Cell division*

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Abstract

The *C. elegans* embryo is a powerful model system for studying the mechanics of metazoan cell division. Its primary advantage is that the architecture of the syncytial gonad makes it possible to use RNAi to generate oocytes whose cytoplasm is reproducibly (typically >95%) depleted of targeted essential gene products via a process that does not depend exclusively on intrinsic protein turnover. The depleted oocytes can then be analyzed as they attempt their first mitotic division following fertilization. Here we outline the characteristics that contribute to the usefulness of the *C. elegans* embryo for cell division studies. We provide a timeline for the first embryonic mitosis and highlight some of its key features. We also summarize some of the recent discoveries made using this system, particularly in the areas of nuclear envelope assembly/ dissassembly, centrosome dynamics, formation of the mitotic spindle, kinetochore assembly, chromosome

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1. The *C. elegans* embryo as a system to study cell division

The *C. elegans* embryo is a powerful model system for studying the mechanics of metazoan cell division. Its primary advantage is that the syncytial gonad makes it possible to use RNA interference (RNAi) to generate oocytes whose cytoplasm is reproducibly (>95%) depleted of targeted essential gene products. Introduction of dsRNA rapidly catalyzes the destruction of the corresponding mRNA in many different systems. However, depletion of pre-existing protein is generally a slow process that depends on the half-life of the targeted protein. In contrast, in the *C. elegans* gonad, the protein present when the dsRNA is introduced is depleted by the continual packaging of maternal cytoplasm into oocytes (Figure 1). Since depletion relies on the rate of embryo production instead of protein half-life, the kinetics tend to be similar for different targets. By 36-48 hours after introduction of the dsRNA, newly formed oocytes are typically >95% depleted of the target protein.

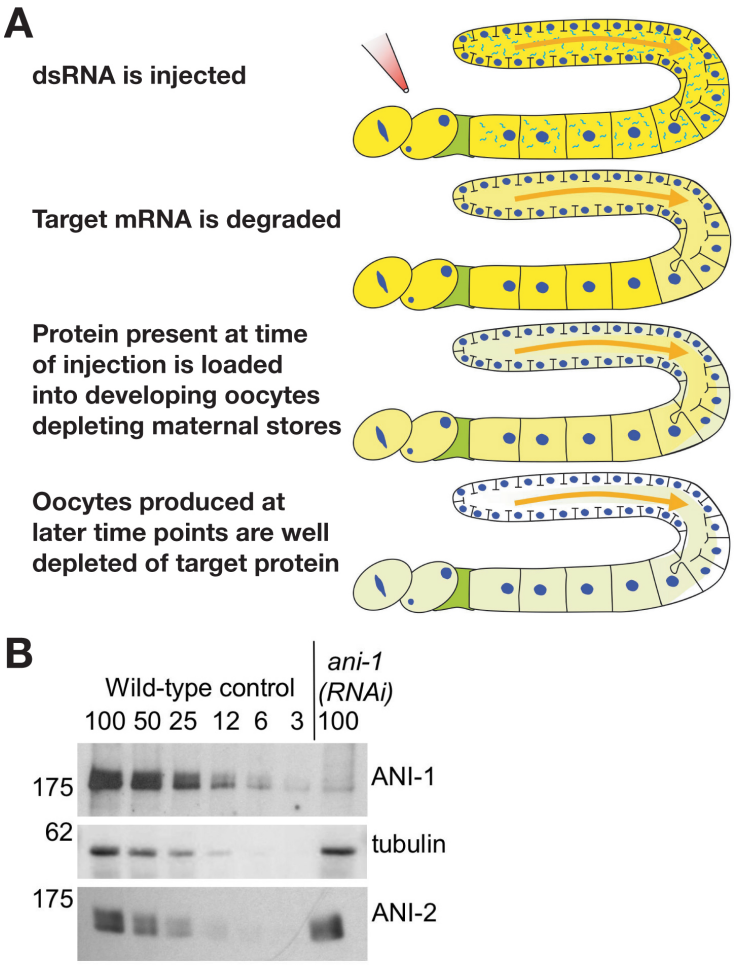

Figure 1. The generation of oocytes depleted of target proteins by RNAi in *C. elegans* does not require intrinsic protein turnover. (A) The gonad is a syncytial tube lined with nuclei in various stages of meiotic prophase. The meiotic nuclei contribute mRNA that is translated to generate the protein that is loaded into the developing oocytes. The meiotic nuclei and all of the developing oocytes except the final 4-5 (Maddox et al., 2005) are diffusionally connected to the rachis (the central cytoplasmic core of the syncytial gonad). Introduction of a dsRNA triggers the degradation of the corresponding mRNA. However, the syncytial gonad and connected oocytes still contain the target protein that was present at the time of injection. Maternal stores are depleted by the continual packaging of gonad cytoplasm into developing oocytes. By 36 to 48 hours after introduction of the dsRNA oocytes are typically >95% depleted of the target protein. (B) Quantitative western blot of worms injected with a dsRNA against ANI-1, a protein required for cortical contractility in the early embryo (Maddox et al., 2005). Serial dilutions of identically processed control worms were loaded to quantify the depletion level. ANI-1 is ~97% depleted in the injected worms, whereas levels of two control proteins, α-tubulin and a related actin binding protein, ANI-2, are unaffected. Figure courtesy of Amy Maddox.

Several additional advantages contribute to the usefulness of the *C. elegans* embryo as a model system. Of particular importance is their rapid and highly stereotypical mitotic divisions; the time between the onset of DNA condensation and the completion of furrow ingression during cytokinesis is approximately 14 minutes. The invariant

nature of the first few divisions also facilitates the development of methods to assess the consequences of molecular perturbations. Quantitative methods have already been developed to monitor pronuclear migration (Figure 4; see also Albertson, 1984; O'Connell, 2000), cortical flows (for examples see Cheeks et al., 2004; Hird and White, 1993; Munro et al., 2004), chromosome segregation and spindle elongation during anaphase (for examples see Cheeseman et al., 2004; Grill et al., 2001; Labbe et al., 2004), and the asymmetric positioning of the spindle within the embryo (for examples see Colombo et al., 2003; Labbe et al., 2004; Tsou et al., 2002). Assay development has been accelerated by the emergence of microparticle bombardment mediated transformation (Praitis et al., 2001) and the availability of vectors containing regulatory sequences that direct efficient germline expression (Strome et al., 2001), which together have led to the generation of a large number of strains expressing fluorescent fusion proteins in the early embryo. Analysis of the mechanical consequences of depleting essential cell division proteins is also facilitated by the relatively weak DNA damage (Brauchle et al., 2003) and spindle checkpoints (Encalada et al., 2004), which allow the embryo to proceed through the cell cycle despite dramatic defects in nuclear structure, spindle assembly, chromosome segregation and centrosome function.

Genetic and RNAi-based approaches have identified a large number of loci important for cell division. Mutants in proteins required for cell division have been uncovered in screens of collections of nonconditional maternal effect and temperature sensitive mutations that result in embryonic lethality (for some examples see Encalada et al., 2000; Golden et al., 2000; Gönczy et al., 1999; Kemphues et al., 1988; O'Connell et al., 1998). The ability to reproducibly deplete oocytes of target proteins by RNAi, which can be performed by feeding, soaking or injection of hermaphrodites (see Reverse genetics), has also led to a series of genome-wide screens that identified a set of ~2100 genes required for embryonic viability (Fernandez et al., 2005; Fraser et al., 2000; Gönczy et al., 2000; Kamath et al., 2003; Maeda et al., 2001; Piano et al., 2000; Rual et al., 2004; Simmer et al., 2003; Sõnnichsen et al., 2005). Filming of embryos depleted of each of these 2100 gene products using differential interference contrast (DIC) microscopy has defined a set of 660 genes whose inhibition results in detectable defects during the first two cell divisions (Gönczy et al., 2000; Piano et al., 2000; Sõnnichsen et al., 2005; Zipperlen et al., 2001). Roughly half of these genes are specifically required for cell division processes such as chromosome segregation or cytokinesis, whereas the other half contributes to cell maintenance, via roles in processes such as translation and mitochondrial function (Sõnnichsen et al., 2005). The embryonic lethality resulting from RNAi of some of the 1440 genes for which no DIC defect was observed may be due to cell division defects that remain undetected, either due to incomplete penetrance of the RNAi or failure to score the resulting defects by DIC (for example subtle defects in chromosome segregation are very difficult to detect using this assay). Alternatively, depletion of many of these gene products may cause embryonic lethality due to developmental defects that preclude hatching.

2. A timeline of events during the first mitotic division of the *C. elegans* embryo

Due to its accessibility to RNAi-based molecular perturbation, the first embryonic division that ensues following fertilization has been the most intensively studied. In this section, we provide a brief timeline for the events between fertilization and the completion of the first cytokinesis (outlined schematically in Figure 2; for reviews see Cowan and Hyman, 2004; Pelletier et al., 2004; Schneider and Bowerman, 2003).

Prior to fertilization, *C. elegans* oocytes are arrested in meiotic prophase with nuclei containing two copies of the diploid genome packaged into recombined bivalent chromosomes. The two rounds of meiotic chromosome segregation that generate the haploid oocyte pronucleus are completed in the zygote after the oocytes are fertilized. During each meiotic division, chromosome segregation is accomplished by a small acentriolar meiotic spindle that forms in the embryo anterior. During anaphase of meiosis I and again in meiosis II, the meiotic spindle associates with the cortex in an end-on fashion, and a highly asymmetric cytokinesis-like event extrudes a polar body (Figure 2; Albertson and Thomson, 1993; Clark-Maguire and Mains, 1994; Yang et al., 2003). In addition to the haploid pronucleus, the sperm brings a pair of centrioles into the oocyte, which lacks centrioles due to their degradation during oogenesis. As meiosis completes, the haploid oocyte and sperm-derived pronuclei, located at opposite ends of the embryo increase in size, becoming visible by DIC microscopy. After entering the oocyte, the sperm-derived centriole pair recruits pericentriolar material and acquires the ability to nucleate microtubules (O'Connell, 2000; Pelletier et al., 2004). Subsequently, the two sperm-derived centrioles separate, forming two centrosomes positioned on either side of the paternal pronucleus. Coincident with chromosome condensation during mitotic prophase, the pronuclei migrate towards each other. After the pronuclei meet, the nuclear-centrosome complex moves to the center of the embryo and rotates to align with the long axis of the embryo (Albertson, 1984; Hyman and White, 1987). The miotitc spindle begins to move towards the embryo posterior during metaphase (Labbe et al., 2004; Oegema et al., 2001), and asymmetric elongation during anaphase contributes to its posterior displacement (Albertson, 1984; Grill et al., 2001). Since the cleavage furrow bisects the mitotic spindle, this displacement results in an asymmetric first

cleavage (For more on the mechanisms that generate this asymmetry see Asymmetric cell division and axis formation in the embryo).

