Depletion of a Cks homolog in *C. elegans* embryos uncovers a post-metaphase role in both meiosis and mitosis

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In eukaryotic cells, the key regulators of cell-cycle transitions are the cyclin-dependent kinases (CDKs). The best studied CDK is a component of the M-phase promoting factor (MPF), which promotes entry into and progression through meiosis and mitosis. One of the enduring mysteries of the MPF complex has been the role of Cks/Suc1, a highly conserved member of the cell-cycle machinery in eukaryotes [1,2]. Cks has been proposed to be involved in activation of MPF [3], general interactions of MPF with its mitotic substrates [4] and/or inactivation of MPF [5,6]. We identified two Cks homologs in the genome of *Caenorhabditis elegans* and used RNA-mediated interference (RNAi) to investigate their roles in development. Whereas cks-2(RNAi) embryos display no apparent defects, cks-1(RNAi) embryos display defects in both meiosis and mitosis. Specifically, cks-1(RNAi) embryos fail to exit M phase properly. We propose that Cks-1 has an essential role in the inactivation of MPF during early *C. elegans* embryogenesis.

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Results and discussion

The *C. elegans* genome contains two homologs of Cks/Suc1, termed *cks-1* and *cks-2* [7] (see Supplementary material). To eliminate the maternal load of *cks-1* or *cks-2*, double-stranded (ds) RNA was prepared from full-length cDNA and injected into adult hermaphrodites. This method of RNA-mediated interference is an extremely potent and specific way of reducing gene expression, and has been shown to phenocopy strong or null mutations in maternally expressed genes [8,9]. Within 12 hours after injection of *cks-1* dsRNA, 100% of the progeny displayed defects in completion of maternal meiosis and embryonic mitoses (Figures 1–4) and arrested later in embryogenesis. In contrast, injection of *cks-2* dsRNA resulted in no detectable defects. Co-injection of *cks-1* and *cks-2* dsRNA did not alter the phenotype caused by *cks-1* dsRNA alone.

*cks-1(RNAi)* embryos initiate, but are defective in completing, the maternal meiotic divisions, which normally occur after fertilization and result in the maternal pronucleus and two polar bodies. *cks-1(RNAi)* embryos initiated the meiotic divisions, as evidenced by formation of a meiotic spindle (Figure 2k). The meiotic spindle persisted, however, while the centrosomes adjacent to the paternal pronucleus began to nucleate astral microtubules for the first mitotic spindle (Figures 1f,g,2m,n). To determine whether *cks-1(RNAi)* embryos can complete meiosis I, we incubated live embryos in the DNA dye DAPI, which normally stains the first polar body but not the second polar body nor DNA inside the embryo (Figure 3a). *cks-1(RNAi)* embryos did not display staining of a polar body (Figure 3b), like the meiosis mutant *mei-1* (Figure 3c). This indicates that RNAi defects occur as early as meiosis I. To analyze the fate of the maternal chromosomes, we stained fixed embryos with DAPI and also observed histone tagged with green fluorescent protein (GFP–histone) in living *cks-1(RNAi)* embryos. The maternal DNA became separated into multiple micronuclei (Figure 4h–j) and/or masses of condensed DNA (data not shown), of which only a subset migrated to join the paternal pronucleus (Figure 4h–j). Some of the maternal DNA was eventually pinched off into an unusually large polar body (Figures 1f–h,2o,p). Taken together, our results indicate that *cks-1(RNAi)* embryos are able to initiate but not correctly execute the meiotic divisions. Our observation of at least some meiotic chromosome separation (Figures 2n, 4h) suggests that the meiotic chromosomes do progress past metaphase.

