3409**, *ne1490**
MLC-4	*mlc-4*(C56G7.1)	nonmuscle myosin II regulatory light chain	Regulates the ability of myosin II to form filaments and interact with actin; localizes to the contractile ring during cytokinesis as well as the other cortical contractile structure	
LET-502	*let-502*(C10H11.9)	Rho-binding kinase (ROK)	Rho-binding serine/threonine kinase; promotes myosin II contractility by increasing the phosphorylation of MLC-4; localizes to the contractile ring	
MEL-11	*mel-11*(C06C3.1)	Myosin phosphatase targeting subunit (MYPT)	Regulatory subunit of myosin phosphatase; inhibits cortical contraction by de-phosphorylating the regulatory light chain of myosin II; LET-502 and MEL-11 colocalize in cleavage furrows	
RHO-1	*rho-1*(Y51H4A.3)	RhoA	Small GTPase that connects signaling by the anaphase spindle to assembly and ingression of a cortical contractile ring; localizes to the furrow	
RGA-3/4	*rga-3(K09H11.3)*; *rga-4(Y75B7AL.4)*	Not identified	Rho GTPase activating proteins regulating RHO-1 in the early embryo	
ECT-2	*let-21*(T19E10.1)	Ect2	Guanine nucleotide exchange factor; activates RHO-1; uniformly distributed over the cortex but presumably activated at the furrow	
CYK-1	*cyk-1*(F11H8.4)	formins	A member of the formin family of proteins, promotes actin assembly in response to activation of Rho family GTPases; localizes to the cleavage furrow and is required to initiate furrow ingression	*or596**
PFN-1	*pfn-1*(Y18D10A. 20)	profillin	One of three *C. elegans* homologs of the actin binding protein profilin	
ANI-1	*ani-1*(Y49E10.19)	Anillin	One of the three *C. elegans* anillins; required for contractile events in the early embryo	
UNC-59;UNC-61	*unc-59*(W09C5.2); *unc-61*(Y50E8A.4)	septins	*C. elegans* homologs of the septins form nonpolar membrane-associated filaments	
NOP-1	*nop-1(F25B5.2)*	Not identified	Contributes to RHO-1 activation	
UNC-60A	*unc-60*(C38C3.5)	cofilin	Regulates actin filament dynamics	
ZEN-4	*zen-4*(M03D4.1)	kinesin-6 family member MKLP1	Interacts with CYK-4 to form the centralspindlin complex; localizes microtubule bundles in the spindle midzone and midbody	*or153**
CYK-4	*cyk-4*(K08E3.6)	MgcRacGAP	Interacts with ZEN-4 to form the centralspindlin complex; localizes microtubule bundles in the spindle midzone and midbody	*or749**
AIR-2	*air-2*(B0207.4)	Aurora B	Mitotic serine threonine kinase part of the chromosome passenger complex (CPC)	*or207**
ICP-1CelINCENP	*icp-1*(Y39G10AR. 13)	INCENP	Part of the chromosome passenger complex (CPC)	*or663**
BIR-1	*bir-1*(T27F2.3)	Survivin	Part of the chromosome passenger complex (CPC)	
CSC-1	*csc-1*(Y48E1B.12)	Borealin, Dasra A/B	Part of the chromosome passenger complex (CPC)	
SPD-1	*spd-1*(Y34D9A.4)	PRC1	Microtubule bundling factor; localizes to microtubule bundles in the spindle midzone	

2016; Khaliullin *et al.* 2018; Sugioka and Bowerman 2018), the contact-induced reduction in cortical flow likely arises because cell contact generates a drag that prevents the cortex in that region from flowing in response to ring generated tension. This contact-induced cessation of flow leads to an anisotropy in cortical flow that is proposed to generate a cell-surface torque that rotates the cell division axis (Sugioka and Bowerman 2018). In addition, cortical flows perpendicular to the long axis of the cell have also been observed. In the one-cell stage embryo, this causes the cortex in the anterior and posterior halves of the embryo to counter-rotate relative to each other. Counter-rotating cortical flows also are observed in subsequent divisions (Naganathan *et al.* 2014; Sugioka and Bowerman 2018), and recent theoretical work has provided a potential explanation for how force and torque generation at the molecular scale could give rise to these larger-scale rotational flows (Naganathan *et al.* 2014).

