Mutual antagonism between the Anaphase Promoting Complex and the Spindle Assembly Checkpoint contributes to mitotic timing in *Caenorhabditis elegans*

Alexandra Bezler and Pierre Gönczy

Swiss Institute for Experimental Cancer Research (ISREC)
School of Life Sciences, Swiss Federal Institute of Technology (EPFL)
Lausanne, Switzerland

pierre.gonczy@epfl.ch
Running Title:

**APC/C contributes to mitotic timing through the SAC**

Keywords:

*Caenorhabditis elegans*, embryo, anaphase promoting complex, spindle checkpoint, *such-1*

Corresponding author:

**Pierre Gönczy**

pierre.gonczy@epfl.ch

EPFL SV 1526
Station 19
CH-1015 Lausanne
Switzerland

Phone: +41 21 693 07 11
Fax: +41 21 693 07 20
SUMMARY

The anaphase promoting complex/cyclosome (APC/C) triggers the separation of sister chromatids and exit from mitosis across eukaryotic evolution. The APC/C is inhibited by the spindle assembly checkpoint (SAC) until all chromosomes have achieved bipolar attachment, but whether the APC/C reciprocally regulates the SAC is less understood. Here, we report the characterization of a novel allele of the APC5 component SUCH-1 in C. elegans. We find that some such-1(t1668) embryos lack paternally contributed DNA and centrioles and assemble a monopolar spindle in the one-cell stage. Importantly, we show that mitosis is drastically prolonged in these embryos, as well as in embryos that are otherwise compromised for APC/C function and assemble a monopolar spindle. This increased duration of mitosis is dependent on the SAC, since inactivation of the SAC components MDF-1/MAD1 or MDF-2/MAD2 rescues proper timing in these embryos. Moreover, partial depletion of the E1 enzyme uba-1 significantly increases mitosis duration upon monopolar spindle assembly. Taken together, our findings raise the possibility that the APC/C negatively regulates the SAC and, therefore, that the SAC and the APC/C have a mutual antagonistic relationship in C. elegans embryos.
INTRODUCTION

Mitosis is tightly regulated in time and space to ensure equal distribution of sister chromatids and cytoplasmic constituents to daughter cells. Mistakes in chromosome segregation can cause aneuploidy and contribute to tumor progression. Therefore, a thorough knowledge of the mechanisms regulating mitosis is key for understanding proliferation control.

Bipolar attachment of sister chromatids to the mitotic spindle is crucial for their faithful segregation. Once bipolar attachment is achieved, the APC/C triggers the metaphase-anaphase transition and mitotic exit. The APC/C is a multisubunit complex that functions as an E3 ligase which ubiquitinates substrates, thereby marking them for destruction by the 26S proteasome (reviewed in Peters 2006). The recognition of substrates during mitosis is mediated primarily by the APC/C-associated coactivator Cdc20 (Visintin et al. 1997). Degradation of the APC/C substrates securin and cyclin B triggers the metaphase-anaphase transition and initiates mitotic exit, respectively (Cohen-Fix et al. 1996; Murray and Kirschner 1989; Murray et al. 1989). To ensure that cells do not enter anaphase until all chromosomes have achieved bipolar attachment, the spindle assembly checkpoint (SAC) monitors microtubule attachment and tension at kinetochores (Li and Nicklas 1995; Rieder et al. 1994). As long as the SAC is engaged, the SAC component MAD2 is present at kinetochores (Chen et al. 1996; Li and Benezra 1996), resulting in the sequestration of Cdc20 in a complex with MAD2 and/or BubR1 (Hwang et al. 1998; Kim et al. 1998; Nilsson et al. 2008; Tang et al. 2001). Therefore, the APC/C cannot efficiently ubiquitinate substrates, thus preventing their
degradation and hence delaying progression through mitosis (Hwang et al. 1998; Kim et al. 1998). When complete bipolar chromosome attachment is achieved, MAD2 departs from kinetochores (Chen et al. 1996; Li and Benezra 1996), thus allowing Cdc20 to function as an APC/C co-activator and promote mitotic progression. Cells can also undergo what has been termed “mitotic slippage” and eventually progress through mitosis without satisfying the SAC (Rieder and Maiato 2004).

Despite the holocentric nature of C. elegans chromosomes (reviewed in Oegema and Hyman 2006), the SAC components and their function are conserved in nematodes. Thus, the checkpoint proteins MDF-1/MAD1 and MDF-2/MAD2 are required for viability and for germ cell arrest after nocodazole treatment (Kitagawa and Rose 1999). In early embryos, however, chemical or mutational disruption of the spindle does not result in arrest, but instead leads to a merely 1.5- to 2-fold delay during mitosis (Encalada et al. 2005; Essex et al. 2009). The mechanisms underlying such differential responses are only partially understood.

The relationship between the SAC and the APC/C is not fully elucidated either. Cdc20 is turned over in an APC/C-dependent manner during SAC engagement, both in budding yeast and in human cells (Nilsson et al. 2008; Pan and Chen 2004). It has thus been proposed that Cdc20 ubiquitination and degradation maintain SAC engagement in human cells (Ge et al. 2009; Nilsson et al. 2008). Accordingly, modest overexpression of Cdc20 inactivates the SAC in budding yeast, further indicating that low Cdc20 levels must be maintained for proper SAC function (Pan and Chen 2004). In apparent contradiction with this view, ubiquitination of Cdc20 is needed to
inactivate the SAC in human cells (Garnett et al. 2009; Reddy et al. 2007). Overall, although the SAC and the APC/C have been studied extensively biochemically, their relationship remains incompletely understood, in particular in a living organism.

We have taken advantage of the early embryo of C. elegans to study aspects of the regulation of mitosis in vivo. Here, we report the phenotypic analysis of such-1(t1668), a mutation affecting an APC5 subunit. Our findings reveal that some such-1(t1668) embryos lack paternally contributed DNA and centrioles and assemble a monopolar spindle. Importantly, we found a substantial increase in the duration of mitosis in these embryos. Further analysis suggests that this results from prolonged engagement of the SAC. Our findings thus clarify the relationship between the SAC and the APC/C in a developing organism and establish that APC/C activity sets the duration of mitosis through the SAC in C. elegans embryos.
MATERIALS AND METHODS

Worm strains:

*C. elegans* strains of the following genotypes were cultured according to standard procedures (BRENNER 1974). Bristol N2, Hawaiian CB4856, *spd-5*(or213) I (HAMILL et al. 2002) (raised at 16°C and shifted to 25°C for 15 h), *smg-2*(e2008) I (HODGKIN et al. 1989), *emb-27*(g48) II (raised at 16°C and shifted to 22°C for 15 h) (CASSADA et al. 1981), *fzy-1*(h1983) *dpy-10*(e128) II (raised at 16°C and shifted to 25°C for >15 h) (KITAGAWA et al. 2002), *fzy-1*(av15) *unc-4*(e120) II (STEIN et al. 2007), *such-1*(t1668) *unc-32*(e189) III/ qCI *dpy-19*(e1259) *glp-1*(q339); *him-3*(e1147) IV (GÖNCZY et al. 1999) (raised at 15°C or 16 °C unless otherwise indicated). *plg-1*(e2001) III males were used to unambiguously identify mated hermaphrodites owing to the presence of a gelatinous plug (HODGKIN and DONIACH 1997). *fog-2*(q71) V females producing wild-type oocytes were used in crosses to determine the paternal contribution of *such-1*(t1668) (SCHEDL and KIMBLE 1988).

For deficiency mapping, we crossed *such-1*(t1668) *unc-32*(e189)/qCI hermaphrodites to *tDf1*/unc-32(e189) *dpy-18*(e499) III males. We selected wild-type F1 animals and identified among the F2s hemizygous *such-1*(t1668) animals by the absence of Dpy or Dpy-Unc. Some F1 animals were sterile, exhibited an abnormal gonad and a protruding vulva.