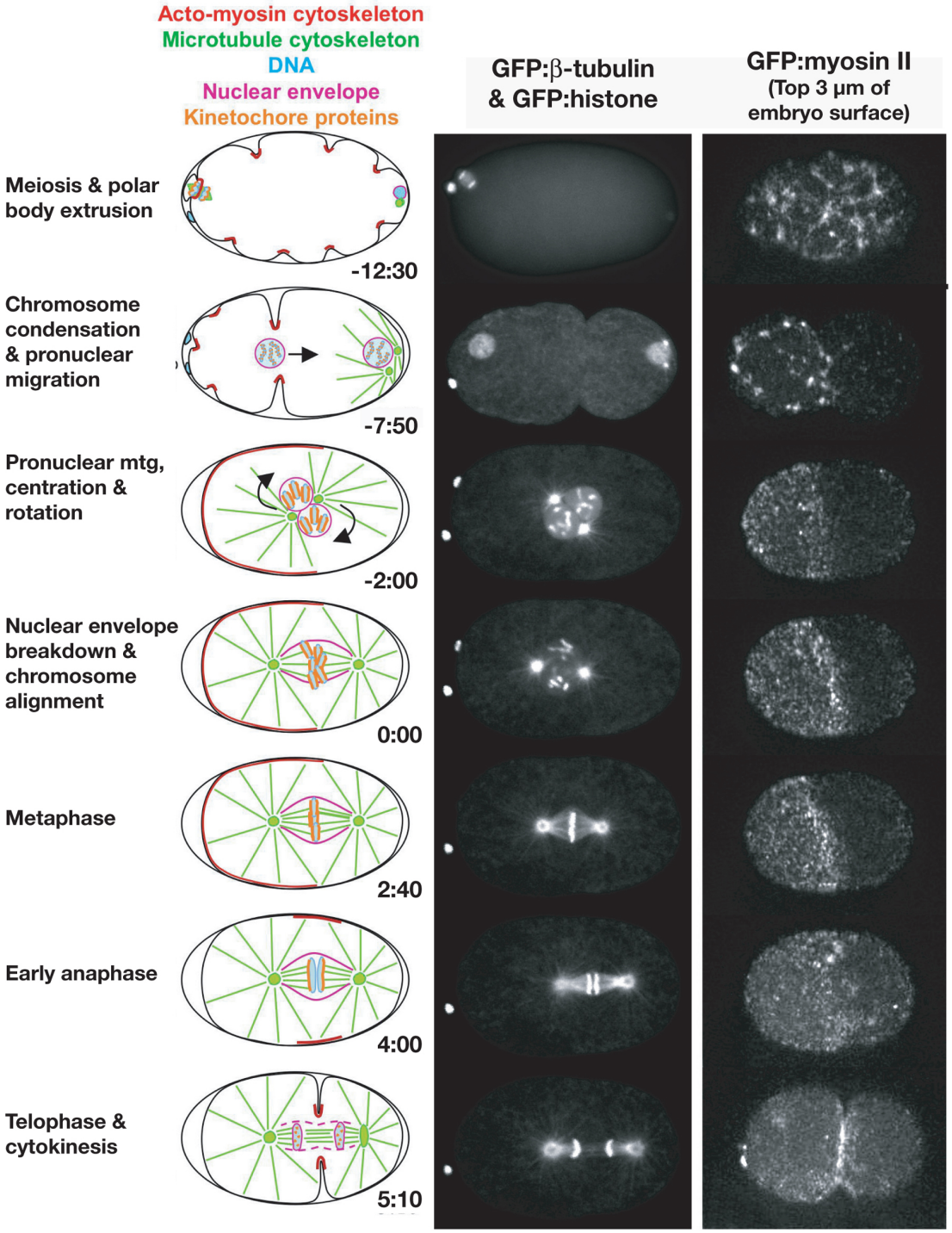

Figure 2. Nuclear envelope dynamics in the *C. elegans* embryo. (Left column) Schematics illustrate the major features of the first division. Approximate times are in minutes:seconds after nuclear envelope breakdown. (Middle column) Images of each stage in a strain expressing GFP: beta-tubulin and GFP: histone to simultaneously visualize the microtubule cytoskeleton and the DNA. The top image of anaphase of meiosis II was taken from a movie collected by wide-field microscopy. All subsequent images are of the same embryo and were collected by spinning disk confocal microscopy (images courtesy of Carrie Cowan). (Right column) Stills from a timelapse sequence of an embryo expressing GFP:myosin II (images courtesy of Amy Maddox; strain provided by Ed Munro). For each time point, three spinning disk confocal images of the embryo surface were collected at 1 µm intervals and projected. During polar body extrusion, ruffles form over the entire cortex. Foci of myosin II are apparent at the base of each of the ingressing ruffles. As polarity is established, myosin II concentrates in an anterior cortical cap that persists into metaphase (Munro et al., 2004). During cytokinesis, an equatorial band of cortical myosin II forms in the plane defined by the spindle midzone. Figure courtesy of Amy Maddox.

3. Nuclear envelope structure and dynamics

The *C. elegans* nuclear envelope is structurally similar to that of vertebrates, consisting of two concentric membranes (outer and inner) enclosing a lumenal space, nuclear pore complexes that mediate bidirectional transport between the cytoplasm and the nucleus, and an underlying laminar network (Figure 3; Cohen et al., 2002). The molecular composition of the *C. elegans* inner nuclear membrane/lamina also resembles that in vertebrates (Table 1). *C. elegans* expresses a single B-type lamin (LMN-1; Liu et al., 2000; Riemer et al., 1993), that forms a meshwork of intermediate filaments beneath the inner nuclear membrane (reviewed in Gruenbaum et al., 2005). The *C. elegans* inner nuclear membrane/lamina also contains three proteins, Ce-emerin, CeMAN-1, and CeLem2, that contain a LEM domain, a defining 40 amino acid motif shared by a family of nuclear envelope proteins (Gruenbaum et al., 2002; Lee et al., 2000; Lin et al., 2000; Liu et al., 2003). LEM family proteins all bind to lamins (reviewed in Lee and Wilson, 2004) and to the small inner nuclear membrane associated protein BAF (Segura-Totten and Wilson, 2004; Zheng et al., 2000). Depletion of LMN-1, Ce-BAF, or simultaneous depletion of the LEM family proteins Ce-Emerin and Ce-MAN-1, results in a similar spectrum of defects in nuclear structure, chromosome condensation, and chromosome segregation (Liu et al., 2000; Liu et al., 2003; Margalit et al., 2005; Zheng et al., 2000).

Table 1. Nuclear envelope proteins

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**TMR**	**Summary of localization and functional analysis**	**Selected references**
Ce-lamin (LMN-1)	*lmn-1* (DY3.2)	YES	Lamin-B	NO	B-type lamin expressed in all cell types; localizes to the nuclear side of the nuclear envelope; required for nuclear morphology, nuclear pore complex distribution, chromosome segregation and the localization of Ce-emerin.	Liu, 2000; Gruenbaum, 2002
Ce-emerin (EMR-1)	*emr-1* (M01D7.6)	NO	Emerin	YES	Integral component of the inner nuclear membrane; contains a LEM domain and interacts with Ce-Lamin; co-depletion with Ce-MAN-1 results in 100% embryonic lethality, chromosome condensation and segregation defects and mislocalization of Ce-BAF.	Gruenbaum, 2002; Liu, 2003
Ce-MAN1 (LEM-2)	*lem-2* (W01G7.5)	PARTIAL Depletion gives 15% lethality	LEM2 and MAN1	YES	Integral component of the inner nuclear membrane; contains a LEM domain and interacts with Ce-Lamin and Ce-BAF *in vitro*. May require Ce-Lamin for its localization.	Liu, 2003
Ce-BAF	*baf-1* (B0464.7)	YES	BAF	NO	10kDa soluble protein highly conserved among metazoans; localization to the NE requires emerin and MAN1; interacts with Ce-MAN1 *in vitro*; required for the proper organization of Ce-lamin, Ce-emerin and Ce-MAN1 on the nuclear envelope.	Liu, 2003; Margalit, 2005
Matefin	*mtf-1 sun-1*	YES	None	NO	Interacts with lamin, but its	Fridkin, 2004;

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**TMR**	**Summary of localization and functional analysis**	**Selected references**
SUN-1	(F57 B1.2)				Localization to the nuclear envelope does not require lamin; expressed in all embryonic cells until mid-embryogenesis and thereafter only in germline cells; required for ZYG-12 localization and the attachment of centrosomes to nuclei.	Malone, 2003
ZYG-12	*zyg-12* (ZK546.1)	YES	SYNE	YES/NO*	Member of the Hook family of proteins; localizes to the nuclear envelope and accumulates around the centrosomes; required for the attachment of centrosomes to nuclei; * TMR present in some isoforms.	Malone, 2003
UNC-83	*unc-83* (W01A11.3)	NO	?	NO	Requires UNC-84 for its localization to the nuclear envelope; mutations disrupt nuclear migration in migrating P cells, hyp7 precursors, and the intestinal primordium.	Starr, 2001
UNC-84	*unc-84* (F54B11.3)	NO	UNC84	YES	Integral nuclear envelope protein required for nuclear migration and anchorage; expressed after the 26-cell stage; localization requires lamin; required to target UNC-83 and ANC-1, which function in nuclear migration and positioning, respectively, to the nuclear membrane; during nuclear positioning, may form a bridging complex with ANC-1 that spans the perinuclear space to connect the NE to actin cytoskeleton.	Starr, 2001; Malone, 1999; Lee, 2002; Starr, 2002
ANC-1	*anc-1* (ZK973.6)	NO	Nesprin/ Nuance	YES	955kDa protein orthologous to vertebrate NUANCE proteins; connects the nuclear envelope to the actin cytoskeleton during nuclear positioning in a UNC-84-dependent manner.	Starr, 2002

The dynamics of nuclear envelope disassembly and reassembly during the first mitotic division of the *C. elegans* embryo are illustrated in Figure 3. LMN-1 leaves the nuclear envelope during prometaphase (Lee et al., 2000; Liu et al., 2000). In contrast, inner nuclear membranes containing Ce-emerin and Ce-MAN-1 remain largely intact and surround the mitotic spindle everywhere except near spindle poles during metaphase and early anaphase, disassembling fully only during mid to late anaphase (Figure 3; Lee et al., 2000; Lee et al., 2002; V. Galy, P. Askjaer and I.W. Mattaj, personal communication). As the remnants of the old nuclear envelopes disperse, the

formation of new nuclear envelopes around the segregated chromatin is detected beginning about 1 minute after anaphase onset (Figure 3; V. Galy, P. Askjaer and I.W. Mattaj, personal communication).