A study analyzing contractile ring constriction during the first four embryonic divisions revealed that the initial rate of ring constriction depends on cell size. The per-unit-length constriction rate is the same for all of these divisions (the mechanistic basis for this is not understood), so larger cells

have a proportionally higher overall initial ingression rate than smaller cells (Carvalho *et al.* 2009). Because the initial constriction rate is proportional to cell size, all early embryonic cells are able to complete cytokinesis in roughly the same amount of time, which may be important for the spatial organization of cell fates during embryogenesis. Photobleaching experiments have shown that once myosin, anillin, and the septins are in the ring, they do not exchange with components in the cytoplasm. Instead, components are lost due to disassembly in proportion to the reduction in ring perimeter (Carvalho *et al.* 2009; Khaliullin *et al.* 2018). Addition of latrunculin A after ingression initiates, to prevent further actin assembly, does not prevent or slow down constriction, suggesting that continuous actin assembly is not needed (Carvalho *et al.* 2009). This result is consistent with upshift experiments utilizing a fast-acting TS allele of the formin CYK-1, which have shown that it is required only during ring assembly and not during constriction (Davies *et al.* 2014). A possible caveat to these experiments is that the latrunculin A experiments were performed before the development of robust methods for eggshell permeabilization, and even fast-acting TS mutant proteins might in some contexts resist inactivation depending on their molecular environment. In contrast to CYK-1 activity, upshift experiments utilizing a fast-acting NMY-2 allele indicate that myosin II is required throughout ingression (Liu *et al.* 2010; Davies *et al.* 2014). Understanding exactly how myosin II drives ring constriction is currently an important challenge.

While most studies of contractile ring dynamics have focused on factors that promote furrow ingression, a recent study identified an inhibitory role for HMR-1, a transmembrane protein and the sole classical cadherin in *C. elegans* (Padmanabhan *et al.* 2017). HMR-1 mediates cell-cell adhesion and has essential roles in gastrulation, cell polarity, and epidermal morphogenesis. However, it also has cell-cell adhesion-independent roles during early embryogenesis. Clusters of HMR-1 localize to the cell cortex in the one-cell zygote and other early blastomeres, where they impede actomyosin flow and limit NMY-2 levels. During cytokinesis, HMR-1 is excluded from the ingressing contractile ring, and HMR-1 knockdown results in faster ingression. Conversely, HMR-1 overexpression slows ingression, independently of NMY-2 and the HMR-1 extracellular domain. The cytoplasmic catenin-binding domain in HMR-1 is required for this inhibitory influence on furrow ingression, and knockdown of the *C. elegans* β-catenin HMP-2 also leads to more rapid ingression. These results suggest that HMR-1 interacts through HMP-2 with actin filaments, independently of microfilament assembly, to somehow reduce the rate of contractile ring constriction.

A final intriguing characteristic of contractile ring dynamics in the early embryo is the pronounced asymmetry of ingression, which occurs first and more extensively from one side. This phenomenon has been observed in other settings, but in *C. elegans*, it requires the ring components anillin and septin (A. S. Maddox *et al.* 2007). During asymmetric constriction, the ring components NMY-2, anillin, septin, and microfilaments are all more concentrated on the side that has ingressed the farthest, suggesting that component accumulation may represent a record of how far that side of the ring has ingressed (A. S. Maddox *et al.* 2007; Khaliullin *et al.* 2018). Ingression and the accumulation of contractile ring components is symmetric in embryos depleted of the septins or anillin (A. S. Maddox *et al.* 2007), which is required to recruit the septins to the ring (Maddox *et al.* 2005). In septin-inhibited embryos, the contractile ring is more sensitive to loss of the Rho kinase LET-502, with double mutants exhibiting synthetic decreases in both furrowing rate and the completion of cytokinesis. Thus asymmetric furrowing may serve to make cytokinesis more resilient during perturbations that might otherwise prevent successful development (A. S. Maddox *et al.* 2007), although the synthetic defect could reflect contributions made by these factors to other processes. Finally, codepletion of both anillin and the nematode specific RhoA regulator NOP-1 (see below) restores contractile ring asymmetry, indicating that other factors can also contribute to this process (Tse *et al.* 2012).