For timing the metaphase-anaphase transition, we generated a strain carrying H2B-mCherry (MCNALLY et al. 2006) in a *such-1*(t1668) *unc-32*(e189) / qCI background; embryos from homozygous *such-1*(t1668) hermaphrodites fertilized by *plg-1*(e2001) sperm with 100 % bipolar spindles were analyzed.
The strain such-1(h1960) III; unc-46(e177) mdf-1(gk2) V (TARAILO et al. 2007) was used to generate homozygous such-1(h1960) animals. For all experiments with such-1(h1960), L4 larva were shifted to 25°C for 15 h. We crossed homozygous such-1(h1960) males to fzy-1(av15) unc-4(e120) II (STEIN et al. 2007) or MDF-2-GFP (ESSEX et al. 2009) hermaphrodites. The mutation in such-1(h1960) destroys an HpaII restriction site, which was followed in all crosses by PCR and RFLP. Transgenic lines were maintained at 24°C.

In all experiments with such-1(t1668), L4 hermaphrodites were selected and kept separate at the indicated temperature to avoid fertilization by sibling males. For progeny tests, we quantified eggs laid after removal of the parents and hatched larva 24-48 h later. We found that such-1(t1668) is 98.5% embryonic lethal at 15°C and 100% at 25°C (N>1000 in each case). For simplicity, results from progeny tests carried out at 15°C-25°C were pooled.

We found also that progeny from such-1(t1668) heterozygous mutant animals develop slightly slower than wild-type, indicative of a semi-dominant effect on zygotic development.

For experiments involving mating, animals were kept at 20°C. To address the paternal contribution to embryonic lethality, we generated the strain such-1(t1668) /qCI dpy-19(e1259) glp-1(q339). Single males from this strain where mated with several fog-2 females, embryonic lethality determined, and subsequently homozygous such-1(t1668) males identified by sequencing. Matings for complementation analysis and deficiency mapping were carried out at 20°C and animals then shifted to 25°C. such-1(h1960) fails to complement such-1(t1668) for embryonic lethality; additionally, such-
1(h1960)/such-1(t1668) embryos undergoing monopolar mitosis induced by spd-5(RNAi) are delayed during mitosis (43 min 10 sec +/- 2 min 39 sec N=7) like either homozygous mutant such-1 parental strain.

**Mapping:**
Unc-Emb and Unc-nonEmb recombinants were recovered from strains heterozygous for CB4856 and unc-32(e189) such-1(t1668). SNP mapping (WICKS et al. 2001) was then used to narrow the region between 10.75 cM and 11.51 cM with SNPs snp_Y66D12A[1] and haw47495. Sequencing of such-1 DNA from such-1(t1668) worms revealed a single nucleotide (A) deletion in exon 6 at position 1155. RT-PCR for such-1 was performed with gene-specific primers from nucleotide 1 until nucleotide 1461. In a second PCR step, this template was amplified with primers spanning exons 6 and 7 to reveal the ratio of the two mRNA species.

**RNAi-mediated inactivation:**
RNAi-mediated inactivation by feeding was performed by selecting L4 larva and treating them as follows: spd-5(RNAi) >15 h at 25°C (RUAL et al. 2004), except for spd-5(RNAi) in emb-27(g48) which was carried out at 22°C; mdf-1(RNAi) (from nucleotides 325 through 1522 of the gene amplified from genomic DNA and cloned in L4440 (TIMMONS and FIRE 1998)) and mdf-2(RNAi) (from nucleotides 75 through 575 of the gene amplified from genomic DNA and cloned in L4440) 48 h at 16°C; partial uba-1(RNAi) 13.5 h- 19 h at 25°C (RUAL et al. 2004), partial fzy-1(RNAi) 24 h at 25°C (RUAL et al. 2004), gfi-3(RNAi) >30 h at 24°C (KAMATH et al. 2003).
We generated an RNAi feeding vector containing the first 1461 nucleotides of the such-1 cDNA cloned in L4440 (TIMMONS and FIRE 1998). Feeding L1/L2 larva at 25°C with such-1(RNAi) results in 100% embryonic lethality (N=105) but no meiotic arrest. such-1(h1960) was reported to cause 46 % embryonic lethality at 25°C (TARAILO et al. 2007). In our hands, such-1(h1960) shifted as L4 is 10 % and 90 % embryonic lethal at 24°C (N=309) and 25°C (N=264), respectively. such-1(h1960) is not a paternal-effect allele, since the extent of embryonic lethality is not different among self-progeny and when such-1(h1960) are mated with plg-1(e2001) males (80% +/- 3% SEM, N=443 versus 83% +/- 3% SEM, N=212) and since such-1(h1960) males do not cause significant embryonic lethality after fertilization of oocytes from fog-2(q71) females (3% lethality +/- 6% SEM; N=175). We investigated potential redundancy between such-1 and gfi-3; gfi-3(RNAi) alone does not affect viability but increases embryonic lethality in such-1(h1960) at 24°C to 80 % (N=166).

**Time-lapse Microscopy:**

Embryos were analyzed by time-lapse DIC microscopy on a Zeiss Axioskop 2 with 100x A-plan objective (NA 1.25 oil) at 23°C with a frame-rate of 10 sec (GÖNCZY et al. 1999). Images were acquired with Scion Image and a Kappa CF 8/4 camera. M phase duration was measured from the first signs of nuclear envelope breakdown (NEBD; initial “shrinking” of the NE) until the first signs of nuclear envelope reformation (NER). Due to the inclusion of the first signs of NEBD and the fact that we did not use cytokinesis to time mitotic exit, M phase duration reported here for wild-type embryos is slightly longer than in
the literature (BRAUCHLE et al. 2003; ENCALADA et al. 2005; ESSEX et al. 2009; HACHET et al. 2007). Note that average times are given in the text in each case, with standard error of mean and number of embryos examined indicated in the corresponding figure panels. The calculated average duration was rounded up to the nearest 10 sec (except for movies with 30 sec time intervals, where the rounding up was to the nearest 30 sec). For *spd-5* depletion, only embryos with a monopolar spindle were quantified.

Dual time-lapse fluorescence and DIC microscopy was carried out on a Zeiss Axioplan 2, 63x Plan-Apochromat (NA 1.4 oil DIC) equipped with a RT monochrome spot camera 2.1.1 and Metamorph software (SONNEVILLE and GÖNCZY 2004), with a frame rate of 10 sec, except *such-1(h1960) spd-5(RNAi)* embryos that were imaged every 30 sec. The duration of MDF-2-GFP localization on chromosomes was quantified in wide-field recordings from the first signs of NEBD until the enrichment of the GFP signal on chromosomes disappeared.

For illustrating the MDF-2-GFP data (Fig. 6), embryos were imaged on an LSM700 Zeiss confocal microscope with a 40x Plan-Apochromat (NA 1.3 oil) objective, capturing a single optical slice of 3 μm at a time interval of 10 sec for *spd-5(RNAi)* and of 10 sec (early mitosis) up to 10 min (during the prolonged mitosis) for *such-1(h1960) spd-5(RNAi)* embryos.

**Indirect Immunofluorescence:**

Fixation and indirect immunofluorescence were performed essentially as described (GÖNCZY et al. 1999). The following primary antibodies were used: mouse α-tubulin (DM1A, Sigma; 1/300), mouse SP-56 (a generous gift from
Susan Strome (WARD et al. 1986; 1/100), rabbit SAS-4 (LEIDEL and GÖNCZY 2003; 1/600), rabbit TAC-1 (BELLANGER and GÖNCZY 2003; 1/300).