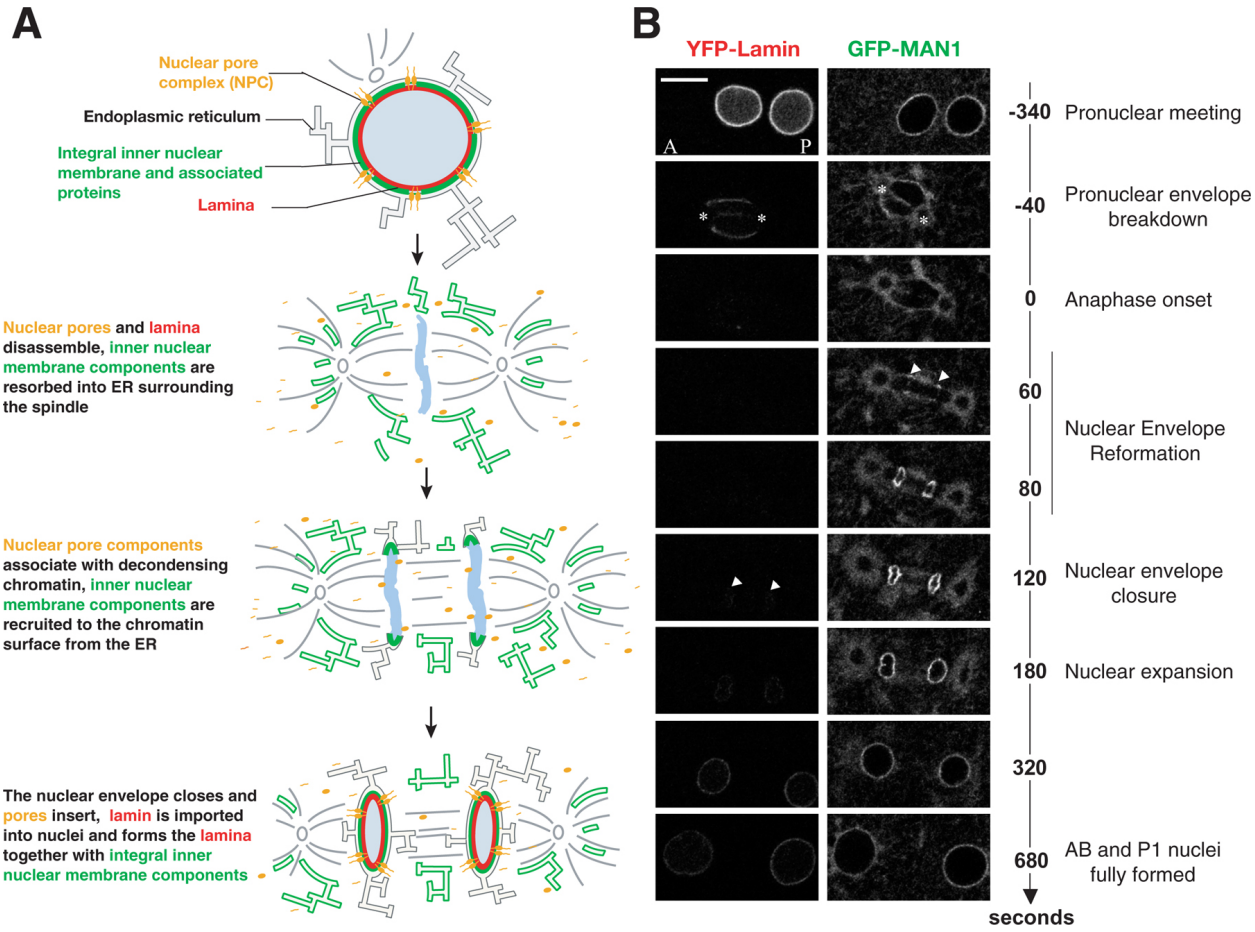

Figure 3. Nuclear envelope dynamics in the *C. elegans* embryo. Figure courtesy of Vincent Galy. (A) Schematics illustrate the cycle of nuclear envelope breakdown and reassembly. (B) Still images of the first mitotic division of wild-type embryos expressing YFP-Lamin (left) and GFP-MAN1 (right). Times on the right are relative to first metaphase to anaphase transition. Arrowheads indicate the reappearance of GFP-MAN1 around the chromatin at t=60 sec and YFP-Lamin at t=120 sec. White stars mark the positions of the centrosomes. Note the persistence of membranes containing GFP-MAN1 around the mitotic spindle and centrosomes. Scale bar = 10µm.

The molecular composition of the nuclear pore complexes (NPCs) is also similar to that in vertebrates. *C. elegans* orthologs of at least one component of each vertebrate NPC sub-complex have been identified (Galy et al., 2003; Kuznetsov et al., 2002). 17 genes encoding 19 nucleoporins are essential for embryonic viability. Depletion of 14 of these proteins results in defects in nuclear morphology and, in some cases, to reduced nuclear size consistent with a defect in nucleo-cytoplasmic transport (Galy et al., 2003). Nuclear envelope assembly also requires the small GTPase, Ran and the nuclear transport receptor importin-γ (IMB-1; Askjaer et al., 2002; Bamba et al., 2002; Walther et al., 2003). High concentrations of RanGTP in the vicinity of chromatin are thought to promote the dissociation of importin-γ from nucleoporins to trigger NPC assembly on the chromatin surface.

4. Pronuclear migration

The site of sperm entry defines the embryo posterior (Goldstein and Hird, 1996). As the pronuclei become visible by DIC, the sperm-derived pronucleus and its associated centrosome(s) sit on the posterior cortex. The oocyte-derived pronucleus forms following two rounds of meiotic chromosome segregation, typically in the embryo anterior. The two pronuclei migrate towards each other coincident with chromosome condensation during the first mitotic prophase. Pronuclear migration consists of movement of the oocyte pronucleus towards the sperm pronucleus and movement of the sperm pronucleus away from the cortex towards the embryo center (Albertson, 1984; O'Connell et al., 2000). Initially, the oocyte pronucleus moves ~ 12 µm towards the posterior at a slow rate (~ 3.5 µm/min). As it approaches the sperm pronucleus, the oocyte pronucleus accelerates, moving an additional 10 µm

at ~5-10 times its initial rate (Figure 4; Albertson, 1984; O'Connell et al., 2000). The sperm pronucleus begins its migration later than its female counterpart and travels at a slow rate of ~ 3.5 µm/min till it meets the oocyte pronucleus near the embryo center (~7 µm).

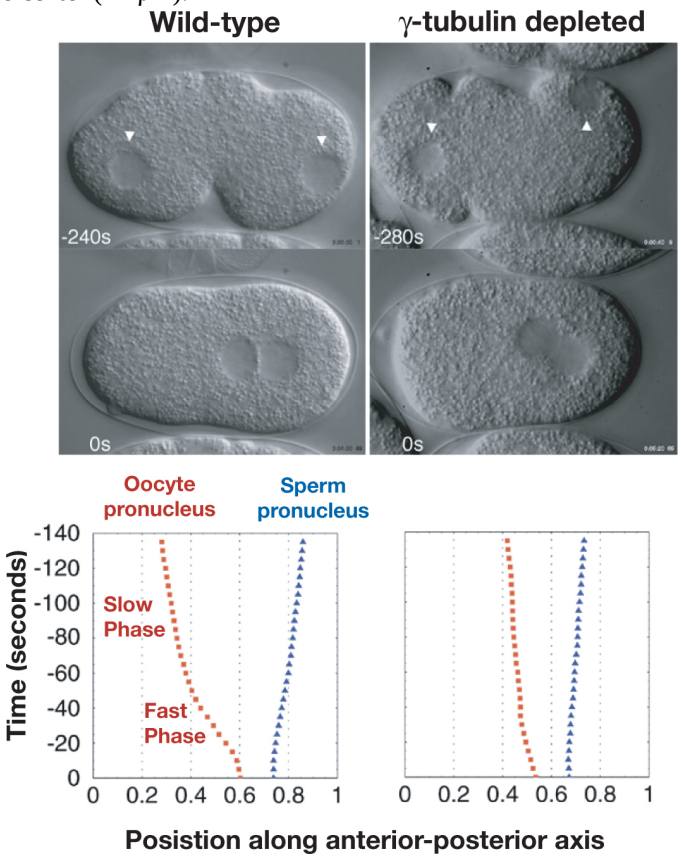

Figure 4. Kinetics of pronuclear migration in the *C. elegans* embryo. Timelapse DIC sequences of 20 wild-type and 16 gamma-tubulin depleted embryos were collected. The average position of the oocyte-derived and sperm-derived pronuclei along the anterior-posterior axis of the embryo is plotted (x-axis) as a function of time (y-axis). Times are with respect to pronuclear meeting. The sperm pronucleus moves towards the embryo anterior at a uniform slow rate. The oocyte pronucleus initially moves towards the embryo posterior at a similar slow rate (Slow phase), but then speeds up prior to nuclear meeting (Fast phase). Pronuclear migration depends on the timing of formation and size of centrosomal microtubule asters. In embryos, depleted of gamma-tubulin centrosomal microtubule asters form later than in wild-type (Hannak et al., 2002) and the fast phase of pronuclear migration is not observed. A similar phenotype has been characterized in embryos mutant for the centrosomal protein SPD-2, in which the centrosomal microtubule asters are highly attenuated (O'Connell et al., 2000). Figure courtesy of Eva Hannak and Stephan Grill.

Rapid movement of the oocyte pronucleus towards the sperm pronucleus and pronuclear meeting, both require an intact microtubule cytoskeleton (Strome and Wood, 1983). Two nuclear envelope proteins, ZYG-12 and SUN-1 recruit dynein to pronuclei, and are required for centrosomes to maintain their association with nuclei (Table 1; Malone et al., 2003). The centrosomes separate around the sperm pronucleus in a dynein dependent manner (Gönczy et al., 1999). As the pronuclei move towards each other, dynein on the oocyte pronucleus is thought to come into contact with microtubules emanating from the centrosomal asters associated with the sperm pronucleus. The fast phase of pronuclear migration and pronuclear meeting is thus mediated by nuclear envelope associated dynein pulling on the two centrosomal microtubule asters (Cowan and Hyman, 2004; Gönczy et al., 1999; Hamill et al., 2002; Malone et al., 2003; O'Connell et al., 2000; Schmidt et al., 2005).

In addition to the migration of the female pronucleus towards the male pronucleus, the migration and centration of the male pronucleus within the embryo has also been analyzed to distinguish between two possible models: (1) a “pushing mechanism,” in which the male pronucleus is pushed away from the cortex by the polymerization of astral microtubules and, (2) a “pulling mechanism” in which the male pronucleus is pulled by minus-end-directed motors anchored throughout the cytoplasm. Comparisons between simulations and actual migration indicate that the second “pulling” model is the primary mechanism (Kimura and Onami, 2005). Although microtubule-based mechanisms for pronuclear movement are the best characterized, some slow pronuclear movement is still observed in embryos in which the microtubule cytoskeleton is compromised, or which lack centrosomes or fail to recruit dynein to nuclei (Cowan and Hyman, 2004; Gönczy et al., 1999; Hamill et al., 2002;

Malone et al., 2003; O'Connell et al., 2000; Schmidt et al., 2005). Consistent with these observations, correlative evidence suggests that cortical flows may also contribute to the slow phase of pronuclear migration (Hird and White, 1993).

5. Centrosome assembly and duplication

Centrosomes consist of a single centriole or centriole pair surrounded by pericentriolar material that nucleates and anchors microtubules. Like the centrioles in *Drosophila* embryos (Callaini and Riparbelli, 1990), *C. elegans* centrioles are composed of nine singlet microtubules symmetrically positioned around a central tube (Figure 5B; Albertson, 1984; Kirkham et al., 2003; O'Connell et al., 2001; Wolf et al., 1978). Each cylindrical centriole is approximately 200– 250 nm in length and 175 nm in diameter. In contrast, vertebrate centrioles typically have nine triplet microtubules (Marshall, 2001; Preble et al., 2000). Consistent with this structural difference, the *C. elegans* genome lacks homologs of delta- and epsilon-tubulin, two tubulin family members required for the formation of triplet microtubules (Dutcher, 2003).