Two mitotic spindle signals influence contractile ring assembly

Signals from the anaphase mitotic spindle control contractile ring assembly, coordinating chromosome segregation with cytokinesis to ensure proper inheritance of the genome (von Dassow 2009). Two signals from the spindle appear to mediate ring assembly in a partially redundant fashion, one signal emanating from the spindle poles and another from the central spindle (Figure 9). The existence of two signals was first noted when a late cytokinesis defect, caused by mutational loss of the central spindle (see below), and simultaneous loss of other gene functions that promote spindle elongation but, by themselves, do not prevent cytokinesis, led to a synthetic loss of ingression (Dechant and Glotzer 2003). Experimental manipulation of the mitotic spindle in wild-type one-cell stage embryos subsequently provided a more explicit documentation of two spindle signals (Bringmann and Hyman 2005). Asymmetric laser severing of the spindle at anaphase onset, between one pole and the two sets of separating chromosomes attached to the other pole, led to excessive pole separation and the appearance of two furrows, one positioned midway between the two poles and another positioned over the displaced central spindle, closer to the attached pole. Knockdown of contractile ring components led to loss of both furrows, while knockdown of factors required for central spindle assembly led to loss of only the midzone-positioned furrow. Further support for distinct pole and midzone spindle signals came from analysis of cytokinetic furrowing in mutant one-cell stage embryos with destabilized astral microtubules and posteriorly displaced and transversely oriented mitotic spindles (Werner *et al.*, 2007). In these mutants, two furrows also form: one from the posterior pole that bisects the transversely oriented spindle and depends on the spindle midzone, and a second

circumferential furrow positioned toward the anterior pole, distal to the displaced spindle, that forms independently of the midzone. Finally, delayed spindle elongation and pole separation, resulting from knockdown of the TPX2 ortholog TPXL-1, only delays furrowing, while knockdown of TPXL-1 combined with spindle midzone disruption results in its complete absence (Lewellyn *et al.* 2010). These studies all indicate that signals from both the spindle poles and the central spindle influence contractile ring assembly and position.

Molecular pathways that mediate furrow signaling from the spindle poles

Genetic approaches have identified factors that mediate the influence of spindle poles on contractile ring assembly (Figure 9). The cortically localized Gα proteins GOA-1 and GPA-16 and their regulators GPR-1/2 and LET-99, which influence mitotic spindle positioning, were shown to be required for furrowing in a spindle midzone-defective mutant, or after laser ablation of the spindle midzone (Bringmann *et al.* 2007). The spindle poles position a band of LET-99 at the future site of furrowing, and LET-99 prevents the accumulation of GPR-1/2 at the furrowing site. GPR-1/2 localize to the cortex where they regulate pulling forces on astral microtubules that position the mitotic spindle and control its elongation (Rose and Gonczy 2014). GPR-1/2 could therefore exert their effects on cytokinesis via their influence on spindle structure or through direct effects on the cortex, or from a combination of the two. Further insight into the role of G protein–signaling comes from the simultaneous knockdown of GPR-1/2 and TPXL-1: in addition to the delay in furrowing, a broader more diffuse contractile ring assembles and multiple furrows form. This phenotype may arise due to the prolonged delay in pole separation resulting from the double inhibition combined with the loss of a TPXL-1–dependent mechanism that clears contractile ring proteins from the poles (see below), suggesting that G protein–mediated signaling from the poles restricts furrowing to a single site (Lewellyn *et al.* 2010).

The contractile ring scaffolding protein anillin/ANI-1 also has been implicated in spindle pole control of furrow formation (Tse *et al.* 2011). ANI-1 knockdown in one-cell stage *zyg-9(-)* mutants—with a posteriorly displaced and transversely oriented mitotic spindle—eliminates the anterior pole-dependent furrow that forms, but not the posterior furrow induced by the displaced spindle midzone. Anillin is required for the formation of large cortical NMY-2 foci, with astral MTs inhibiting the cortical localization of anillin, thereby positioning anillin, large NMY-2 foci and a contractile ring toward the anterior pole once cortical microtubule density is sufficiently low. These results are to some extent consistent with the findings that simultaneous GPR-1/2 and TPXL-1 knockdown results in a broader distribution of anillin and the formation of multiple furrows (Lewellyn *et al.* 2010), and that G protein–mediated signaling by GOA-1 and GPA-16, and their regulators GPR-1/2, contribute to pole signaling (Bringmann *et al.* 2007).