Secondary antibodies were goat anti-mouse coupled to Alexa 488 (1/1000) and goat anti-rabbit coupled to Alexa 568 (1/1000) (Molecular Probes). DNA was revealed with ~1 µg/ml Hoechst 33258 (Sigma). Images were acquired on a LSM 700 Zeiss (embryos) or SP2 Leica (sperm) confocal microscope. Maximal intensity projections of selected confocal planes are shown and were generated in ImageJ.
RESULTS

Aberrant centrosome number and DNA content in *such-1(t1668)* embryos:

Mitosis can be analyzed with high temporal and spatial resolution in one-cell stage *C. elegans* embryos using time-lapse DIC microscopy. In the wild-type (Movie 1), there is one male and one female pronucleus (Fig. 1A, - 5:00). The female pronucleus forms at the presumptive anterior and then moves towards the male pronucleus, which is located at the posterior, associated with the centrosomes. The two joined pronuclei and the accompanying centrosomes then move to the cell center. The two centrosomes serve as microtubule-organizing centers (MTOCs) that organize bipolar spindle assembly following nuclear envelope breakdown (NEBD), thus ensuring faithful chromosome segregation (Fig. 1A, + 2:30; Fig. 2A). Cytokinesis and nuclear envelope reformation (NER) mark the end of mitosis, resulting in the generation of two daughter cells, each with a full complement of chromosomes (Fig. 1A, + 9:00).

*such-1(t1668)* is a recessive mutant allele identified in a collection of EMS-induced parental effect lethal mutations (GÖNCZY *et al.* 1999). The number of eggs laid by *such-1(t1668)* homozygous mutant animals is on average 92 +/- 5 SEM (N=17 adults), compared to 255 +/- 13 SEM (N=9 adults) for heterozygous siblings. We conducted time-lapse DIC microscopy to investigate the nature of the defect in embryos from homozygous *such-1(t1668)* hermaphrodites (hereafter referred to as *such-1(t1668)* embryos for simplicity). This enabled us to define three classes of *such-1(t1668)* embryos
(N=38 embryos). Class I embryos (Movie 2; 29%) have one male and one female pronucleus and assemble a bipolar spindle (Fig. 1B; Fig. 2B). Some class I embryos harbor more than two MTOCs and consequently assemble a multipolar spindle (data not shown; legend of Fig. 1). Class II embryos (Movie 3; 55%) possess a female pronucleus, but no male pronucleus, and are thus haploid (Fig 1C, - 5:00). Nevertheless, MTOCs are present at the embryo posterior and the female pronucleus migrates toward them as in the wild-type. The single pronucleus and associated centrosomes then move to the cell center and during mitosis the two MTOCs assemble a bipolar spindle (Fig 1C, + 2:30). Occasional class II embryos have more than two MTOCs and assemble a multipolar spindle (data not shown; legend of Fig. 1). Most interestingly, class III embryos (Movie 4; 16%) also have a female pronucleus but no male pronucleus and are thus haploid (Fig. 1D, - 5:00). Here, however, no MTOC is apparent and the female pronucleus drifts slowly towards the cell center. Bipolar spindle assembly does not occur during mitosis. Instead, a monopolar configuration is observed, which will be referred to as monopolar mitosis hereafter (Fig 1D, + 2:30; Fig 2C). This phenotype is reminiscent of that observed in spd-5 mutant embryos, which cannot nucleate robust microtubules from their defective centrosomes (Hamill et al. 2002). Similarly, we found that microtubules appear to be nucleated around the chromatin in class III such-1(t1668) embryos (Fig 2C). Class III embryos fail to divide after M phase exit (Movie 4).

To further investigate the apparent lack of centrosomes in class III embryos, we addressed whether these embryos contain centrioles using antibodies against the centriolar protein SAS-4 (Kirkham et al. 2003; Leidel
and Gönčzy 2003). In the wild-type, each spindle pole contains two juxtaposed centrioles (Fig. 2A). Class I and II *such-1(t1668)* embryos are indistinguishable from the wild-type with respect to SAS-4 distribution (Fig 2B). By contrast, SAS-4 foci are invariably absent in class III *such-1(t1668)* embryos (Fig. 2C; 27/27 embryos). Accordingly, foci of the centrosomal protein TAC-1 are also absent from class III embryos (Fig. S1C).

Taken together, these findings establish that some *such-1(t1668)* embryos lack a male pronucleus as well as centrioles and undergo monopolar mitosis. Furthermore, these results demonstrate that centrioles are dispensable for fertilization.

**such-1(t1668) sperm has aberrant centriole numbers and missing DNA:**

Since sperm contributes the DNA making up the male pronucleus as well as the sole centrioles to the zygote, we addressed whether some *such-1(t1668)* sperm exhibit abnormal DNA contents and centriole numbers.

Wild-type sperm contain highly condensed DNA associated with a pair of centrioles marked by SAS-4 (Fig. 3A). These two centrioles usually appear as a single focus, due to their small size and proximity, which is usually below the resolution limit of the light microscope. *such-1(t1668)* sperm display an array of phenotypes with respect to DNA contents and centriole number that we classified by analogy with the embryonic phenotypes (N= 87 sperm). Class I sperm (32%) contain DNA and one or two separate SAS-4 foci, presumably corresponding to two or four centrioles (Fig. 3B; data not shown). This subset of mutant sperm probably results in class I embryos, which are diploid and assemble a bipolar or a multipolar spindle. In class II sperm
(47%), the DNA is absent, and one focus of SAS-4 is usually present (Fig. 3C). This subset of such-1(t1668) sperm presumably results in class II haploid embryos lacking solely the male pronucleus. Class III sperm (21%) lack DNA, as well as centrioles (Fig. 3D), and thus likely give rise to class III such-1(t1668) embryos.

Given the suggestive parallel between the defects in such-1(t1668) sperm and embryos, as well as the related proportions of each phenotypic class, we addressed whether the observed embryonic lethality is of paternal origin. In homozygous such-1(t1668) self-fertilizing hermaphrodites, mutant oocytes are fertilized by mutant sperm, yielding 100% embryonic lethality (Fig. 3E). By contrast, we found that embryonic lethality is rescued when such-1(t1668) mutant oocytes are fertilized with wild-type sperm contributed by plg-1(e2001) males (Fig. 3E). Moreover, such-1(t1668) males crossed to spermless fog-2(q71) females cause 100% embryonic lethality in the offspring (Fig. 3E). We conclude that such-1(t1668) sperm causes paternal-effect embryonic lethality.

While the missegregation of DNA and centrioles likely occurs during sperm meiosis, the rescue of viability by wild-type sperm indicates that both meiotic divisions in such-1(t1668) oocytes occur normally. Despite the full rescue by wild-type sperm, the duration of mitosis in the resulting embryos is on average 33% longer than in the wild-type (described below), a slight extension which is tolerated by the developing organism (see also TARAILO et al. 2007).
Molecular lesion in *such-1(t1668)*:

To identify the molecular nature of the *such-1(t1668)* allele, we initially carried out deficiency mapping. We found that the deficiency *tDf1* fails to complement *such-1(t1668)* and that *such-1(t1668)/tDf1* animals have an abnormal gonad and are almost sterile, precluding analysis of the resulting embryos. Additionally, some hemizygous animals have a protruding vulva, indicating that SUCH-1 has additional functions later during development. We conclude that *such-1(t1668)* is a reduction-of-function allele of a gene located on the right arm of chromosome III. Further SNP mapping placed *such-1(t1668)* within a 119 kbp interval between 10.77 cM and 11.51 cM (Fig. 4A). Of 20 predicted genes within this region, *such-1* was a plausible candidate since it encodes an APC5 protein, which is a subunit of the APC/C (TARAILO et al. 2007). Indeed, some hypomorphic APC/C alleles exhibit phenotypes partly reminiscent of the ones reported here (GOLDEN et al. 2000; SADLER and SHAKES 2000). We found that animals transheterozygous between *such-1(t1668)* and the previously identified allele *such-1(h1960)* give rise to 91% (+/- 3% SEM, N=1068) embryonic lethality, whereas the progeny of *such-1(t1668)* or *such-1(h1960)* heterozygous animals is viable (data not shown; TARAILO et al. 2007). Thus, *such-1(h1960)* fails to complement *such-1(t1668)* in terms of embryonic lethality.