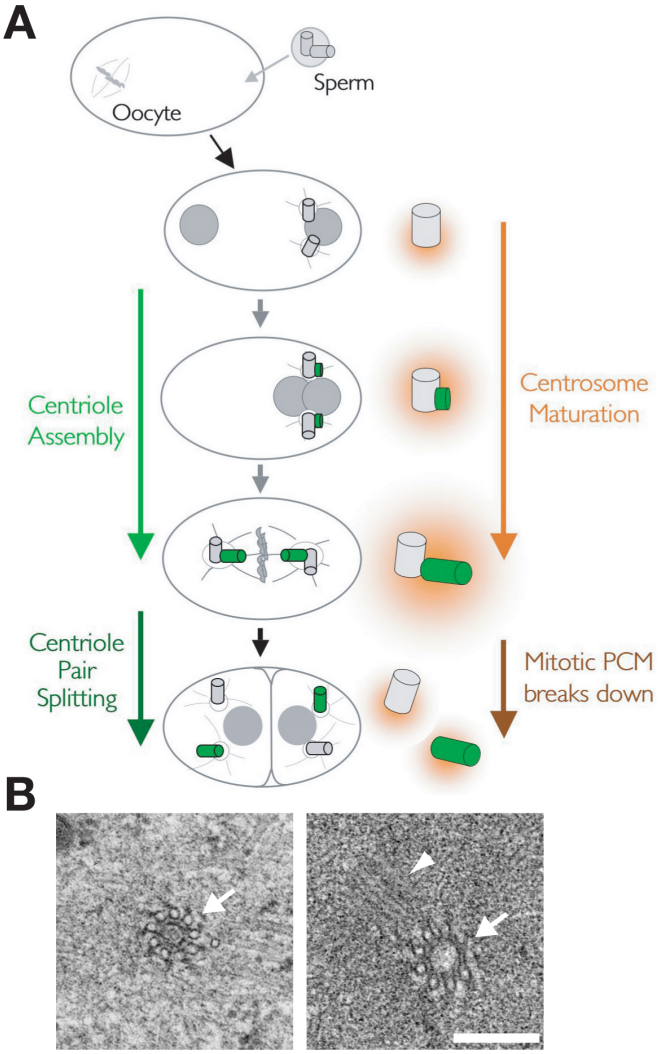

Figure 5. Mitotic kinetochores in the *C. elegans* embryo. (A) Schematic of the first cycle of centrosome duplication that immediately follows fertilization. Figure and electron micrographs courtesy of Amy Maddox and Thomas Müller-Reichert, respectively. Centrosome duplication consists of alternating cycles of new centriole assembly and splitting of the centriole pairs. A pair of centrioles (grey) enters the egg with the sperm during fertilization. The sperm centrioles acquire pericentriolar material (PCM; orange) in the egg and begin to nucleate microtubule asters. New daughter centrioles (green) assemble adjacent to each of the sperm centrioles so that by metaphase each centrosome contains two full-length centrioles, one inherited from the sperm and one that formed in the embryo cytoplasm. As the embryo enters mitosis, the amount of PCM around the centrioles and the number of microtubules nucleated by the centrosomes increases in a process called centrosome maturation. The centriole pairs split in late anaphase/telophase and the mitotic PCM breaks down, so that each daughter cell inherits a pair of small centrosomes each containing a single centriole. (B) Two images of prometaphase/metaphase centrioles with singlet microtubules in either cross section (arrows) or a longitudinal orientation (arrowhead) are shown. Bar is 250 nm.

During fertilization, the amoeboid sperm brings a pair of centrioles into the egg. After entering the egg, the centrioles recruit pericentriolar material and separate around the sperm-derived nucleus, forming two centrosomal microtubule asters (Figure 5; O'Connell, 2000; Pelletier et al., 2004). After they separate, a new daughter centriole begins to form adjacent to each sperm centriole. The centrosomes recruit additional pericentriolar material as the embryo enters mitosis in a process called centrosome maturation, increasing about 5 fold in size and nucleating capacity by metaphase (Hannak et al., 2001). Coincident with the recruitment of additional PCM, the daughter centrioles elongate, reaching full length by metaphase. In late anaphase/telophase the centriole pairs split. Concurrent with cytokinesis, the mitotic PCM disassembles, releasing two small centrosomes into each daughter cell.

Pericentriolar material is thought to consist of a proteinacious matrix, called the “centromatrix”, that recruits other PCM components (Bornens, 2002; Palazzo et al., 2000). Three centrosomal proteins, SPD-5, SPD-2 and AIR-1 (Aurora-A kinase), are required for the assembly of pericentriolar material (see Table 2 for a list of *C. elegans* centrosome/centriole components). SPD-5 is a coiled-coil protein thought to be a component of the centromatrix. In *spd-5* embryos, no PCM forms around the centrioles, and no centrosomal microtubule asters are observed (Hamill et al., 2002). Severe defects in PCM assembly are also observed when SPD-2 or the aurora A kinase homolog AIR-1 are inhibited (Hannak et al., 2001; Kemp et al., 2004; O'Connell et al., 2000; Pelletier et al., 2004; Schumacher et al., 1998). In these embryos, centrosomal microtubule asters form, but are very small, and additional PCM fails to accumulate around the centrioles during mitotic entry.

Table 2. Centrosome proteins

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
SAS-4	*sas-4* (F10E9.8)	YES	CPAP	Centriole protein required for centriole assembly; incorporated into centrioles during their assembly and does not subsequently exchange with the cytoplasmic pool; partial depletion leads to the formation of defective centrioles that recruit less than wild-type levels of PCM.	Kirkham, 2003; Leidel, 2003
SAS-5	*sas-5* (F35B12.5)	YES	?	Centriole protein required for centriole assembly; physically interacts with SAS-6; centriolar SAS-5 shuttles continuously between centrioles and the cytoplasm; requires ZYG-1 and SAS-6, but not SAS-4 to localize to centrioles; required for the localization of SAS-4 and SAS-6 to centrioles. Like SAS-4, partial depletion of SAS-5 leads to the formation of defective centrioles that recruit less than wild-type levels of PCM.	Delattre, 2004; Dammermann, 2004; Leidel, 2005
SAS-6	*sas-6* (Y45F10D.9)	YES	HsSAS-6; DKFZP761 A078	Centriole protein required for centriole assembly; like SAS-4, is recruited to centrioles once per cell cycle and does not subsequently exchange; physically interacts with SAS-5; requires ZYG-1 and SAS-5, but not SAS-4 to be	Dammermann, 2004; Leidel, 2005

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
				recruited to centrioles; required for the localization of SAS-4 and SAS-5. Like SAS-4, partial depletion of SAS-5 leads to the formation of defective centrioles that recruit less than wild-type levels of PCM. The human homolog of SAS-6 localizes to centrioles and is required for centriole duplication.	
ZYG-1	*zyg-1* (F59E12.2)	YES	?	Centriole protein required for centriole assembly; localizes to centrioles throughout the cell cycle, but in contrast to SAS-4-6 is not detected on centrioles in sperm; required to target SAS-4, SAS-5 and SAS-6 to centrioles; atypical protein kinase that autophosphorylates in vitro, but *in vivo* substrates remain unknown; *zyg-1* mutation interacts genetically with a mutation in spd-2.	O'Connell, 2001; Kemp, 2004; Delattre, 2004; Dammermann, 2004
SPD-2	*spd-2* (F32H2.3)	YES	FLJ10352	Bi-functional protein required for new centriole formation and for the assembly of the PCM around the centrioles; localizes to both centrioles and the PCM; *spd-2* mutation interacts genetically with mutations in both *zyg-1* and *spd-5*; the SPD-2 domain shares homology to the ASP protein family.	O'Connell, 2000; Kemp, 2004; Pelletier, 2004
SPD-5	*spd-5* (F56A3.4)	YES		PCM component required for PCM assembly; required to recruit all tested PCM proteins; in SPD-5 depleted embryos centrosomal microtubule asters are absent and spindle assembly fails; depleted embryos also exhibit a severe defect in new centriole assembly, possibly due to failure to recruit gamma-tubulin to promote the formation of centriolar microtubules; a *spd-5* mutation interacts genetically with mutations in *spd-2* and dynein heavy chain.	Hamill, 2002; Kemp, 2004; Dammermann, 2004
AIR-1	*air-1* (K07C11.2)	YES	Aurora A	PCM component required for PCM assembly; localization appears more peripheral than that of gamma-tubulin, extending out	Schumacher, 1998; Hannak, 2001

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				along centrosomal microtubules; required to recruit additional PCM during centrosome maturation; in depleted embryos centrosomes remain small and spindle assembly fails.	
gamma-tubulin	*tbg-1* (F58A4.8)	YES	gamma-tubulin	Normal levels of SPD-5 are observed in depleted embryos indicating that it is not required for PCM formation; in depleted embryos, centrosomal microtubule asters fail to form during interphase, but robust asters assemble as embryos enter mitosis; although unknown mechanisms support partial assembly of mitotic centrosomal asters, the rate of centrosomal microtubule nucleation is severely compromised in depleted embryos indicating that the kinetically dominant pathway for the nucleation of centrosomal microtubules is gamma-tubulin dependent; depleted embryos also exhibit a severe defect in new centriole assembly.	Bobbinec, 2000; Strome, 2001; Hannak, 2002
CeGrip-1	*gip-1* (H04J21.3)	YES	GCP3	PCM component; orthologues form a heterotrimeric complex with gamma-tubulin in *Xenopus*, *Drosophila*, and *S. cerevisiae*; depletion prevents recruitment of gamma-tubulin to centrosomes; depletion phenotype is essentially identical to that observed in gamma tubulin depleted embryos.	Hannak, 2002
CeGrip-2	*gip-2* (C45G3.3)	YES	GCP2	PCM component; orthologues form a heterotrimeric complex with gamma-tubulin in *Xenopus*, *Drosophila*, and *S. cerevisiae*; depletion prevents recruitment of gamma-tubulin to centrosomes; depletion phenotype is essentially identical to that observed in gamma tubulin depleted embryos.	Hannak, 2002
ZYG-9	*zyg-9* (F22B5.7)	YES	XMAP-215	PCM component; physically interacts with TAC-1; this interaction is required for the efficient localization of the ZYG-9/TAC-1 complex to centrosomes; important regulator of microtubule dynamics;	Matthews, 1998; Le Bot, 2003; Srayko, 2003; Bellanger, 2003

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				depletion phenotype is similar to that resulting from treatment of embryos with low doses of nocodozole which destabilize microtubules, pronuclear migration fails and a short spindle forms in the embryo posterior.	
TAC-1	*tac-1* (Y54E2A.3)	YES	TACC-1, 2, 3	PCM component; physically interacts with ZYG-9; TAC-1 and ZYG-9 are dependent on each another for their localization to the centrosome. Depletion phenotype is similar to that resulting from treatment of embryos with low doses of nocodozole which destabilize microtubules, pronuclear migration fails and a short spindle forms in the embryo posterior.	Le Bot, 2003; Srayko, 2003; Bellanger, 2003

Several PCM proteins are required for the activity rather than the assembly of the PCM. The microtubule nucleating activity of the pericentriolar material requires the centrosomal tubulin isoform gamma-tubulin, and two gamma-tubulin associated proteins, CeGrip-1 and CeGrip-2. Embryos depleted of any of these proteins fail to form centrosomal microtubule asters during interphase (Hannak et al., 2002). As depleted embryos enter mitosis, relatively robust microtubule asters form around the centrosomes, suggesting that a gamma-tubulin independent pathway contributes to their assembly (Strome et al., 2001). However, chill and rewarm experiments reveal that the rate of centrosomal microtubule nucleation is highly compromised in gamma-tubulin depleted embryos and spindle assembly fails (Hannak et al., 2002; Strome et al., 2001). ZYG-9 and TAC-1 are two conserved PCM proteins that associate to form a complex that promotes microtubule growth. Embryos depleted of either protein exhibit defects in pronuclear migration, presumably because centrosomal microtubules are too short to capture the oocyte pronucleus, and small spindles form around the sperm chromatin in the embryo posterior (Bellanger and Gönczy, 2003; Le Bot et al., 2003; Matthews et al., 1998; Srayko et al., 2003).

Seven *C. elegans* proteins have been shown to contribute to new centriole assembly. Four of these, SAS-4 (Kirkham et al., 2003; Leidel and Gönczy, 2003), SAS-5 (Dammermann et al., 2004; Delattre et al., 2004; Schmutz and Spang, 2005), SAS-6 (Dammermann et al., 2004; Leidel et al., 2005), and the atypical protein kinase ZYG-1 (O'Connell et al., 2001), localize to centrioles and are specifically required for centriole formation. Depletion of any of these proteins by RNAi results in specific failure of centrosome duplication. In this characteristic phenotype (O'Connell et al., 2001), the two sperm centrioles separate after fertilization (as in wild-type) and organize the two centrosomes that form the poles of an apparently normal spindle during the first mitotic division. However, since daughter centrioles fail to form adjacent to each of the sperm centrioles, only one centriole instead of the normal two are released into each daughter cell when the centrosomes break down in telophase. Since each daughter cell contains only one centriole, it can form only one centrosome, and monopolar spindles are observed in both cells at the two-cell stage. SPD-2 is a bi-functional protein that localizes to centrioles as well as the PCM and is required for centriole assembly in addition to its role in PCM recruitment (Kemp et al., 2004; Pelletier et al., 2004). Embryos depleted of two pericentriolar material proteins, SPD-5 and gamma-tubulin, also exhibit severe defects in centriole assembly, leading to the idea that the PCM contributes to centriole assembly by recruiting gamma-tubulin, which may promote the assembly of the microtubules that make up the centriolar cylinders, as well as those nucleated by the PCM (Dammermann et al., 2004).