Observations from different model systems indicate that mitotic spindle poles and their astral microtubules inhibit the cortical accumulation of contractile ring components at the two poles of a dividing cell, thereby limiting their circumferential enrichment to the cortical region encircling the central spindle (von Dassow 2009). In *C. elegans*, reducing astral microtubule contact with the cortex via nocodazole treatment, combined with knocking down a ubiquitin ligase that targets the microtubule-severing complex katanin for proteasomal degradation after the completion of oocyte meiosis, results in dramatic furrowing throughout the entire cell cortex, consistent with such an inhibitory role for astral microtubules (Kurz *et al.* 2002; Pintard *et al.* 2003). The mechanism underlying this inhibition of cortical actomyosin contractility by astral microtubules has recently been shown involve both the TPX2 ortholog TPXL-1 and the Aurora A kinase AIR-1 (Mangal *et al.* 2018). Knockdown of TPXL-1 results in a failure to clear contractile ring components from the anterior cortex, although clearing from the posterior cortex in wild-type embryos is less apparent. This loss of anterior cortical contractile ring components is not due to the delayed spindle elongation associated with TPXL-1 knockdown, as it also is observed after simultaneous kinetochore disruption that results in premature and excessive pole separation. AIR-1 has been previously implicated as part of the pole signaling that influences contractile ring assembly (Motegi *et al.* 2006), and expression of a TPXL-1 mutant predicted to be defective in activating AIR-1 fails to rescue anterior cortical clearing after knockdown of endogenous TPXL-1. Moreover, TPXL-1, like AIR-1, localizes to spindle poles and astral microtubules (Figure 9B), and the localization of AIR-1 to astral microtubules depends on TPXL-1. Thus astral microtubule-associated TPXL-1 appears to act through AIR-1 to mediate the clearing of contractile ring components from the anterior cortex, presumably through the diffusion of active AIR-1 to the cortex, where it may phosphorylate unknown targets to promote removal of contractile ring components (Mangal *et al.* 2018).

In summary, these studies in *C. elegans* have identified mechanisms by which the spindle poles influence contractile ring assembly, but whether these influences constitute a single signaling pathway or the combined effects of multiple parallel pathways is not clear. Higher resolution imaging of contractile ring components in the different contexts that have been used to study pole signaling might advance our understanding of the different gene requirements identified thus far.

NOP-1: a nematode-specific regulator of cytokinesis contributes to spindle pole signaling

NOP-1, a protein that thus far appears to be nematode-specific, has a nonessential but important role during cytokinesis (Fievet *et al.* 2012; Morton *et al.* 2012; Tse *et al.* 2012; Zhang and Glotzer 2015). The *nop-1* gene (no pseudocleavage), which encodes a serine-rich protein with no conserved domains, was named for the absence of the pseudocleavage

furrow during the first embryonic mitosis, a prominent but transient invagination of the cell membrane that accompanies polarization of the zygote along the anterior–posterior body axis (Rose *et al.* 1995). Although homozygous *nop-1* mutants are viable, ~20% of *nop-1* mutant embryos fail to hatch (Rose *et al.* 1995), indicating that NOP-1 has important roles. NOP-1 contributes to the actomyosin-dependent establishment of anterior–posterior polarity in the one-cell stage embryo (Tse *et al.* 2012). Additionally, although cytokinesis is not defective in *nop-1* mutants, eliminating NOP-1 function in a hypomorphic *ect-2* mutant that also completes cytokinesis leads to a synthetic loss of furrowing and cytokinesis (Zonies *et al.* 2010). These results suggest that NOP-1 contributes to RhoA activation, although it is not essential for it under normal conditions (Tse *et al.* 2012). Importantly, double mutants lacking both NOP-1 and CYK-4 are completely defective in furrowing and exhibit a synthetic loss of cortical NMY-2, microfilaments and active RhoA (detected using a fragment of anillin fused to GFP that serves as a biosensor for active RhoA) midway between the spindle poles during anaphase. Furthermore, in mutants with a posteriorly displaced and transversely oriented mitotic spindle in the one-cell embryo, NOP-1 is fully required for the anterior spindle pole-dependent furrowing and partially required for the midzone-dependent posterior furrowing. Normally, exit from mitosis triggers RhoA activation and cortical contractility for cytokinesis (Green *et al.* 2012). NOP-1 may represent a nematode-specific adaptation that allows RhoA activation during a window of cortical contractility before mitosis to facilitate the establishment of polarity. To summarize, in the absence of NOP-1, cytokinesis depends entirely on signaling by the central spindle, indicating that NOP-1 plays a role in pole signaling during the first embryonic cytokinesis, although whether it has a similar role in subsequent cell divisions is not known.