Sequencing of *such-1* in *such-1(t1668)* revealed a single nucleotide deletion in exon 6, which causes a frameshift creating a premature stop codon in exon 7 (Fig. 4B, schematic). Since transcripts with premature stop codons are targeted for degradation by nonsense-mediated decay (NMD) (PULAK and ANDERSON 1993), *such-1* mRNA from *such-1(t1668)* should be significantly
diminished. To test this prediction, we amplified exons 6-7 from wild-type and such-1(t1668) mutant animals using RT-PCR. As shown in Fig. 4B, wild-type animals yield a clear amplification product corresponding to exons 6-7, without the intervening intron (lane 1, bottom). This amplification product is significantly diminished in such-1(t1668) animals (lane 2, bottom). Inhibition of NMD using a mutation in smg-2 restores the levels of this amplification product (lane 4, bottom), confirming that the such-1(t1668) transcript containing a stop codon is usually degraded by NMD. such-1(t1668) smg-2(e2008) animals give rise to an embryonic lethality of 59 % +/- 13 % SEM (N=566) at 20°C, compared to 100% for such-1(t1668) (see Fig. 3E). Thus, increasing mutant SUCH-1 levels rescues viability, indicating that the protein is at least partially functional.

Furthermore, RT-PCR analysis and sequencing revealed that such-1(t1668) animals accumulate a longer transcript that retains the intron between exons 6 and 7 (Fig. 4B, schematic and lane 2, top). This retention corrects the frameshift from exon 7 onwards such that this transcript is prevalent in such-1(t1668). Trace amounts of this longer transcript are also present in the wild-type (Fig. 4B, lane 1, top). The longer transcript in such-1(t1668) is predicted to yield a protein in which 21 amino acids encoded by exon 6 are altered due to the frameshift, and 20 extraneous amino acids are encoded by the intron. However, the N- and C- termini of SUCH-1 are unchanged, perhaps accounting for the residual function in such-1(t1668) animals.
**Prolonged M phase in *such-1(t1668)* embryos with monopolar mitosis:**

Further analysis of *such-1(t1668)* embryos shed new light on the mechanisms regulating mitosis in developing *C. elegans* embryos.

In wild-type one-cell stage embryos, the time from the beginning of NEBD until NER is on average 5 min 30 sec, as measured by DIC time lapse microscopy (Fig. 5A). Previous work established that severe loss of APC/C function provokes metaphase arrest in meiosis I, whereas partial reduction of APC/C function does not prevent the execution of meiosis but delays the metaphase-anaphase transition and exit from mitosis in one-cell stage embryos (Furuta et al. 2000; Golden et al. 2000; McCarthy Campbell et al. 2009; Shakes et al. 2003; Tarailo et al. 2007). To address whether there is a delay in the metaphase-anaphase transition in *such-1(t1668)* embryos, we analyzed embryos derived from mutant oocytes fertilized by wild-type sperm, which all set up a bipolar spindle. Using mCherry-H2B (McNally et al. 2006) to monitor chromosomes, we found that the time separating NEBD from the metaphase-anaphase transition is 2 min 20 sec on average in the wild-type (+/- 5 sec SEM, N=10). By contrast, this increases to 3 min 40 sec in the *such-1(t1668)* background (+/- 16 sec SEM, N=9). Thus, the metaphase-anaphase transition is slightly delayed in *such-1(t1668)* embryos with a bipolar spindle, as anticipated for a hypomorphic allele of an APC/C component.

As predicted also, we found that all *such-1(t1668)* embryos exhibit prolonged M phase duration. Importantly in addition, we found that the extent of this prolongation depends on the phenotypic class of *such-1(t1668)* embryos. Since anaphase cannot be scored in embryos with a monopolar
spindle, the duration of mitosis in the following sections refers to the time separating NEBD from NER in DIC time-lapse recordings. As reported in Fig. 5A, the duration of mitosis is prolonged only slightly in class I and class II embryos that assemble a bipolar spindle (6 min 50 sec and 7 min 20 sec, respectively), which corresponds to a 1.3 fold delay compared to the wild-type. Strikingly, M phase lasts 39 min 10 sec in class III \textit{such-1(t1668)} embryos that undergo monopolar mitosis, corresponding to a 7-fold increase compared to the wild-type (Fig. 5A). By contrast to the effects on mitosis, the duration of interphase is similar to the wild-type in all three classes of \textit{such-1(t1668)} embryos (Fig. S2). Overall, we conclude that class III \textit{such-1(t1668)} embryos are compromised in their capacity to exit mitosis.

\textbf{Prolonged M phase in APC/C mutants with monopolar mitosis:}

We set out to investigate the mechanisms leading to the substantial prolongation of mitosis in class III \textit{such-1(t1668)} embryos. To address whether it is the lack of bipolar spindle assembly in class III \textit{such-1(t1668)} embryos that causes this prolongation, we sought to prevent bipolar spindle assembly also in class I and class II embryos. To this end, we depleted in addition SPD-5, a coiled-coil protein required for centrosome assembly and hence bipolar spindle formation (Hamill \textit{et al.} 2002). Embryos lacking SPD-5 function, obtained through RNAi-mediated depletion or using the mutant \textit{spd-5(or213)}, enter mitosis with two non-functional centrosomes. In the absence of MTOCs, microtubules are nucleated instead around the chromatin, thus mimicking the monopolar configuration of \textit{such-1(t1668)} class III embryos (Fig. 2D; Movie 5, compare to Fig. 2C and Movie 4). The duration of M phase
in $\text{spd-5(RNAi)}$ embryos is slightly increased compared to the wild-type, to $6 \text{ min 10 sec}$ (Fig. 5B). Importantly, we found that $\text{such-1(t1668) spd-5(RNAi)}$ embryos all exhibit an increase in M phase duration to $41 \text{ min 30 sec}$ (Fig. 5B). We conclude that monopolar mitosis provokes a substantial prolongation of mitosis in $\text{such-1(t1668)}$ embryos. To test whether the delay is of maternal origin, we timed M phase duration in $\text{such-1(t1668) spd-5(RNAi)}$ embryos fertilized by wild-type sperm. In this case, monopolar mitosis lasts $37 \text{ min 50 sec +/- 6 min 13 sec}$ ($N=12$), similar to the timing in self-fertilized embryos. Thus, the prolonged M phase upon monopolar mitosis is of maternal origin.

Next, we addressed whether prolonged M phase duration in $\text{such-1(t1668)}$ embryos upon monopolar mitosis is allele specific. We analyzed $\text{such-1(h1960)}$, another reduction-of-function allele that exhibits a minor increase in M phase duration upon bipolar spindle assembly ($7 \text{ min}$; Fig. 5B) (TARAILO et al. 2007). Strikingly, we found that $\text{such-1(h1960) spd-5(RNAi)}$ embryos, which all undergo monopolar mitosis, spend $49 \text{ min 10 sec}$ in mitosis (Fig. 5B). Therefore, increased M phase duration upon monopolar mitosis is a common consequence of compromising SUCH-1 function.

Since SUCH-1 is an APC/C component, we addressed whether prolonged M phase is a general response to monopolar mitosis among hypomorphic APC/C mutants. To this end, we used $\text{emb-27(g48)}$, a temperature sensitive allele of the APC/C subunit APC6 (CASSADA et al. 1981; GOLDEN et al. 2000). $\text{emb-27(g48)}$ embryos all arrest in metaphase of meiosis I at the restrictive temperature of $25^\circ\text{C}$ (CASSADA et al. 1981; GOLDEN et al. 2000). To circumvent this arrest, we used the semi-permissive temperature of $22^\circ\text{C}$, which enables progression through meiosis and mitosis and results in
10% embryonic lethality (N=80). As expected from a reduction of APC/C function, the duration of mitosis in such embryos with a bipolar spindle is slightly increased, to 6 min 10 sec (Fig. 5B). *emb-27(g48) spd-5(RNAi)* embryos instead assemble a monopolar spindle and remain in M phase for 48 min 40 sec (Fig. 5B). Taken together, these findings reveal that whereas reduction of APC/C function alone causes merely a minor prolongation of M phase, assembling a monopolar spindle under such conditions results in a drastic increase in the duration of mitosis.