6. Formation of mitotic chromosomes

The formation of mitotic chromosomes begins when cohesin is loaded onto chromosomes and establishes cohesion between the duplicated chromosomes (sister chromatids) during DNA replication in S phase. Proteolytic cleavage of one of the cohesin subunits later in the cell cycle is thought to release the linkage between the sister chromatids to allow their segregation during anaphase (Haering and Nasmyth, 2003). The components of the *C. elegans* cohesin complex have been identified and depletion has revealed roles in mitotic chromosome segregation and the pairing of homologous chromosomes during meiosis (Table 3; Chan et al., 2003; Mito et al., 2003; Pasierbek et al., 2003).

Table 3. Mitotic chromosome proteins

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
HIM-1	*him-1*; *smc-1*; (F28B3.7)	YES	SMC-1	Component of the cohesin complex; concentrated in the nucleus during interphase; by the metaphase to anaphase transition levels in the vicinity of the chromatin are reduced and protein appears excluded from the compacted chromatin.	Chan, 2003
SMC-3	*smc-3*; (Y47D3A.26)	YES	SMC-3	Component of the cohesin complex; concentrated in the nucleus during interphase; by the metaphase to anaphase transition levels in the vicinity of the chromatin are reduced and protein appears excluded from the compacted chromatin.	Chan, 2003
SCC-1	*scc-1*; (F10G7.4)	YES	SCC-1	Component of the cohesin complex; concentrated in the nucleus during interphase; by the metaphase to anaphase transition levels in the vicinity of the chromatin are reduced and protein appears to be excluded from the compacted chromatin; depletion results in a defect in mitotic chromosome segregation; the severity of the chromosome segregation defects is enhanced by simultaneous RNAi of *him-1*.	Chan, 2003; Mito, 2003
SCC-3	*scc-3*; (F18E2.3)	YES	SCC-3	Component of the cohesin complex; concentrated in the nucleus during interphase; by the metaphase to anaphase transition levels in the vicinity of the chromatin are reduced and protein appears to be excluded from the compacted chromatin; depletion results in defects in chromosome segregation during mitosis and	Chan, 2003; Pasierbek, 2003; Moore, 2005

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				homologous pairing during meiosis.	
TIM-1	*tim-1*; *csg-5*; (Y75B8A.22)	YES	mTim1	Component of the cohesin complex; concentrated in the nucleus during interphase; by the metaphase to anaphase transition levels in the vicinity of the chromatin are reduced and protein appears excluded from the compacted chromatin; RNAi of *tim-1* in conjunction with *him-1* results in more severe defects in chromosome segregation than either alone; required to recruit non-SMC cohesin subunits to chromatin before or during pre-meiotic S phase and to stabilize homologous chromosome associations during synapsis and sister chromatid cohesion in diplotene/ diakinesis.	Chan, 2003
MIX-1	*mix-1*; (M106.1)	YES	SMC-2	Component of the condensin complex; required for mitotic chromosome condensation; chromosomal localization does not require kinetochore assembly; depletion results in severe chromatin bridging during anaphase; required for X-chromosome dosage compensation (repression).	Lieb, 1998; Hagstrom, 2002; Kaitna, 2002; Chan, 2004
SMC-4	*smc-4*; (F35G12.8)	YES	SMC-4	Component of the condensin complex; required for mitotic chromosome condensation; chromosomal localization does not require kinetochore assembly; depletion leads to severe chromatin bridging during anaphase.	Hagstrom, 2002
HCP-6	*hcp-6*; (Y110A7A.1)	YES	CAP-D3, Heat (IIA)	Component of the condensin complex; required for mitotic chromosome condensation; chromosomal localization requires kinetochore assembly (fails to localize in CeCENP-C RNAi); required for mitotic chromosome condensation; depletion leads to severe chromatin bridging during anaphase.	Stear and Roth, 2002; Chan, 2004
	F55C5.4	YES	CAP-G2; Heat (IIB)	Predicted component of the condensin complex based on sequence homology; no published localization or detailed depletion	Ono, 2003

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				data.	
KLE-2	C29E4.2	YES	CAP-H2; Kleisin γ (IIC)	Component of the condensin complex; required for mitotic chromosome condensation; depletion leads to severe chromatin bridging during anaphase.	Schleiffer, 2003
AIR-2	*air-2*; *let-603*; *stu-7*; *cyk-6*; (B0207.4)	YES	Aurora B	Aurora/Ip11- related serine/threonine protein kinase; chromosomal passenger protein, localizes to chromosomes between prophase and telophase and to microtubule bundles in the spindle midzone between anaphase and telophase; forms a complex with BIR-1, ICP-1, and CSC-1 and requires all three to localize to chromosomes; required for histone H3 phosphorylation, chromosome alignment and segregation, formation of the spindle midzone and cytokinesis.	Schumacher, 1998; Hsu et al., 2000; Kaitna, 2000; Severson, 2000; Oegema, 2001; Rogers 2002; Kaitna, 2002; Romano, 2003
ICP-1, CeINCENP	*icp-1*; (Y39G10A R.13)	YES	INCENP	Chromosomal passenger protein, localizes to chromosomes between prophase and telophase and microtubule bundles in the spindle midzone between anaphase and telophase; required for chromosome alignment and segregation, formation of the spindle midzone and cytokinesis; chromosomal localization requires BIR-1 and CSC-1, but not AIR-2. Kinetochore assembly does not require ICP-1 and inhibiting kinetochore assembly by depletion of CENP-A (HCP-3) does not block the recruitment of ICP-1 to chromosomes.	Kaitna, 2000; Kaitna, 2002; Oegema, 2001; Romano, 2003
BIR-1	*bir-1*; (T27F2.3)	YES	Survivin	Chromosomal passenger protein, localizes to chromosomes between prophase and telophase and microtubule bundles in the spindle midzone between anaphase and telophase; chromosomal localization requires CSC-1 and ICP-1, but not AIR-2; required for chromosome alignment and segregation, formation of the spindle midzone and cytokinesis.	Fraser, 1999; Kaitna, 2000; Speliotes, 2000; Oegema, 2001; Romano, 2003
KLP-19	*klp-19*	YES	kinesin-4; Kif4	Localizes to the chromatin between the kinetochores; depletion results in aberrant	Powers, 2004

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
				chromosome motions during prometaphase, chromosome misalignment and the formation of multiple chromatin bridges during anaphase; thought to reduce the frequency with which individual diffuse kinetochores become attached to microtubules emanating from both spindle poles (merotelic attachment) by interacting with microtubules to stabilize an orientation in which the two kinetochores directly face the spindle poles.	

Two additional protein complexes, condensin and the chromosomal passenger complex, also have critical roles in the formation and segregation of mitotic chromosomes. In contrast to vertebrates, which have two complexes, condensins I and II, that mediate spatially distinct aspects of condensation (Hirano, 2004), *C. elegans* has only condensin II (Table 3, Hagstrom et al., 2002; Ono et al., 2003). Chromosome condensation is delayed in condensin depleted embryos, but DNA compaction is ultimately achieved, indicating the existence of condensin-independent mechanisms that can compact mitotic chromatin. However, sister chromatid pairs formed in the absence of condensin are structurally defective and cannot be efficiently separated from each other by the mitotic spindle (Hagstrom et al., 2002; Kaitna et al., 2002; Chan et al., 2004). Concurrent with, but largely independent of condensation, kinetochores assemble to create chromosomal attachment sites for spindle microtubules (described in greater detail below). Like condensin, the chromosomal passenger protein complex (including the aurora B kinase, AIR-2, BIR-1, ICP-1 and CSC-1) is recruited to mitotic chromosomes as they form and is required for their proper segregation, (Schumacher et al., 1998; Severson et al., 2000; Kaitna, 2000; Oegema, 2001; Kaitna et al., 2002; Rogers 2002; Romano 2004; Hsu et al., 2000). Although it does not appear to be required for kinetochore assembly in *C. elegans*, the passenger complex plays important roles in chromosome structure and likely functions to correct aberrant kinetochore-microtubule attachments (reviewed in Vagnarelli and Earnshaw, 2004).

7. Kinetochore assembly

Eukaryotes can be divided into two groups based on the architecture of their mitotic chromosomes. Monocentric organisms assemble kinetochores on a single localized chromosomal site defined by the presence of dedicated centromeric chromatin. In contrast, holocentric organisms, including *C. elegans*, assemble diffuse kinetochores along the entire poleward face of each sister chromatid (Figure 6A). Holocentric chromosome architecture is present in widely divergent and highly successful metazoan lineages (including nematodes, hemipteran insects, and lower plants) that constitute a large part of the earth's biomass, and *C. elegans* has emerged as an important model system for studying holocentric chromosome architecture (reviewed in Dernburg, 2001; Maddox et al., 2004).

Despite differences in the extent of the chromosomal length occupied by the kinetochore, the molecular composition of kinetochores in *C. elegans* and monocentric organisms is very similar (Table 4). Importantly, mitotic kinetochores in both monocentric and holocentric organisms assemble on a base of specialized centromeric chromatin defined by the presence of nucleosomes containing the histone H3 variant CENP-A (Buchwitz et al., 1999; Sullivan, 2001). Depletion of the *C. elegans* homolog of CENP-A leads to a characteristic "kinetochore-null" phenotype in which chromosomes fail to distribute over the spindle equator and to segregate (Oegema et al., 2001). This defect results from the failure to assemble kinetochores that can interact with spindle microtubules. To date only 4 proteins have been identified whose depletion gives a kinetochore-null defect: CENP-A^{HCP-3}, CENP-C^{HCP-4}, KNL-3 and KNL-1, which can be placed in a linear assembly hierarchy (Figure 6B) with CENP-A^{HCP-3} at the top (Moore and Roth, 2001; Oegema et al., 2001; Desai et al., 2003; Cheeseman et al., 2004). KNL-3 and KNL-1 are components of a 10-protein complex that is critical to forming the outer domains of the kinetochore that interact

with spindle microtubules (Figure 6B; Cheeseman et al., 2004; Desai et al., 2003). A number of other kinetochore proteins have been identified whose depletion results in less severe chromosome segregation defects (for examples see Howe et al., 2001; Moore et al., 1999; for a detailed summary see Table 4). The reproducible phenotypes obtained following protein depletion have been useful in grouping proteins that function together in the context of the kinetochore (Figure 6B; Cheeseman et al., 2004).