Centralspindlin and cytokinesis: the role of the CYK-4 GAP domain

The signaling by the spindle midzone that specifies contractile ring assembly critically depends on a protein complex comprising the kinesin-6 ZEN-4 and the Rho family GTPase-activating protein CYK-4 (Raich *et al.* 1998; Jantsch-Plunger *et al.* 2000; Severson *et al.* 2000; Powers *et al.* 2004). This widely conserved complex was dubbed centralspindlin due to its role in cross-linking antiparallel microtubules between the separating chromosomes to promote assembly of the central spindle during anaphase (Mishima *et al.* 2002). ZEN-4 and CYK-4 form parallel homodimers that associate to form a heterotetramer that can cross-link antiparallel microtubules (Mishima *et al.* 2002; Pavicic-Kaltenbrunner *et al.* 2007). ZEN-4 and CYK-4 are mutually dependent on each other for localization to the spindle midzone, and genetic suppression studies confirm the functional importance of their interaction (Jantsch-Plunger *et al.* 2000). Centralspindlin also requires the upstream-acting Aurora B kinase AIR-2 (Severson *et al.* 2000) and other members of the chromosomal passenger complex (CPC) (Kaitna *et al.* 2000; Hutterer *et al.* 2009; Lewellyn *et al.* 2011; Basant *et al.* 2015), to localize to the central spindle. A direct interaction between centralspindlin and the microtubule cross-linking protein SPD-1/PRC further promotes central spindle stability (Lee *et al.* 2015). Centralspindlin is also a target of cell-cycle regulation, with the cyclin-dependent kinase CDK-1 inhibiting centralspindlin assembly (Mishima *et al.* 2004). In the absence of either ZEN-4 or CYK-4, cytokinetic furrowing initiates in a NOP-1–dependent fashion (Tse *et al.* 2012), but only ingresses halfway and ultimately regresses. Temperature upshifts with conditional alleles indicate that both CYK-4 and ZEN-4 are also required late in cytokinesis (Severson *et al.* 2000; Davies *et al.* 2014).

Across systems, including *C. elegans*, there is good evidence that CYK-4 homologs play a role in the local activation of the small GTPase RhoA to promote contractile ring assembly (Motegi *et al.* 2006; Loria *et al.* 2012; Zhang and Glotzer 2015; Basant and Glotzer 2018). Consistent with a role in activating RhoA, CYK-4 orthologs have been shown to bind ECT-2 via a region in their N-terminal half, positioned between the coiled-coil and C1 domains, and this binding is proposed to relieve autoinhibition of ECT-2 to activate RhoA for contractile ring assembly (Somers and Saint 2003; Yüce *et al.* 2005; Burkard *et al.* 2009; Wolfe *et al.* 2009; Loria *et al.* 2012; Zhang and Glotzer 2015; Basant and Glotzer 2017). It is surprising that a GAP domain, expected to inactivate small GTPases by stimulating their GTP hydrolysis activity, acts to promote RhoA signaling (Basant and Glotzer 2017). Recent work has shown that mutations that specifically disrupt the Rho GTPase binding interface of the GAP domain compromise RhoA activation during cytokinesis (Zhang and Glotzer 2015). To explain this, it was proposed that the CYK-4 GAP interacts directly with the GEF domain to activate it, However, although pulldown experiments have suggested that the CYK-4 GAP domain can interact with the ECT-2 GEF domain, *in vitro* tests have so far failed to provide evidence that GAP domain binding can activate the ECT-2 GEF (Zhang and Glotzer 2015).

Centralspindlin also functions in the syncytial *C. elegans* germline (Zhou *et al.* 2013; K. Y. Lee *et al.* 2018), where it localizes to stable intercellular bridges. In the hermaphrodite germline, CYK-4 is required for a cytokinesis-like event that closes these intercellular bridges to cellularize oocytes (K. Y. Lee *et al.* 2018). As in cytokinesis, CYK-4 is required for RhoA activation in the germline. Interestingly, although ZEN-4 also is present in the germline, it is not essential for oocyte cellularization, which may reflect the fact that contractile signaling is not patterned by midzone-like microtubule-based structures in this context. In the germline, the Rho GTPase binding interface of the GAP domain plays an essential role in targeting CYK-4 to intercellular bridges, likely via binding to active membrane-associated RhoA (K. Y. Lee *et al.* 2018). The adjacent lipid-binding C1 domain is also independently required for targeting (Zhang and Glotzer 2015; K. Y. Lee *et al.* 2018). This has led to the proposal that the conserved C1-GAP region of CYK-4 constitutes a targeting module that

functions in a fashion similar to the targeting mechanism recently identified for anillin, in which its C2, Rho-binding, and PH domains all form low-affinity interactions that collectively target anillin to the membrane (Sun *et al.* 2015). In the context of RhoA activation, positive feedback might promote RhoA activation, with activated RhoA generated by CYK-4 recruiting additional CYK-4 through its C1-GAP module. Whether the C1-GAP module has a similar role in targeting centralspindlin to the membrane during cytokinesis is not yet clear, as the prominent localization of CYK-4 to midzone microtubules makes the membrane-associated population difficult to assess.