**Ubiquitination influences M phase duration upon monopolar mitosis:**

The APC/C is an E3 ligase, which functions at the terminal step of a cascade forming ubiquitin chains on substrates (reviewed in Peters 2006). Prior to that step, ubiquitin is activated by an E1 enzyme and transferred to an E2 enzyme, which cooperates with the E3 ligase to ubiquinate substrates. We set out to test whether increased M phase duration upon monopolar mitosis is provoked by insufficient ubiquitination of APC/C substrates. If this were the case, then interfering with the ubiquitination cascade should also prolong the duration of monopolar mitosis. To test this prediction, we depleted by RNAi *uba-1*, the sole gene encoding an E1 activating enzyme in *C. elegans* (Kulkarni and Smith 2008). Due to its widespread requirement, strong RNAi-mediated inactivation of *uba-1* causes sterility and metaphase arrest during meiosis I (Jones et al. 2002; Maeda et al. 2001; Sonnichsen et al. 2005). To allow entry into mitosis, we used partial inactivation conditions, which results in M phase lasting 6 min 30 sec upon bipolar spindle assembly (Fig. 5C). Importantly, we found that the duration of M phase in *uba-1(RNAi) spd-5(RNAi)* embryos...
5(or213) embryos, which all undergo monopolar mitosis, is increased to 14 min 50 sec (Fig. 5C). Overall, we conclude that ubiquitination by the APC/C participates in setting the duration of M phase upon monopolar mitosis.

The prolonged M phase is likely transmitted via the APC/C coactivator FZY-1/Cdc20:

We then asked how the APC/C could provoke such a delay upon monopolar mitosis. Since the APC/C functions with its coactivator FZY-1/Cdc20 during mitosis, we addressed whether FZY-1/Cdc20 participates in prolonging M phase in APC/C mutants in a monopolar setting. As for other APC/C components and UBA-1, strong RNAi-mediated inactivation of FZY-1 causes metaphase arrest in meiosis I (KITAGAWA et al. 2002). Therefore, we again used partial RNAi-mediated inactivation, which results in mitosis lasting 7 min 40 sec upon bipolar spindle assembly (Fig. 5D). When FZY-1 was inactivated in this manner in spd-5(or213) embryos, the duration of mitosis is lengthened to 10 min 10 sec (Fig. 5D). Similarly, whereas M phase lasts 8 min 10 sec in the weak reduction-of-function allele fzy-1(h1983), it lasts 10 min 30 sec in fzy-1(h1983) spd-5(RNAi) embryos (Fig. 5D). These slight prolongations upon FZY-1 partial inactivation are statistically significant (legend of Fig. 5) and indicate that prolonged M phase duration is at least partially dependent on FYZ-1.

As a further test of the requirement for FYZ-1 function in this delay, we used the mutant allele fzy-1(av15), which has been isolated as a suppressor of the hypomorphic APC/C mutant mat-3(or180) (STEIN et al. 2007). It has been proposed that this mutation may interfere with MDF-2 binding, thus
mimicking the loss of SAC signaling (STEIN et al. 2007). If FZY-1 is required for prolonged M phase during monopolar mitosis, then fzy-1(av15) might be expected to alleviate this effect. As shown above, such-1(h1960) spd-5(RNAi) one-cell stage embryos spend 49 min 10 sec in mitosis (Fig. 5B). By contrast, such-1(h1960) fzy-1(av15) spd-5(RNAi) embryos undergoing monopolar mitosis spend only 8 min 20 sec in M phase (Fig. 5D). This finding further supports the notion that FZY-1/Cdc20 is involved in prolonging M phase duration in APC/C mutants.

**Prolonged M phase in APC/C mutants results from excess activation of the spindle assembly checkpoint:**

Next, we investigated whether the substantial increase in M phase duration in such-1 embryos upon monopolar mitosis is caused by SAC engagement. This is of particular interest because the slight delay observed in such-1(h1960) embryos that assemble a bipolar spindle is independent of the SAC (TARAILO et al. 2007). As in all eukaryotes, the MAD2 checkpoint protein MDF-2 accumulates on unattached kinetochores in *C. elegans* and can be used as readout for SAC activation (CHEN et al. 1996; ESSEX et al. 2009; KITAGAWA and ROSE 1999; LI and BENEZRA 1996). In wild-type one-cell stage embryos, MDF-2-GFP enters the nucleoplasm at prometaphase and remains present as a diffuse cloud until the onset of anaphase (ESSEX et al. 2009). In line with previous observations in embryos undergoing monopolar spindle assembly, we found that MDF-2-GFP is detected on chromatin during 2 min 10 sec on average in spd-5(RNAi) embryos (Fig. 6A, 6B) (ESSEX et al. 2009). Strikingly, we found that MDF-2-GFP chromatin localization is prolonged to 29
min in *such-1(h1960)* spd-5(RNAi) embryos (Fig. 6B, 6B), which corresponds to a 13 fold increase. Thus, prolonged M phase duration in APC/C mutants upon monopolar mitosis correlates with significant persistence of MDF-2-GFP on chromatin.

To test whether it is indeed SAC engagement that provokes prolonged M phase duration in APC/C mutants upon monopolar mitosis, we depleted the *C. elegans* checkpoint components MDF-1 (a homologue of MAD1) or MDF-2 (Kitagawa and Rose 1999). Importantly, we found that the duration of monopolar mitosis in *such-1(h1960) mdf-1(gk2) spd-5(RNAi)* is reduced to 10 min (Fig. 6C). Similarly, *mdf-1(RNAi)* or *mdf-2(RNAi)* rescues the prolongation of M phase in *such-1(t1668)* class III embryos to 9 min 50 sec and 10 min, respectively (Fig. 6C). The slight remaining prolongation compared to *spd-5(RNAi)* embryos may reflect an MDF-1/MDF-2 independent delay, as observed previously in other contexts in worms, flies and humans (Orr *et al.* 2007; Tarailo *et al.* 2007; Williamson *et al.* 2009).
Aberrant sperm, spindle assembly and centriole number:

We found that such-1(t1668) sperm contains aberrant DNA contents and centriole numbers. These phenotypes are partly reminiscent of the absence of paternal DNA and the presence of additional centrioles observed in some alleles of emb-27, emb-30, mat-1, mat-2 and mat-3, which all encode homologues of APC/C subunits (Furuta et al. 2000; Golden et al. 2000; Sadler and Shakes 2000). Given that the embryonic lethality of such-1(t1668) embryos is of paternal origin, the sperm defects fully explain the range of phenotypes observed in the resulting embryos. Thus, like other APC/C components, the APC5 subunit SUCH-1 is important for faithful segregation of DNA, centrioles and perhaps other organelles during the meiotic divisions of male germ cells. In contrast, the female meiotic divisions seem normal in such-1(t1668) mutant animals, given notably that wild-type sperm is sufficient to rescue lethality. Thus, even though the analysis of animals with more complete inactivation of function may alter this conclusion, it appears that SUCH-1 has different requirements in the male and the female germline.

C. elegans mutant embryos that possess centrioles but cannot nucleate microtubules from their defective centrosomes do not assemble a bipolar spindle (Hamill et al. 2002). Here, we establish that the same is true for embryos that lack centrioles all together. In other systems, including Xenopus egg extracts, Drosophila neuroblasts and vertebrate cells, a bipolar spindle can assemble in the absence of centrosomes owing to nucleation of
microtubules around chromatin and their subsequent organization into a bipolar array (Basto et al. 2006; Heald et al. 1996; Khodjakov et al. 2000). This mechanism is critical for assembling the acentrosomal meiotic spindle, but can be also active in cells with centrosomes (Heald et al. 1997; Khodjakov et al. 2000). In early C. elegans embryos, by contrast, although some microtubules are nucleated in the vicinity of chromatin, they do not get organized into a bipolar array. Thus, even though the acentrosomal pathway of spindle assembly is active during the female meiotic divisions in C. elegans, it is either absent or inefficient during the mitotic division that follows.