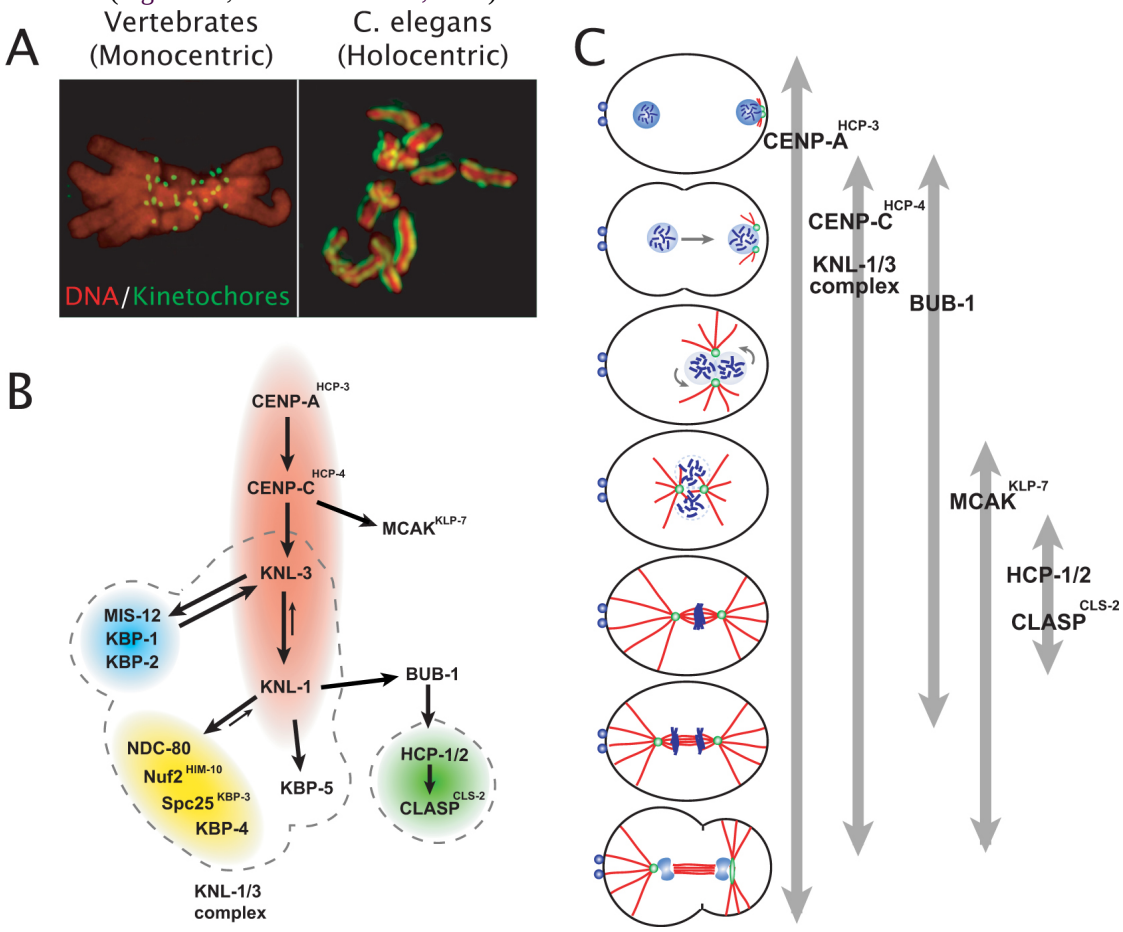

Figure 6. Mitotic kinetochores in the *C. elegans* embryo. (A) Images comparing the localized kinetochores in a vertebrate tissue culture cell with monocentric chromosomes to the diffuse kinetochores in a *C. elegans* embryo with holocentric chromosomes. (B) Schematic illustrating the hierarchy for mitotic kinetochore assembly based on pair-wise depletion and localization assays, phenotypic analysis, and biochemical purifications. Dotted lines indicate groups of proteins that have been shown to co-purify from *C. elegans* extracts in immunoprecipitations and tagged protein isolations. The colored ovals group proteins together whose individual depletions result in a similar phenotype. (Red) "Kinetochore Null"/KNL proteins whose depletion is characterized by a failure to assemble kinetochores that are competent to form spindle microtubule attachments. Consequently, segregation is severely defective and spindle poles separate prematurely. (Blue) "MIS" proteins whose depletion results in relatively subtle chromosome segregation defects. In MIS embryos, kinetochore assembly occurs but at a slower rate and to a reduced extent relative to wild-type. (Yellow) "NDC" proteins whose depletion results in a chromosome alignment and segregation defects of intermediate severity, relative to the KNL and MIS classes. In NDC embryos, attachments that can sustain tension fail to form. Consequently, spindle poles separate prematurely. (Green) HCP/CLASP proteins, whose depletion causes sister chromatids to co-segregate to the same spindle pole. This defect likely arises from an inability to polymerize microtubules at kinetochores. (C) Schematic illustrating the temporal window during the first mitotic division when each of the indicated proteins localizes to kinetochores. Figure and images courtesy of Susan Kline-Smith and Arshad Desai, respectively.

Table 4. Kinetochore proteins

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
CeCENP-A, HCP-3	*hcp-3*; (F58A4.3)	YES	CENP-A	Histone H3-variant; thought to localize to chromatin throughout the cell cycle; found in nuclear foci during interphase and concentrated	Buchwitz, 1999; Oegema, 2001

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
				on the chromatin in a stripe that runs along the poleward face of each sister chromatid during mitosis; CeCENP-A is required for the localization of all other kinetochore components that have been tested; depletion results in failure to align and segregate mitotic chromosomes and premature spindle elongation due to the inability of the kinetochores to attach to spindle microtubules; a highly homologous gene (F54C8.2) is likely also targeted by dsRNAs generated against CeCENP-A. The relative abundance of CeCENP-A versus F54C8.2 is not known. *C. briggsae* does not have 2 genes encoding CENP-A like proteins, suggesting that F54C8.2 may have arisen from a recent duplication.	
CeCENP-C, HCP-4	*hcp-4*; (T03F1.9)	YES	CENP-C	Kinetochore localization (prophase to telophase) requires CeCENP-A; CeCENP-C is required for the localization of all kinetochore proteins except CeCENP-A; associates with the KNL-1/3 complex and may act as an adaptor to connect centromeric chromatin to the outer kinetochore; plays a role in the resolution of CeCENP-A chromatin into two paired “lines” on the replicated chromosome; depletion results in failure to align and segregate mitotic chromosomes and premature spindle elongation.	Moore, 2001; Oegema, 2001; Desai, 2003; Cheeseman, 2004
KNL-3	*knl-3* (T10B5.6)	YES	?	Kinetochore localization (prophase to telophase) requires CeCENP-A and CeCENP-C; KNL-3 levels at the kinetochore are reduced in embryos depleted of MIS-12, KBP-1, KBP-2 and KNL-1; depletion results in failure to align and segregate mitotic chromosomes and premature spindle elongation; component of the 10-protein KNL-1/3 complex required to assemble an outer kinetochore that can make microtubule attachments.	Cheeseman, 2004
KNL-1	*knl-1*	YES	AF15q14	Kinetochore localization (prophase	Desai, 2003

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
	(C02F5.1)			to telophase) requires CeCENP-A, CeCENP-C and KNL-3; KNL-1 levels at the kinetochores are reduced in embryos depleted of NDC-80 and HIM-10; depletion results in failure to align and segregate mitotic chromosomes and premature spindle elongation. Component of the 10-protein KNL-1/3 complex required to assemble an outer kinetochore that can make microtubule attachments.	Cheeseman, 2004
MIS-12	*mis-12* (Y47G6A.24)	YES	Mis12	Kinetochore localization (prophase to telophase) requires KNL-3 but not KNL-1; relatively weak chromosome missegregation phenotype seen in depleted embryos consistent with a delay in the formation of chromosome-spindle attachments; identified by sequence homology to human and fission yeast Mis12 and as a component of the KNL-1/3 complex.	Cheeseman, 2004
KBP-1	*kbp-1* (R13F6.1)	YES	?	Localizes to kinetochores from prophase to telophase; relatively weak chromosome missegregation phenotype seen in depleted embryos consistent with a delay in the formation of chromosome-spindle attachments; identified as a component of the KNL-1/3 complex.	Cheeseman, 2004
KBP-2	*kbp-2* (F26F4.13)	YES	?	Localizes to kinetochores from prophase to telophase; relatively weak chromosome missegregation phenotype seen in depleted embryos consistent with a delay in the formation of chromosome-spindle attachments; identified as a component of the KNL-1/3 complex.	Cheeseman, 2004
NDC-80	*ndc-80* (W01B6.9.1)	YES	Ndc80/ HEC	Localizes to kinetochores from prophase to telophase; kinetochore localization requires KNL-1 and HIM-10 in addition to chromatin-proximal components; depletion results in chromosome missegregation and premature spindle elongation; phenotype is less severe than a kinetochore-null but more severe than that of	Desai, 2003 Cheeseman, 2004

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				MIS-12, suggests defect in the ability to form microtubule attachments that can withstand tension; member of the NDC-80 subcomplex, which is part of the larger KNL-1/3 complex.	
HIM-10 Nuf2^{HIM-10}	*him-10* (R12B2.4)	YES	Nuf2	Localizes to kinetochores from prophase to telophase; kinetochore localization requires KNL-1 and NDC-80, in addition to chromatin-proximal components. Depletion disrupts kinetochore ultrastructure, resulting in chromosome missegregation and premature spindle elongation; phenotype is less severe than a kinetochore-null but more severe than that of MIS-12, suggests defect in the ability to form microtubule attachments that can withstand tension; member of the NDC-80 subcomplex, which is part of the larger KNL-1/3 complex.	Howe, 2001 Desai, 2003 Cheeseman, 2004
KBP-3	*kbp-3* (F26H11.1)	YES	Spc25	Localizes to kinetochores from prophase to telophase; depletion results in chromosome missegregation and premature spindle elongation; phenotype is less severe than a kinetochore-null but more severe than that of MIS-12, suggests defect in the ability to form microtubule attachments that can withstand tension; member of the NDC-80 subcomplex, which is part of the larger KNL-1/3 complex.	Cheeseman, 2004
KBP-4	*kbp-4* (Y92C3B.1)	YES	?	Localizes to kinetochores from prophase to telophase. Depletion results in chromosome missegregation and premature spindle elongation; phenotype is less severe than a kinetochore-null but more severe than that of MIS-12, suggests defect in the ability to form microtubule attachments that can withstand tension; identified as a KNL-1/3-associated protein.	Cheeseman, 2004
KBP-5	*kbp-5* (C34B2.2)	NO	?	Localizes to kinetochores from prophase to telophase. Identified as a KNL-1/3-associated protein.	Cheeseman, 2004
HCP-1	*hcp-1*	YES*	CENP-F?	Functionally redundant proteins	Moore, 1999; Desai,

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
HCP-2	(ZK1055.1) *hcp-2* (T06E4.1)	*When co-depleted		that localize to the region of the mitotic spindle and to kinetochores between late prometaphase and early anaphase; kinetochore localization requires CeCENP-A/C, KNL-1, and BUB-1, but not the NDC-80 subcomplex; depletion perturbs chromosome alignment and segregation. HCP-1 and 2 physically associate with CLASP^{CLS-2}.	2003; Stear, 2004; Encalada, 2004; Cheeseman, 2005
CLASP^{cls-2} (CLS-2)	*cls-2* (R107.6)	YES	CLASP	Microtubule- associated protein that localizes to spindle poles, the region of the miotic spindle and kinetochores; prominent kinetochore localization is seen at metaphase. Kinetochore localization requires KNL-1 and HCP-1/2, but not the NDC-80 subcomplex. Depletion perturbs chromosome alignment and segregation. CLASP^{CLS-2} associates with HCP-1/2.	Desai, 2003; Cheeseman, 2005
CeMCAK (KLP-7)	*klp-7* (K11D9.1)	YES	MCAK	Kinesin-13 microtubule depolymerase that localizes to kinetochores between early prometaphase and telophase, and to spindle poles throughout mitosis; kinetochore localization requires CeCENP-A and CeCENP-C, but not KNL-1 or KNL-3; depletion results in snapping of the anaphase spindle, suggesting excessive astral pulling forces or a defect in spindle midzone formation/ stability.	Oegema, 2001; Grill, 2001; Desai, 2003; Powers, 2004
LIS-1	*lis-1* (T03F6.5)	YES	LIS1	Microtubule associated protein that localizes to the cell cortex, nuclear periphery, kinetochores, and microtubule asters; kinetochore localization requires CeCENP-C, but not dynein; depletion results in multiple defects in spindle positioning, pronuclear migration, and centrosome separation, leading to gross missegregation of chromosomes.	Cockell, 2004
CZW-1	*czw-1* (F20D12.4)	YES	Zw10	Predicted kinetochore protein based on sequence homology; partial depletion results in chromosome missegregation;	Starr, 1998