In addition to the potential contributions of the CYK-4 GAP domain to RhoA activation, the CYK-4 GAP domain may also contribute to cytokinesis by inactivating Rac. Across systems, biochemical experiments have revealed that CYK-4 is a substantially better GAP for Rac and Cdc42 than RhoA (Touré *et al.* 1998; Jantsch-Plunger *et al.* 2000; Kawashima *et al.* 2000; Minoshima *et al.* 2003; Bastos *et al.* 2012). Work in *Xenopus* epithelia has also revealed a dramatic increase in active Rac when the CYK-4 ortholog is inhibited (Breznau *et al.* 2015), providing evidence that CYK-4 orthologs do inactivate Rac *in vivo* in some contexts. In *C. elegans*, inhibition of the Rac1 GTPase CED-10 can suppress cytokinesis defects resulting from *cyk-4* inhibition (Canman *et al.* 2008; Zhuravlev *et al.* 2017). The idea that the GAP activity of CYK-4 could contribute to cytokinesis by inactivating Rac originated with the isolation of recessive, TS, separation-of-function mutations in *cyk-4* that do not disrupt central spindle assembly or centralspindlin localization, but nevertheless result in the same compromised furrow ingression observed after more complete reduction of CYK-4 function. The cytokinesis defect caused by these special *cyk-4* alleles can be partially rescued by reducing CED-10/Rac1 or the Rac1 target Arp2/3 (Canman *et al.* 2008; Zhuravlev *et al.* 2017). Because CED-10/Rac1 promotes the Arp2/3-dependent assembly of branched cortical microfilaments, downregulation of CED-10/Rac1 is proposed to decrease or prevent branched microfilament assembly such that the compromised cytokinesis furrow can ingress to completion. Notably, the rate of ring constriction is not rescued by CED-10/Rac1 inhibition, and a forward genetic screen for suppressors of a separation of function *cyk-4* allele, which specifically interferes with furrowing and not with central spindle assembly, identified gain-of-function alleles of the RhoA GEF *ect-2*. Together with the finding that CYK-4 GAP activity is required for furrowing even in the absence of CED-10/Rac1, these observations are more consistent with CYK-4 promoting RhoA activation (Zhang and Glotzer 2015; Basant and Glotzer 2018). Furthermore, knockdown of Arp2/3 does not affect the cortical compression that accompanies furrow ingression (Khaliullin *et al.* 2018), further supporting a primary role for CYK-4 as a RhoA activator. Thus, although inhibition of CED-10/Rac1 can rescue cytokinesis defects in *cyk-4* mutant embryos, it is still unclear whether CED-10/Rac1 is inhibited by CYK-4 in the context of normal cytokinesis to facilitate furrowing.

In summary, there is good evidence that the CYK-4 GAP domain contributes to the role of CYK-4 in Rho activation either by promoting its targeting to the membrane or by directly interacting with the ECT-2 GEF domain to activate it. The CYK-4 GAP domain may also have a second nonmutually exclusive function in inactivating CED-10/Rac1 to facilitate furrowing (Figure 10). More work is needed to understand how CYK-4 activates RhoA, and to assess the functional importance of the ability of the CYK-4 GAP to inactivate Rac1.

Further contributions from the spindle midzone to the regulation of cytokinesis

Molecular genetic studies of cytokinesis in *C. elegans* have focused largely on identifying the mechanisms by which the spindle poles and centralspindlin signal to the cortex to regulate contractile ring assembly. However, other activities that are not as directly associated with these two cues from the anaphase mitotic spindle have also been identified. First, assembly of the spindle midzone in animal models depends on two other factors in addition to centralspindlin: the CPC and the microtubule cross-linking protein PRC1. Both of these spindle midzone factors also contribute to cytokinesis in *C. elegans* (von Dassow 2009; Green *et al.* 2012). The CPC consists of four proteins: the Aurora B kinase (AIR-2 in *C. elegans*) and INCENP, Survivin, and Borealin (ICP-1, BIR-2, and CSC-1 in *C. elegans*). Depletion of any one of the CPC components reduces the rate of furrow ingression, disrupts the spindle midzone and centralspindlin localization at the midzone, and results in chromosome segregation defects and a late failure in cytokinesis (Kaitna *et al.* 2000; Hutterer *et al.* 2009; Lewellyn *et al.* 2011; Basant *et al.* 2015). Furthermore, AIR-2 phosphorylates the ZEN-4 subunit of centralspindlin, and this phosphorylation event releases centralspindlin from a complex with the 14-3-3 protein PAR-5, allowing for centralspindlin to assemble into clusters at the spindle midzone that appear to mediate its influence on cytokinesis (Guse *et al.* 2005; Basant *et al.* 2015). Thus AIR-2 appears to act upstream of centralspindlin to influence its localization and function. Consistent with such a pathway relationship, TS alleles indicate that AIR-2 acts earlier in cytokinesis than CYK-4 and ZEN-4 (Severson *et al.* 2000; Davies *et al.* 2014). An analysis of contractile ring component dynamics and the genetic interactions of AIR-2 and centralspindlin with other ring components and regulators suggests that AIR-2 may have additional roles in cytokinesis beyond influencing the localization and function of centralspindlin (Lewellyn *et al.* 2011). However, the nature of these additional roles is not known. Because knockdown of PAR-5 bypasses AIR-2 requirements for furrowing (Basant *et al.* 2015), AIR-2 appears to promote cytokinesis primarily by dissociating centralspindlin from PAR-5 such that it can assemble into its active oligomeric form (Basant and Glotzer 2018).