Many cells possess the capacity to form centrioles de novo, for instance following ablation of resident centrioles in human cells or in parthenogenetic activated oocytes (Khodjakov et al. 2002; Miki-Noumura 1977; Szollosi and Ozil 1991). Our analysis indicates that this is not the case in early C. elegans embryos, at the least during the first two cell cycles, since we did not observe de novo centriole formation by time-lapse DIC microscopy among class III embryos.

**APC5 related subunits in C. elegans:**

Across eukaryotic evolution, the complete loss of APC/C function blocks the cell cycle at the metaphase-anaphase transition. Accordingly, newly fertilized C. elegans embryos lacking APC/C function arrest during metaphase of meiosis I (Davis et al. 2002; Furuta et al. 2000; Golden et al. 2000). By contrast, both meiotic divisions are completed in embryos of the two extant such-1 alleles. However, neither such-1(t1668) nor such-1(h1960) is a molecular null, and the partial rescue of embryonic viability in such-1(t1668)
smg-2(e2008) animals suggests that the corresponding mutant protein retains residual activity. More importantly, there are two genes encoding APC5 proteins in *C. elegans*, *such-1* and *gfi-3* (Davis et al. 2002; Tarailo et al. 2007). Both corresponding transcripts are expressed in the gonad and the embryo (Yuji Kohara personal communication; http://nematode.lab.nig.ac.jp). Although *gfi-3(RNAi)* increases the embryonic lethality conferred by *such-1(h1960)* animals, it does not confer meiotic arrest (data not shown). However, subjecting *such-1* mutant animals simultaneously to *gfi-3(RNAi)* and *such-1(RNAi)* results in the vast majority of embryos being arrested in meiosis I (Stein et al., this issue of *Genetics*), indicating that the two APC5 components act largely redundantly at this stage.

Therefore, in contrast to the situation in Drosophila, where some APC/C substrates are turned over in embryos harboring a genetic null mutation in the sole APC5 gene *ida* (Bentley et al. 2002), the available evidence indicates that in *C. elegans* APC5 is essential for canonical APC/C function.

**Mutual antagonism between the Anaphase Promoting Complex and the Spindle Assembly Checkpoint:**

In most systems, engagement of the SAC results in a drastic delay of mitotic progression. By contrast, in *C. elegans* embryos, SAC engagement causes only a two-fold prolongation in M phase progression (Encalada et al. 2005; Essex et al. 2009), similar to the minor delay observed in other early embryonic systems (Minshull et al. 1994). Moreover, partial inactivation of the APC/C also results in merely a marginal delay during mitosis in *C. elegans*
embryos (TARAILO et al. 2007). Importantly, we found that engaging the SAC in embryos with compromised APC/C function results in a substantial prolongation of M phase, which lasts approximately 7 times longer than in the wild-type. At least two models could explain the mechanism leading to prolonged M phase duration in APC/C mutants. First, the SAC, which negatively regulates the APC/C, may further compromise the already crippled APC/C. Since APC/C governs mitotic exit, the combination of SAC engagement and compromised APC/C may provoke the extended duration of mitosis. In this first model, one might expect that the overall delay should correspond to the additive effect of these two factors. However, whereas the mitotic delay compared to the wild-type is 2-fold upon SAC engagement and 1.3-fold in such-1(t1668) embryos with a bipolar spindle, it is 7-fold in such-1(t1668) mutant embryos with an engaged checkpoint. Because of this synergistic effect, we favor a second model in which the APC/C negatively regulates the SAC in C. elegans embryos (Fig. 7A), as has been proposed from biochemical and cell biological experiments in human cells (REDDY et al. 2007). During SAC engagement in the wild-type, the ubiquitination by the APC/C of substrates such as securin and cyclin B is inhibited, due to sequestration of Cdc20 by a MAD-2- and/or BubR1-containing complex. At the same time, Cdc20 is turned over, and this continuous degradation is dependent on the SAC as well as on the APC/C (NILSSON et al. 2008; PAN and CHEN 2004; REDDY et al. 2007). Once the SAC is inactivated, Cdc20 becomes available to fully activate the APC/C, thus enabling efficient ubiquitination of mitotic substrates. Both Cdc20 accumulation and Cdc20 liberation from the MAD2- and/or BubRI-containing complex have been
proposed to be instrumental for full APC/C activation at this stage (Nilsson et al. 2008; Pan and Chen 2004). Our observations support the notion that the APC/C participates in inactivating the SAC (Reddy et al. 2007), and thus that the SAC and the APC/C have a mutual antagonistic relationship in C. elegans embryos (Fig. 7A). When APC/C is compromised, this negative feedback mechanism is dampened, leading to prolonged SAC engagement and extended mitosis (Fig. 7B).

**Mechanisms of SAC inactivation by the APC/C:**

How does the APC/C participate in inactivating the SAC in C. elegans embryos? We found that reduction of ubiquitination by partial depletion of the E1 enzyme UBA-1 also provokes excess SAC engagement upon monopolar spindle assembly. The relevant ubiquitination event may influence the function or the half-life of the substrate. Regardless, our observations are in line with those in human cells, where ubiquitination of substrates promotes mitotic exit after SAC engagement (Garnett et al. 2009; Reddy et al. 2007; Summers et al. 2008; Williamson et al. 2009). In this context, the APC/C together with the E2 UbcH10 monoubiquitinates substrates, which are subsequently elongated by the E2 Ube2S, leading to substrate degradation (Garnett et al. 2009; Summers et al. 2008; Williamson et al. 2009; Wu et al. 2010). Interestingly, Ube2S depletion does not affect cell cycle progression or cyclin B degradation in unperturbed cells, but becomes essential for SAC inactivation when microtubules are disrupted (Garnett et al. 2009). This raises the possibility that Ube2S is a specific SAC silencing E2 enzyme in humans. A Ube2S homolog has been identified but not yet studied in Drosophila, but sequence
analysis did not reveal a *C. elegans* counterpart (Jones et al. 2002), raising the possibility that other mechanisms are at play in this respect in nematodes.

Which critical substrates might be ubiquitinated by the APC/C to inactivate the SAC in *C. elegans*? A plausible candidate is Cdc20 itself. Cdc20 is ubiquitinated by the APC/C and thereby targeted for degradation during SAC engagement in human cells (Nilsson et al. 2008; Pan and Chen 2004; Reddy et al. 2007). Moreover, Cdc20 ubiquitination reduces binding to MAD2 (Reddy et al. 2007), and this has been proposed to constitute a mechanism whereby the checkpoint is inactivated. However, the fact that a non-ubiquitylatable form of Cdc20 drives checkpoint-arrested human cells out of mitosis challenged these views (Nilsson et al. 2008), such that the exact contribution of Cdc20 ubiquitination remains to be resolved.

Our findings underscore the essential role of FZY-1/Cdc20 during prolonged SAC activation in *C. elegans* embryos. The *fzy-1*(*av15*) allele used to suppress the drastic M phase delay was proposed to affect a MAD2 binding domain (Stein et al. 2007), which likely is critical for communicating the status of the SAC to the APC/C. Because Cdc20 levels are cell-cycle regulated and its overexpression overrides the SAC in yeast, it appears that Cdc20 levels or activity control the strength of SAC engagement (Pan and Chen 2004; Prinz et al. 1998; Schott and Hoyt 1998). In early *C. elegans* embryos, FZY-1/Cdc20 levels might be more stable, as both interphase and M phase cells stain brightly for FZY-1 (AB and PG, unpublished observation; Kitagawa and Rose 1999). In these rapidly dividing cells, degradation of FZY-1/Cdc20 at the end of the cell cycle might not occur; instead, there might be a slow decline, as observed for cyclin B in *Drosophila* syncytial embryos (Edgar et al. 1994).
In conclusion, our findings lead us to propose that the APC/C negatively regulates the SAC in early *C. elegans* embryos, suggesting that mutual antagonism between the APC/C and the SAC modulates the duration of mitosis during the cleavage divisions of a developing organism.
ACKNOWLEDGMENTS

We are grateful to Ralf Schnabel for having generated such-1(t1668) mutant animals (Gönczy et al. 1999). We thank Arshad Desai, Andy Golden, Frank McNally, Diane Shakes, Susan Strome and Ann Rose for worm strains and antibodies, Karine Baumer for generation of RNAi constructs, Yuji Kohara for expression data, Andy Golden also for suggestions and sharing results prior to publication, Diane Shakes for helpful suggestions, as well as Virginie Hachet, Tamara Mikeladze-Dvali, Viesturs Simanis and Adam Williamson for making useful comments to improve the manuscript. Some nematode strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). Supported by a Boehringer Ingelheim Fonds PhD student fellowship (to AB) and the Swiss National Science Foundation (grant 3100A0-122500/1 to PG).
FIGURE LEGENDS

FIGURE 1

Phenotypes of *such-1(t1668)* embryos:

Still images from time-lapse DIC recordings; time relative to onset of NEBD in min:sec. Anterior or polar body position is left in this and other figures. Scale bar = 10 µm. Asterisks: pronuclei (female left, male right); black arrowheads: spindle poles; white arrowhead: center of monopolar configuration. See also Movies 1-4.