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				penetrant depletion results in sterility	
ROD-1	*rod-1* (F55G1.4)	YES	Rod	Predicted kinetochore protein based on sequence homology; depletion results in chromosome missegregation.	Scaerou, 2001
BUB-1	*bub-1* (R06C7.8)	YES	Bub1	Mitotic checkpoint pathway serine/threonine protein kinase; kinetochore localization (prophase to metaphase) requires CeCENP-A, CeCENP-C, and KNL-1; depletion results in misalignment and missegregation of chromosomes.	Oegema, 2001; Desai, 2003; Encalada, 2004
BUB-3	Y54G9A.6	NO	Bub3	Predicted mitotic checkpoint pathway protein based on sequence homology.	
MDF-1	*mdf-1* (C50F4.11)	NO	Mad1	Mitotic checkpoint pathway protein based on sequence homology; mutant worms fail to thrive due to accumulated chromosomal abnormalities; required to delay the embryonic cell cycle in response to spindle defects.	Kitagawa, 1999; Encalada, 2004
MDF-2	*mdf-2* (Y69A2AR.30)	NO	Mad2	Mitotic checkpoint pathway protein with similar functions as MDF-1; depletion of MDF-2 also overrides the mitotic arrest induced by anoxia, suggesting that survival under very low oxygen levels is promoted by activation of the mitotic checkpoint.	Kitagawa, 1999; Nystul, 2003; Encalada, 2004
SAN-1	*san-1* (ZC328.4)	NO	BubR1	Mitotic checkpoint pathway protein that localizes to kinetochores after nuclear envelope breakdown. SAN-1 is the homologue of budding yeast MAD3 protein (their vertebrate orthologue, BubR1, has a serine/threonine kinase domain fused to the Mad3 homology region). SAN-1 was identified in a genetic screen for anoxia-sensitive mutants, highlighting a link between survival under very low oxygen levels and mitotic arrest mediated by the mitotic checkpoint pathway.	Nystul, 2004

In addition to its role in directing kinetochore assembly, CENP-C has also been implicated in sister kinetochore resolution, the process by which the kinetochores on the two sister chromatids resolve from one another, coming to rest on opposite sides of the mitotic chromosome (Moore and Roth, 2001). Defects in sister kinetochore resolution in CENP-C depleted embryos can be suppressed by RNAi or mutation of cohesin subunits suggesting that resolution of the sister kinetochores during the assembly of mitotic chromosomes is normally facilitated by loss of cohesion between the sister centromeres (Moore et al., 2005). In addition to kinetochore-localized proteins, the chromokinesin KLP-19, which localizes to the chromatin between the diffuse kinetochores, is also critical for chromosome segregation. Interactions between KLP-19 and spindle microtubules are postulated to generate a pushing force that rotates chromosomes to orient the sister kinetochores to face opposite spindle poles (Table 3; Powers et al., 2004), thereby reducing the possibility that a single kinetochore will become incorrectly attached to microtubules coming from both spindle poles.

8. Assembly of the mitotic spindle

In vertebrates and *Drosophila*, spindle assembly results from the superposition of two distinct mechanisms: (1) an inside out chromatin-based mechanism in which Ran-GTP produced in proximity to chromatin activates factors that promote the assembly and self-organization of microtubules to form a bipolar spindle, and (2) an outside in mechanism in which centrosomal microtubule asters reinforce spindle bipolarity and position the spindle within the cell (Gadde and Heald, 2004). In contrast, *C. elegans* appears to lack a significant inside out pathway and primarily utilizes an outside in centrosome-based mechanism to form its spindles. Consistent with this idea, Eg5, a mitotic kinesin that promotes anti-parallel microtubule sliding and plays an essential role in the chromatin-based pathway in vertebrates and *Drosophila*, is not required for spindle assembly in *C. elegans* (Bishop et al., 2005). Conversely, whereas centrosomes are not essential for spindle assembly in *Drosophila* or vertebrates (Khodjakov et al., 2000; Megraw et al., 1999; Rebollo et al., 2004; Vaizel-Ohayon and Schejter, 1999), spindles fail to form in *C. elegans* embryos that lack functional centrosomes (Hamill et al., 2002; Kemp et al., 2004; Pelletier et al., 2004) and the majority of characterized *C. elegans* proteins that are required for spindle assembly (Sõnnichsen et al., 2005) are known centrosome components. Interestingly, essentially perfect “half spindles” form in embryos that have a single centrosome instead of the normal two (Kirkham et al., 2003; O'Connell et al., 2001), indicating that the mechanisms that regulate spindle length in the *C. elegans* embryo are largely independent of bipolarity. Experiments in which the size of mitotic centrosomes is modulated by varying the levels of centriole components have also revealed an interesting positive correlation between half-spindle length and centrosome size (Delattre et al., 2004; Kirkham et al., 2003).

Concomitant with nuclear envelope break down, centrosomal microtubules penetrate the nuclear space and begin to interact with chromosomes. Spindle assembly is completed in the subsequent ~2.5 minute period between nuclear envelope breakdown and anaphase onset (see Figure 2). Tomographic reconstruction of a *C.elegans* mitotic spindle from electron micrographs indicates that *C. elegans* mitotic spindles consist primarily of microtubules that connect centrosomes to kinetochores. These kinetochore microtubules do not form bundles that resemble the kinetochore fibers present in vertebrate and *Drosophila* cells, probably because of the holocentric nature of *C. elegans* chromosomes (O'Toole et al., 2003). Assembly of a stable mitotic spindle requires kinetochores that can form stable bipolar microtubule attachments. In the absence of functional kinetochores, the two spindle poles are abruptly pulled apart at a time that corresponds to the onset of cortical pulling forces on the two centrosomes that normally asymmetrically position the spindle within the embryo (Oegema et al., 2001). The abrupt separation of the spindle poles in the absence of functional kinetochores is similar to that brought about by severing the spindle with a UV microbeam (Grill et al., 2001), confirming the critical role of kinetochores in maintaining spindle integrity.

9. Chromosome segregation

Chromosome segregation typically consists of two components: (1) anaphase A, in which the chromosomes move towards the spindle poles and, (2) anaphase B in which the spindle poles separate from each other with the chromosomes in tow. In *C. elegans* the majority of chromosome movement is due to anaphase B (Oegema et al., 2001), powered by a combination of cortical forces that pull on the centrosomal microtubule asters to separate them, and pushing forces generated by the array of overlapping microtubules, called the central spindle, that forms between the separating chromosomes. If the mitotic spindle is severed during anaphase using a UV micro beam, the two spindle poles separate with increased velocity (Grill et al., 2001). This experiment demonstrates the existence of pulling forces during the first mitotic division and suggests that the central spindle normally acts to limit the rate of pole separation. However, in embryos in which G protein signaling that mediates cortical pulling forces is disrupted,

spindles still elongate (Colombo et al., 2003) albeit at a slower rate, indicating that other spindle intrinsic forces contribute to anaphase spindle elongation.

10. Cytokinesis

During cytokinesis, signals from the anaphase spindle trigger the assembly of an equatorial cortical contractile ring enriched in actin and myosin II. Constriction of the contractile ring changes the shape of the cell to facilitate cell division (reviewed in Glotzer, 2005). The components of the microtubule and actin cytoskeletons that are known to contribute to cytokinesis in the *C. elegans* embryo can be partitioned into three classes (Table 5). The first class consists of proteins required for cortical acto-myosin contractility (Figure 7). Embryos depleted of proteins in this class have defects in all cortical contractile events including: polar body extrusion, anterior cortical ruffling, pseudocleavage, and the initiation of a furrow during cytokinesis. This class includes the small GTPase RHO-1 (Jantsch-Plunger et al., 2000) and its activating GEF (LET-21; Dechant and Glotzer, 2003), which are thought to be at the top of the signaling cascade that regulates cortical contractility (Glotzer, 2005). Downstream are proteins that regulate the assembly of the contractile ring and its constriction in response to activated Rho. CYK-1 (Severson et al., 2002; Swan et al., 1998), a member of the formin family of proteins, is a Rho effector that promotes the assembly of actin filaments. The assembly and contractility of non-muscle myosin II (NMY-2; Guo and Kemphues, 1996), the motor that powers acto-myosin contractility, is regulated by phosphorylation of its regulatory light chain, MLC-4 (Shelton et al., 1999). Phosphorylation of MLC-4 activates myosin II, allowing it to form bi-polar filaments that can interact with actin to generate cortical ingression (Citi and Kendrick-Jones, 1987). One of the Rho effectors that regulates MLC-4 phosphorylation during cytokinesis is the Rho kinase homolog LET-502 (for details see Table 5; Piekny and Mains, 2002).

Table 5. Cytoskeletal proteins required for cytokinesis

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
NMY-2		YES	non-muscle myosin II	Localizes to the contractile ring during cytokinesis as well as to other cortical contractile structures; motor that powers contraction by moving towards the barbed end of actin filaments; all cortical contractile events fail in depleted embryos including polar body formation, ruffling, and cleavage furrow ingression. NMY-2 is also required for the establishment of polarity.	Guo, 1996; Munro, 2004
MLC-4	*mlc-4* (C56G7.1)	YES	non-muscle myosin II regulatory light chain	Regulates the ability of myosin II to form filaments and interact with actin; localizes to the cortical contractile ring during cytokinesis as well as to other cortical contractile structures; activated by phosphorylation; all cortical contractile events fail in depleted embryos including polar body formation, ruffling, and cleavage furrow ingression; MLC-4 is also required for the establishment of polarity.	Shelton, 1999
LET-502	*let-502* (C10H11.9)	YES	Rho-binding kinase (ROK)	Rho-binding serine/threonine kinase that localizes to the contractile ring; promotes myosin	Piekny, 2002

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				II contractility by increasing the phosphorylation of MLC-4; based on work in other systems, it is thought to do this by both directly phosphorylating MLC-4 and also by phosphorylating and inactivating myosin phosphatase; inhibition of Rho-kinase slows the rate of furrow ingression but does not prevent furrow assembly or ingression, suggesting the existence of redundant MLC-4 kinases; failed cell divisions occur apparently at random in *let-502* embryos, with many divisions being normal.	
MEL-11	*mel-11* (C06C3.1)	YES	Myosin phosphatase targeting subunit (MYPT)	Regulatory subunit of myosin phosphatase; inhibits cortical contraction by de-phosphory-lating the regulatory light chain of myosin II; mutations in *mel-11* result in ectopic furrowing and faster furrow ingression; LET-502 and MEL-11 co-localize in cleavage furrows and their mutations alleviate one another's effects.	Piekny, 2002
RHO-1	*rho-1* (Y51H4A.3)	YES	RhoA	Small GTPase thought to connect signaling by the anaphase spindle to assembly and ingression of a cortical contractile ring; all cortical contractile events fail in depleted embryos including polar body formation, ruffling, and cleavage furrow ingression; has not been localized in *C. elegans* but active Rho has been localized to the contractile ring in other systems.	Jantsch-Plunger, 2000
LET-21	*let-21* (T19E10.1)	YES	Ect2	Guanine nucleotide exchange factor that activates RHO-1; all cortical contractile events fail in depleted embryos including polar body formation, ruffling, and cleavage furrow ingression; has not been localized in *C. elegans*, but in other systems has been localized to microtubule bundles in the central spindle and to the contractile ring.	Dechant, 2003
CYK-1	*cyk-1* (F11H8.4)	YES	formins	A member of the formin family of proteins thought to promote actin assembly in response to activation	Swan, 1998; Severson, 2002