While SPD-1/PRC1, like the CPC and centralspindlin, is required for assembly of the spindle midzone, loss of SPD-1 does not affect furrow ingression or the completion of

cytokinesis, in contrast to PRC requirements in other animal models (Verbrugghe and White 2004). In the absence of SPD-1, the central spindle is disrupted such that the spindle poles separate prematurely and rapidly, as in centralspindlin mutants, but ZEN-4 is detected at the cortex early in furrow formation, suggesting that in the absence of SPD-1/PRC1, centralspindlin at the midzone cortex can still promote furrowing and the completion of cytokinesis. Indeed, the membrane localization of centralspindlin is likely important for RhoA activation even in cells with an intact central spindle (Basant and Glotzer 2018). Although SPD-1/PRC1 is not essential for cytokinesis during the first mitotic division of the embryo, penetrant cytokinesis defects do occur in some later stage embryonic cells (Verbrugghe and White 2004). Thus SPD-1/PRC1 is important for cytokinesis, but not during the initial embryonic cell divisions. Why the requirement for SPD-1 changes over time during early embryogenesis, and how extensively SPD-1/PRC1 is required for cytokinesis later in embryogenesis, are not clear.

Cell polarity and cytokinesis

The actomyosin cytoskeleton is important for both cytokinesis and cell polarity, and a recent study suggests that cell polarity may contribute to cytokinesis in the one-cell stage embryo (Jordan *et al.* 2016). This contribution was initially observed in double mutants with TS mutations in both the formin gene *cyk-1* and the nonmuscle myosin gene *nmy-2*. When grown at a semipermissive temperature that does not prevent cytokinesis in either single mutant, a synthetic cytokinesis failure is observed in the double mutant. Surprisingly, this synthetic cytokinesis defect occurs only upon temperature-upshifts before cytokinesis, during the establishment of anterior–posterior polarity, and not after later upshifts immediately before or during cytokinesis. Furthermore, knockdown of the core polarity proteins PAR-2 or PAR-6 both lead to decreased levels of microfilaments in the contractile ring, and to a synthetic cytokinesis defect in TS *cyk-1* mutants at the semi-permissive temperature. PAR-2 or PAR-6 knockdown also results in a loss of the anterior cortical enrichment of the contractile ring components anillin and septin, while knockdown of anillin or septin do not affect polarity but do lead to increased levels of microfilaments in the contractile ring. Finally, septin or anillin knockdown completely rescues the cytokinesis defect in *par*; *cyk-1* double mutants. Together, the results suggest that anterior–posterior polarity sequesters anillin and the septins to the anterior cortex, limiting their levels at furrow to promote contractile ring assembly. Whether this contribution of the PAR proteins is important only during the division of polarized cells, and whether it contributes to polarized cell divisions later in embryogenesis and during larval development, are not known.