(A) (-5:00): wild-type embryo with one female and one male pronucleus; a pseudocleavage furrow forms. (+2:30): a bipolar spindle assembles during mitosis. (+9:00): the first division gives rise to a larger anterior AB cell and a smaller posterior P₁ cell.

(B-D) *such-1(t1668)* embryos classified according to the number of pronuclei and the presence of centrosomes. All embryos examined contain a single female pronucleus with normal size. N=38; percentages on the right indicate the fraction of embryos within each class.

(B) (-5:00): class I embryos possess one male and one female pronucleus; a pseudocleavage furrow forms. (+2:30): these embryos assemble a spindle that is bipolar (5/11) or multipolar (6/11; not shown, presumably when supernumerary centrioles were contributed by the sperm). (+9:00): all class I embryos divide.

(C) (-5:00): class II embryos have a female pronucleus, but no male pronucleus; pseudocleavage occurs. (+2:30): centrosomes are present and form either a bipolar spindle (10/21) or a multipolar spindle (6/21; not shown).
Sometimes the spindle appears monopolar initially, with strong microtubule nucleation from the MTOC, but resolves within the same prolonged cell cycle into a bipolar spindle (5/21; not shown), possibly reflecting a defect in the timing of centriole disengagement. (+9:00): all class II embryos divide. (D) (-5:00): class III embryos have a female pronucleus, but no male pronucleus; no pseudocleavage occurs. (+2:30): MTOCs are absent and embryos undergo monopolar mitosis, with microtubules nucleated weakly around the chromatin (6/6; see also Fig 2 C). (+9:00): embryos are still in mitosis and fail to divide even after NER and exit from mitosis (see Movie 4).

**FIGURE 2**

*such-1(t1668)* embryos lacking centrioles undergo monopolar mitosis:

Immunofluorescence of mitotic one-cell stage embryos of the indicated parental genotypes. Centrioles (SAS-4, red), microtubules (α-tubulin, green) and Hoechst counterstain to visualize DNA (blue). Insets show 6 fold magnified view of the SAS-4 signal in the center of the asters. Scale bar = 10 µm

(A) wild-type embryo with four centrioles; the two centrosomes nucleate two dense microtubule asters and direct bipolar spindle assembly.

(B) *such-1(t1668)* class I or II embryo (the two classes cannot be distinguished in fixed specimens during mitosis) with four centrioles and a bipolar spindle. 1/28 embryos with a monopolar spindle harbored centrioles and a dense array of microtubules, probably corresponding to the subset of embryos with a putative defect in the timing of centriole disengagement (described in the legend of Fig. 1C).
(C) *such-1(t1668)* class III embryo, which lack centrioles. Microtubules are nucleated around the chromatin, resulting in monopolar mitosis

(D) *spd-5(or213)* embryo, in which centrosome function is compromised despite the presence of centrioles, thus also resulting in monopolar mitosis.

**FIGURE 3**

*such-1(t1668)* sperm with aberrant DNA and centriole content causes embryonic lethality:

(A-D) Immunofluorescence of wild-type and *such-1(t1668)* sperm. Centrioles (SAS-4, red), sperm membranes (SP-56, green) and Hoechst counterstain to visualize DNA (blue). Scale bar = 1µm. Wild-type: N=58; *such-1(t1668)*: N=87; percentages on the right indicate the fraction of *such-1(t1668)* sperm of each class. Sperm with abnormal shapes or decondensed DNA were not considered.

(A) wild-type sperm with highly condensed DNA and one clear SAS-4 focus (corresponding to a pair of tightly apposed centrioles).

(B) *such-1(t1668)* class I sperm with DNA. These sperm contain a single SAS-4 focus (6/28, corresponding to a pair of tightly apposed centrioles), two SAS-4 foci (19/28, presumably corresponding to four centrioles; not shown), or no visible SAS-4 (3/28, not shown).

(C) *such-1(t1668)* class II sperm without DNA but with centrioles. These sperm contain either a single SAS-4 focus (35/41) or two SAS-4 foci (6/41, not shown).

(D) *such-1(t1668)* class III sperm without DNA nor centrioles (18/18); the cell was identified as sperm based on its shape, size and SP-56 staining.
(E) *such-1*(t1668) is paternal effect embryonic lethal. Average embryonic lethality of progeny from wild-type or *such-1*(t1668) hermaphrodites, *such-1*(t1668) hermaphrodites mated with *plg-1*(e2001) males and that exhibited a gelatinous plug on the vulva, as well as *fog-2*(q71) females mated by *such-1*(t1668) males. Error bars: standard error of mean; N= number of embryos from a minimum of 21 hermaphrodites or 4 *such-1*(t1668) males.

**FIGURE 4**

**Molecular characterization of *such-1*(t1668):**

(A) mapping of *such-1*(t1668): SNP mapping positioned *such-1*(t1668) between 10.77 cM and 11.51 cM on the right arm of LGIII. The *such-1* mRNA contains 12 exons.

(B) schematic representation of exons 6 and 7 and of the intervening intron 6 in wild-type and *such-1*(t1668) animals, as well as image of relevant RT-PCR products. In the wild-type, the most prevalent mRNA species is one in which intron 6 is spliced out (lane 1, bottom band, small mRNA). By contrast, in *such-1*(t1668), the most prevalent mRNA species retains intron 6 (lane 2, top band, large mRNA). This change in ratio is due to the fact that the small mRNA is degraded in *such-1*(t1668) embryos by the NMD pathway, since the small mRNA prevails in *such-1*(t1668) *smg-2*(e2008) animals (lane 4).
FIGURE 5

Monopolar mitosis in APC/C mutants substantially increases M phase duration:

Bars represent average M phase duration in one-cell stage embryos of the indicated genotypes measured from the beginning of NEBD until NER in DIC time-lapse recordings. N= number of embryos. Error bars: standard error of mean.

(A) such-1(t1668) class I and class II embryos with a bipolar spindle are delayed only slightly during M phase, while class III embryos are delayed 7-fold.

(B) monopolar mitosis in APC/C mutants substantially increases M phase duration. Note that all mutant embryos are delayed upon spd-5(RNAi).

(C) ubiquitination influences M phase duration in monopolar mitosis. Albeit partial RNAi of uba-1 (in addition to spd-5(or213)) provokes a modest increase in M phase duration, the difference with partial uba-1(RNAi) alone is statistically significant (paired Student’s T-test; p = 1.9 x 10^-5).

(D) partial reduction of FZY-1 function by partial RNAi or with fzy-1(h1983) provokes a modest but statistically significant increase in M phase duration in embryos with a monopolar spindle induced by SPD-5 depletion (paired Student’s T-test compared to fzy-1(RNAi) p= 1.4 x 10^-4; fzy-1(h1983) p= 3.7 x 10^-5). fzy-1(av15) rescues M phase duration in conditions that normally cause a drastic delay (compare to (B)).
FIGURE 6
The substantial increase in M phase duration in APC/C mutants with monopolar mitosis is caused by SAC engagement:
(A) MDF-2-GFP localization in one-cell stage embryos of the indicated genotypes. Note that MDF-2-GFP accumulates for an extended period on chromatin of such-1(t1668) spd-5(RNAi) embryos. Time in min:sec relative to onset of NEBD. Arrowhead points to MDF-2-GFP enrichment on chromatin.
(B) Duration of MDF-2-GFP enrichment on chromatin measured from NEBD.
(C) Inactivation of the SAC through mdf-1 or mdf-2 depletion reduces M phase delay in hypomorphic APC/C mutants.