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
				of Rho family GTPases; localizes to the cleavage furrow and is required to initiate furrow ingression.	
PFN-1	*pfn-1* (Y18D10A.20)	YES	profillin	One of three *C. elegans* homologs of the actin binding protein profilin; the only *C. elegans* profilin that is required in the early embryo; depleted embryos exhibit defects in furrow ingression and the establishment of polarity; thought to function together with CYK-1 to promote actin assembly in response to activation by Rho family GTPases.	Severson, 2002
ANI-1	*ani-1* (Y49E10.19)	YES	Anillin	One of three *C. elegans* homologs of the actin-binding protein anillin; the only anillin homolog that appears to have a role in cortical events in the early embryo; localizes to the contractile ring, as well as to other cortical contractile structures, and is required for the septins to concentrate in the furrow; not essential for embryonic cytokinesis, but required for polar body extrusion, cortical ruffling, and pseudocleavage.	Maddox, 2005
UNC-59; UNC-61	*unc-59* (W09C5.2); *unc-61* (Y50E8A.4)	NO	septins	The only *C. elegans* homologs of the septins, small GTPases that form heteromeric complexes that polymerize to form filaments; UNC-59 and UNC-61 are interdependent for their localization to the cortex and for their localization to the contractile ring; require ANI-1 to become enriched in the contractile ring, but are not required for the targeting of ANI-1; neither is essential for embryonic cytokinesis.	Nguyen, 2000; Maddox, 2005
UNC-60A	*unc-60* (C38C3.5)	YES	cofilin	Promotes actin turnover; concentrates in the cleavage furrow during cytokinesis; depleted embryos frequently exhibit cytokinesis defects; the unc-60 gene generates two ADF/cofilins, UNC-60A and UNC-60B, by alternative splicing that have different functions. UNC-60B is expressed not it embryos, but in somatic/adult	Ono, 2003

***C. elegans* protein**	***C. elegans* gene**	**Required for embryonic viability?**	**Vertebrate orthologue**	**Summary of localization and functional analysis**	**Selected references**
				tissues.	
ZEN-4	*zen-4* (M03D4.1)	YES	kinesin-6 family member MKLP1	Kinesin that physically associates with CYK-4, a GAP for Rho family GTPases, to form a two protein complex called Centralspindlin; localizes to microtubule bundles in the spindle midzone and midbody; required for assembly of the spindle midzone and for the completion of cytokinesis, but not to form a furrow or initiate ingression.	Raich, 1998; Powers, 1998; Severson, 2000; Mishima, 2002
CYK-4	*cyk-4* (K08E3.6)	YES	MgcRacGAP	A GTPase activating protein (GAP), an inhibitor for Rho family GTPases; associates with ZEN-4 to form a protein complex called Centralspindlin; localizes to microtubule bundles in the central spindle and midbody; required for assembly of the central spindle and for the completion of cytokinesis, but not to form a furrow or initiate ingression.	Jantsch-Plunger, 2000; Mishima, 2002
AIR-2	*air-2 let-603 stu-7 cyk-6* (B0207.4)	YES	Aurora B	Mitotic serine/threonine kinase; component of a four-protein chromosomal passenger protein complex that localizes to chromosomes during mitosis and to microtubule bundles in the central spindle and midbody during anaphase/ telophase; required for assembly of the central spindle/midbody and for the completion of cytokinesis, but not to form a furrow or initiate ingression; also required for chromosome segregation (see Table 3).	Schumacher, 1998; Kaitna, 2000; Severson, 2000
ICP-1 CeINCENP	*icp-1* (Y39G10AR.13)	YES	INCENP	Component of a four-protein chromosomal passenger protein complex that localizes to chromosomes during mitosis and to microtubule bundles in the central spindle and midbody during anaphase/ telophase; phosphorylated by AIR-2 and stimulates AIR-2 kinase activity; required for assembly of the central spindle/midbody and for the completion of cytokinesis, but not to form a furrow or initiate ingression; also required for	Kaitna, 2000; Oegema, 2001; Bishop, 2002; Romano, 2004

C. elegans protein	*C. elegans* gene	Required for embryonic viability?	Vertebrate orthologue	Summary of localization and functional analysis	Selected references
				chromosome segregation (see Table 3).	
BIR-1	*bir-1* (T27F2.3)	YES	Survivin	Component of a four-protein chromosomal passenger protein complex that localizes to chromosomes during mitosis and to microtubule bundles in the central spindle and midbody during anaphase/ telophase; required for assembly of the central spindle/midbody and for the completion of cytokinesis, but not to form a furrow or initiate ingression; also required for chromosome segregation (see Table 3).	Fraser, 1999; Kaitna, 2000; Speliotes, 2000; Romano, 2004
CSC-1	*csc-1* (Y48E1B.12)	YES	Borealin, Dasra A/B	Component of a four-protein chromosomal passenger protein complex that localizes to chromosomes during mitosis and to microtubule bundles in the central spindle and midbody during anaphase/ telophase; required for assembly of the central spindle/midbody and for the completion of cytokinesis, but not to form a furrow or initiate ingression; also required for chromosome segregation (see Table 3).	Romano, 2004
SPD-1	*spd-1* (Y34D9A.4)	YES	PRC1	Localizes to microtubule bundles in the spindle midzone and midbody and to nuclei, as well as to short segments along the length of astral microtubules; required to form microtubule bundles in the central spindle; not required for the cytokinesis up to the four-cell stage, but cytokinesis often fails in EMS in depleted embryos.	Verbrugghe, 2004

A second group of proteins is required for the formation of the spindle midzone, an array of microtubule bundles that forms between the separating chromosomes during anaphase (Glotzer, 2005). As cytokinesis progresses, the microtubule bundles in the central spindle compact to form a single structure called the midbody. The cleavage furrow constricts around the midbody, which is thought to promote membrane fusion to complete cytokinesis and generate the two topologically distinct daughter cells (reviewed in Glotzer, 2005). During cytokinesis in embryos depleted of proteins required for central spindle assembly, a furrow initiates between the two asters, but cytokinesis fails to complete because the midbody is absent, and the furrow ultimately regresses. Depleted embryos also have defects in polar body extrusion (highly asymmetric cytokineses that bisect the midbodies of the small meiosis I and II spindles), but are able to form cortical ruffles that concentrate in the embryo anterior during polarity establishment and a pseudocleavage furrow. The microtubule bundles in the spindle midzone are also thought to function early in cytokinesis to signal to the cortex to promote assembly of the

contractile ring. Disruption of the spindle midzone does not prevent furrow formation because this midzone-based signal is redundant with signals from the microtubule asters. In embryos in which the astral signaling mechanism is blocked, the spindle midzone becomes essential for furrow ingression (Dechant and Glotzer, 2003). Proteins required to form the central spindle include: (1) a two protein complex called centralspindlin that includes the kinesin ZEN-4 and CYK-4, a GAP for Rho family GTPases (Jantsch-Plunger et al., 2000; Mishima et al., 2002; Powers et al., 1998; Raich et al., 1998; Severson et al., 2000), and (2) the 4 protein chromosomal passenger protein complex containing the *C. elegans* homolog of the aurora B kinase, AIR-2 (Bishop and Schumacher, 2002; Fraser et al., 1999; Kaitna et al., 2000; Oegema et al., 2001; Romano et al., 2003; Schumacher et al., 1998; Speliotes et al., 2000).

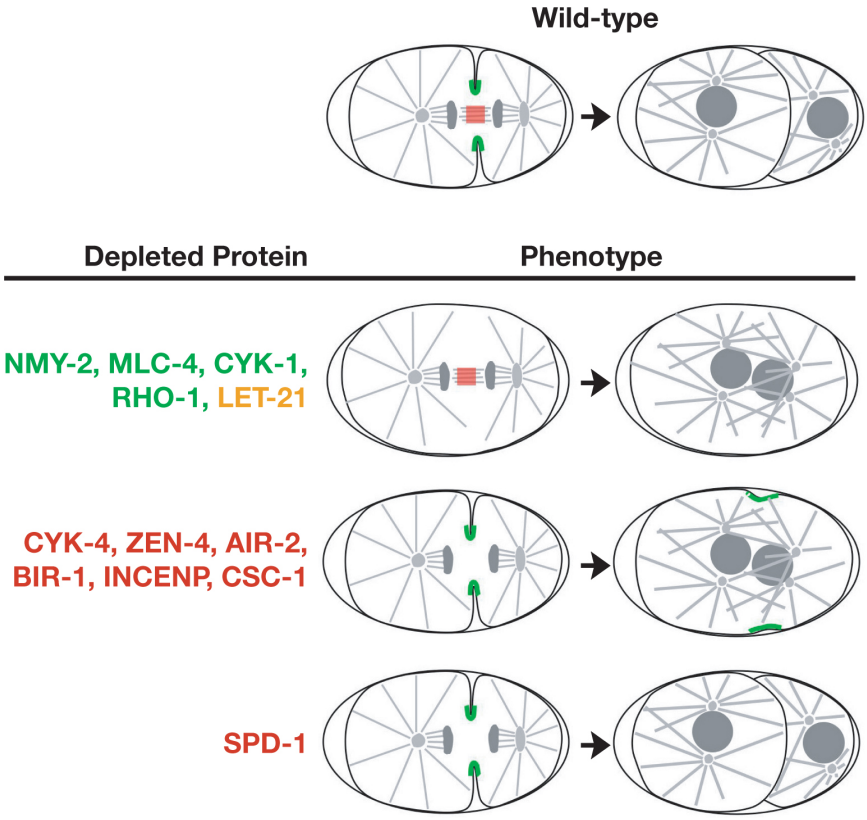

Figure 7. Proteins required for cytokinesis can grouped based on their depletion phenotypes. Proteins that localize to the cortical contractile ring in *C. elegans* are shown in green. Rho has not been localized in *C. elegans* but activated Rho localizes to the furrow in other systems. Proteins that localize to the spindle midzone/midbody in *C. elegans* are shown in red. LET-21 (yellow) has not been localized in *C. elegans*, but is reported to localize to both the contractile ring and the spindle midzone/midbody in other systems. In embryos depleted of LET-21 or the contractile ring components NMY-2, MLC-4, CYK-1 or RHO-1, a normal spindle midzone forms between the separating chromosomes but furrow formation is inhibited and ingression often fails. In embryos depleted of the spindle midzone/midbody components CYK-4 and ZEN-4 or the chromosomal passenger proteins AIR-2, BIR-1, INCENP and CSC-1, a normal spindle midzone fails to form. Cleavage furrows form and ingress, but cytokinesis fails to complete and the furrow ultimately regresses. In embryos depleted of the spindle midzone/midbody component, SPD-1, the first cytokinesis succeeds despite defects in the structure of the spindle midzone/midbody.

A final protein, currently in a class of its own is SPD-1 (Verbrugghe and White, 2004). Like the six proteins listed above, SPD-1 is also required for the formation of microtubule bundles in the spindle midzone. However, in SPD-1 depleted embryos the first cytokinesis does not fail (although later embryonic cytokineses often do). It is not yet clear if this result indicates that the midbody is less compromised in *spd-1* embryos than it is in embryos depleted of or mutant for the other six proteins required for midbody formation—or if centralspindlin and the chromosomal passengers have a separate role in the completion of cytokinesis that is independent of their role in assembling the central spindle/midbody. Three additional proteins, dynamin (Thompson et al., 2002), RAB-11 (Skop et al., 2001) and syntaxin 4 (SYN-4; Jantsch-Plunger and Glotzer, 1999), have been implicated in membrane fusion during abscission, which generates the two topologically distinct daughter cells, however their precise roles in this process remain to be elucidated.

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