Abscission: the last chapter in cytokinesis

During furrow ingression, the contractile ring constricts around the central spindle. Observations across systems suggest that as constriction nears completion the central spindle and contractile ring respectively mature to form the microtubule-based midbody and the cortical midbody ring, which coordinately bring about abscission. Efforts have primarily focused on the role of the microtubule-based midbody in recruiting components, such as the ESCRT filament system that is proposed to bring about the final closure of the intercellular bridge (Fededa and Gerlich 2012; Green *et al.* 2012). However, recent work in *C. elegans* has challenged these ideas, and has highlighted the role of the cortical midbody ring (actin and the septins), rather than midbody microtubules, in abscission (Green *et al.* 2013; König *et al.* 2017)

A detailed analysis of abscission during the first division of the *C. elegans* embryo has been performed using both light and cryo-electron microscopy (Green *et al.* 2013; König *et al.* 2017). Structural views of the intercellular bridge revealed that the interval between the onset of furrow ingression and engulfment of a membrane-bound intercellular bridge remnant into the posterior cell, is ∼15 min (König *et al.* 2017). Furrow ingression to reach the point where the contractile ring closes around the central spindle microtubules to form an intercellular bridge takes ∼3 min. Over the next 4 min, the microtubules passing through the intercellular bridge disassemble. Notably, the microtubules completely disappear from the bridge well before abscission occurs, while the intercellular bridge is still open to the cytoplasm on both sides. Abscission, defined as sealing off of the plasma membrane between the bridge and one of the daughter cells, occurs ∼5–6 min after the microtubules are lost from the bridge. Whether the junction between the anterior cell and the bridge or the posterior cell and the bridge closed first appears to be a random event. Completion of abscission by the second cell leaves a membrane bound intercellular bridge remnant that contains cortical midbody ring components (but no microtubules) in the extracellular space. This membrane bound remnant is ultimately engulfed into the posterior cell about 15 min after the onset of furrow ingression. The fact that midbody microtubules are lost well before abscission occurs suggests that these microtubules are not required for abscission. Consistent with this idea, abscission and internalization of the intercellular bridge remnant occur normally in embryos depleted of SPD-1, which is required for assembly of the microtubule bundles in the central spindle. In SPD-1–depleted embryos, microtubules are not observed to pass through the intercellular bridge even at early stages. In contrast to microtubules, actin filaments are important for abscission, as latrunculin A-treated cells fail to undergo abscission or to engulf the intercellular bridge remnant (König *et al.* 2017)

These studies also suggest that the ESCRT filament system is not required for abscission during embryonic cytokinesis. In ESCRT-inhibited embryos, abscission occurred with normal timing; however, the membrane-bound intercellular bridge remnant was not internalized and instead remained trapped in the extracellular space (Green *et al.* 2013; König *et al.* 2017). The intercellular bridge remnant also became swollen when ESCRT was inhibited, implicating the ESCRT complex in the removal of membrane from this structure before or during its subsequent engulfment by the posterior daughter.

Finally, the membrane remodeling protein dynamin, known to be required late in cytokinesis in the one-cell stage embryo (Thompson *et al.* 2002), localizes to the intercellular bridge during abscission and its knockdown leads to a failure to clear excess membrane from the intercellular bridge before abscission and a subsequent failure in abscission (König *et al.* 2017). These studies have provided substantial insight into the steps by which abscission occurs, but the mechanisms that mediate this process, remain only partially understood. The fate of the engulfed intercellular bridge remnant and its possible roles in subsequent cell cycles also warrant further investigation (Skop *et al.* 2004; Singh and Pohl 2014).

Concluding remarks and perspectives

During the past two decades, tremendous advances have been made in our understanding of the molecular mechanisms that regulate and execute cell division in *C. elegans*. A powerful toolbox of molecular, genetic, and imaging approaches have greatly improved our mechanistic understanding of multiple processes—including kinetochore assembly and function, centrosome duplication and maturation, mitotic spindle assembly, chromosome segregation, and cytokinesis. Throughout this review, we have attempted to highlight topics that require or currently are the focus of further research. Such efforts should benefit from ongoing advances in the development of higher resolution light and electron microscopy methods, and from new technologies, such as optogenetics (Wagner and Glotzer 2016), that will enable higher spatiotemporal resolution in the manipulation of gene functions during cell division. Several other topics that we have not covered due to space limitations will also likely be the focus of future investigation. These include allometry: elucidating how cellular structures modulate and adapt their composition to ensure accurate cell division in differently sized cells (Hara and Kimura 2009; Ladouceur *et al.*, 2017; Lacroix *et al.* 2018), and how cell division processes are coordinated with cell polarity, cell division axis orientation, and cell fate specification to generate appropriate numbers and architectural arrangements of different cell types during development. Importantly, a number of genes required for cell division, such as the polo-like kinase PLK-1, are emerging as important regulators of cell polarity (Noatynska *et al.* 2010; Dickinson *et al.* 2017) and cell fate (Nishi *et al.* 2008; Han *et al.* 2018). The experimental virtues of *C. elegans* ensure that this elegant animal model will occupy a prominent place in research that further advances our understanding of fundamental cell division processes and their relationship to animal development.

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