FIGURE 7
Working model highlighting the mutual antagonism between the SAC and APC/C\textsuperscript{Cdc20}:
(A) In the wild-type, SAC engagement inhibits APC/C\textsuperscript{Cdc20} activity; APC/C\textsuperscript{Cdc20} reciprocally negatively regulates the SAC. Increased APC/C\textsuperscript{Cdc20} activity is required to turn off the SAC and allow sister chromatid segregation and M phase exit.
(B) In conditions where APC/C\textsuperscript{Cdc20} activity is low, the negative regulation from the APC/C\textsuperscript{Cdc20} is weakened, which results in a substantially prolonged engagement of the SAC, and hence increased M phase duration.
SUPPLEMENTAL MATERIAL

SUPPLEMENTAL MOVIES
Time-lapse DIC recordings of the first cell cycle in embryos from hermaphrodites with the indicated genotype. Frames were captured every 10 seconds and the movies are played at 10 frame per second. Embryos are approximately 50 µm-long and oriented with anterior to the left. Elapsed time is indicated in seconds since the beginning of the recording.

Movie 1: wild-type embryo with a male and female pronucleus setting up a bipolar spindle.

Movie 2: class I such-1(t1668) embryo, which is indistinguishable from wild-type.

Movie 3: class II such-1(t1668) embryo with a single female pronucleus and a bipolar spindle.

Movie 4: class III such-1(t1668) embryo with a single female pronucleus. A monopolar spindle forms, and the cell does not divide. The duration of the first cell cycle is ~40 min.

Movie 5: spd-5(RNAi) embryo undergoing monopolar mitosis; the cell cycle is only slightly prolonged.

SUPPLEMENTAL FIGURE S1

such-1(t1668) embryos lacking centrosomes undergo monopolar mitosis:
Immunofluorescence of mitotic one-cell stage embryos of the indicated parental genotypes. Centrosomes (TAC-1, red), microtubules (α-tubulin,
green) and Hoechst counterstain to visualize DNA (blue). Insets show 4 fold magnified view of the TAC-1 signal in the center of the asters. Scale bar = 10 µm.

(A) wild-type embryo with two centrosomes which nucleate two dense microtubule asters and direct bipolar spindle assembly.

(B) such-1(t1668) class I or II embryo (the two classes cannot be distinguished in fixed specimens during mitosis) with two centrosomes and a bipolar spindle. The weaker TAC-1 signal in the wild-type reflects the fact this embryo is at a slightly more advanced stage of the cell cycle.

(C) such-1(t1668) class III embryo, which lacks centrosomes. Microtubules are nucleated around the chromatin, resulting in a monopolar configuration.

SUPPLEMENTAL FIGURE S2

Interphase duration is undistinguishable in wild-type and all classes of such-1(t1668) embryos:

Bars represent average M phase (white) and interphase duration (grey) in embryos of the indicated genotypes. M phase was measured from NEBD until NER in one-cell stage embryos (see also Fig. 5). Interphase was measured in cell cycle 2 from the beginning of NER until NEBD. The time point 0 min corresponds to NER at the end of cell cycle 1. In wild-type, such-1(t1668) class I and such-1(t1668) class II embryos, interphase in the AB cell was measured. For such-1(t1668) class III embryos, recordings were performed on such-1(t1668) animals expressing a transgene (TAC-1-GFP or PAR-6-GFP). N= number of embryos. Error bars: standard error of mean.
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FIGURE 1

- **wild-type**: 100 %
- **class I**: 29 %
- **class II**: 55 %
- **class III**: 16 %

*FIGURE 1*
FIGURE 2

wild-type

such-1(t1668) class I or II

such-1(t1668) class III

spd-5(or213)

α-tubulin/ DNA/ SAS-4
<table>
<thead>
<tr>
<th>Class</th>
<th>Genotypes</th>
<th>Percentages</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wild-type ♂</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>such-1(t1668) ♂</td>
<td>32%</td>
<td>605</td>
</tr>
<tr>
<td></td>
<td>such-1(t1668) ♂ x plg-1(e2001) ♂</td>
<td>47%</td>
<td>514</td>
</tr>
<tr>
<td></td>
<td>fog-2(q71) ♀ x such-1(t1668) ♂</td>
<td>21%</td>
<td>653</td>
</tr>
</tbody>
</table>

**FIGURE 3**

DNA/ SAS-4/ SP-56
such-1 (t1668) is a frameshift mutation caused by a 1155delA deletion in exon 6.

FIGURE 4
<table>
<thead>
<tr>
<th>Condition</th>
<th>A Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>such-1(t1668) class I</td>
</tr>
<tr>
<td></td>
<td>N=8</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>such-1(t1668) class II</td>
</tr>
<tr>
<td></td>
<td>N=5</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>such-1(t1668) class III</td>
</tr>
<tr>
<td></td>
<td>N=9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>B spd-5(RNAi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>such-1(t1668) + spd-5(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=9</td>
</tr>
<tr>
<td></td>
<td>such-1(h1960)</td>
</tr>
<tr>
<td></td>
<td>N=11</td>
</tr>
<tr>
<td></td>
<td>such-1(h1960) + spd-5(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=12</td>
</tr>
<tr>
<td></td>
<td>emb-27(g48)</td>
</tr>
<tr>
<td></td>
<td>N=11</td>
</tr>
<tr>
<td></td>
<td>emb-27(g48) + spd-5(RNAi)</td>
</tr>
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<td></td>
<td>N=4</td>
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</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ceba-1(RNAi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spd-5(or213) + uba-1(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>D fzy-1(RNAi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spd-5(or213) + fzy-1(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=5</td>
</tr>
<tr>
<td></td>
<td>fzy-1(h1983)</td>
</tr>
<tr>
<td></td>
<td>N=9</td>
</tr>
<tr>
<td></td>
<td>fzy-1(h1983) + spd-5(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=15</td>
</tr>
<tr>
<td></td>
<td>fzy-1(av15)</td>
</tr>
<tr>
<td></td>
<td>N=8</td>
</tr>
<tr>
<td></td>
<td>fzy-1(av15) + spd-5(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=7</td>
</tr>
<tr>
<td></td>
<td>such-1(h1960); fzy-1(av15)</td>
</tr>
<tr>
<td></td>
<td>N=6</td>
</tr>
<tr>
<td></td>
<td>such-1(h1960); fzy-1(av15) + spd-5(RNAi)</td>
</tr>
<tr>
<td></td>
<td>N=14</td>
</tr>
</tbody>
</table>

**FIGURE 5**
FIGURE 6

A

MDF-2 - GFP

such-1(h1960) + spd-5(RNAi) + spd-5(RNAi)

B

spd-5(RNAi) + spd-5(RNAi)
such-1(h1960) + spd-5(RNAi)

C

such-1(h1960) + spd-5(RNAi)
such-1(h1960); mdf-1(gk2) + spd-5(RNAi)
such-1(t1668) class III
such-1(t1668) class III + mdf-1(RNAi)
such-1(t1668) class III + mdf-2(RNAi)

MDF-2 - GFP enrichment

M phase duration
FIGURE 7

A

\[
\begin{array}{c}
\text{SAC} \\
\text{APC/C Cdc20} \\
\text{M phase} \\
\text{SAC} \\
\text{APC/C Cdc20}
\end{array}
\]

wild-type

B

\[
\begin{array}{c}
\text{SAC} \\
\text{APC/C Cdc20} \\
\text{M phase} \\
\text{SAC} \\
\text{APC/C Cdc20}
\end{array}
\]

APC/C function compromised

FIGURE 7