Previous analyses of Cks homologs in *Schizosaccharomyces pombe* and *Xenopus* have suggested two different meiotic roles for these proteins. In *S. pombe*, depletion or overexpression of the Cks homolog Suc1 caused a frequent failure of meiosis II, resulting in two-spored ascii [10]. Studies using extracts of unfertilized *Xenopus* eggs suggest that the Cks homolog Xe-p9 participates in releasing oocytes from arrest in meiotic metaphase II [11], which predicts that the Xe-p9 mutant phenotype would be a prolonged meiotic metaphase arrest. We observe a different meiotic phenotype in *C. elegans* CKS-1-depleted embryos. Unlike the predicted *Xenopus* phenotype, *cks-1(RNAi)* embryos do not display meiotic metaphase arrest. Unlike yeast Suc1 mutants, *cks-1(RNAi)* embryos are unable to normally execute the first meiotic division. By analogy with the mitotic defects described below, we think it likely that *cks-1(RNAi)* embryos fail to exit meiosis normally.
Figure 1

Nomarski analysis of live embryos. Early development of (a–e) a wild-type embryo and (f–j) a cks-1(RNAi) embryo was recorded by Nomarski video microscopy. Embryos measure 30 x 50 μm and in all figures are oriented with anterior to the left. Spindle poles are indicated with white arrowheads. (a) In wild type, the maternal pronucleus (left) and the paternal pronucleus (right) have met and are migrating to the center. (b,c) The spindle, seen as a granule-free zone in the center, is oriented along the anterior–posterior (A–P) axis. (d) Cytokinesis is initiated. (e) After a cycle of nuclear envelope reformation and breakdown, spindles form for the second mitotic division. (f,g) In cks-1(RNAi) embryos, no maternal pronucleus forms; the paternal pronucleus and associated MTOCs migrate to the center. The embryo pinches off an anterior body of cytoplasm containing DNA (see Figures 2, 4), which we interpret to be an abnormally large polar body. (h) The paternal nuclear envelope breaks down, and a spindle is apparent. Cytokinesis is initiated but fails. (i) Two spindles form in a common cytoplasm. There were no signs of nuclear envelope reformation before this stage.

Even in the absence of a normal meiosis, cks-1(RNAi) embryos are able to initiate mitosis. The paternally supplied centrosome duplicated and nucleated astral arrays of microtubules adjacent to the paternal pronucleus. These microtubule-organizing centers (MTOCs) migrated and rotated onto the anterior–posterior axis of the embryo and generated a normal-appearing mitotic spindle (Figures 1f–h, 2k,m,o). During this time, the paternal DNA transformed from a diffuse interphase configuration to condensed chromosomes typical of mitotic prophase, after which nuclear envelope breakdown occurred (Figure 1g–i, 4h–k).

We used chromosome morphology in GFP–histone-containing embryos to analyze the time course of mitotic events. During the first cell cycle in cks-1(RNAi) embryos the centrally located chromosomes condensed, assumed a metaphase configuration and underwent anaphase separation (Figure 4k–m). The metaphase and anaphase masses of DNA appeared more ragged than in wild type, however (Figure 4e,f,l,m). More important, the chromosomes stayed condensed and did not return to an interphase morphology (Figure 4n), nuclear envelopes did not reform, and microtubules remained in mitotic configurations (asters and spindles). Despite the failure of RNAi embryos to exit from a mitotic state, MTOCs continued duplicating. This led to embryos with many MTOCs, asters and spindles, but without a correspondingly large amount of DNA, suggesting that few rounds of S phase had

Figure 2

Distribution of tubulin and DNA. (a–j) Wild-type and (k–t) cks-1(RNAi) embryos were fixed and stained with mouse 4A1 anti-tubulin at 1:50 (gift of M. Fuller) (first and third rows) and DAPI (second and fourth rows). (a,b) In wild type, the maternal DNA and meiotic spindle are anterior, and the condensed paternal DNA posterior. (c,d) Maternal meiosis has been completed, polar bodies have been extruded (arrows), pronuclei have formed at opposite ends of the embryo, and the sperm-supplied centrosomes have begun nucleating microtubules. (e,f) The first mitotic spindle has rotated onto the A–P axis, and chromosomes are in metaphase/early anaphase. (g,h) Two-cell embryo undergoing second mitosis. (i,j) Later-stage embryo containing several mitotic spindles and many cells with interphase arrays of microtubules. (k–n) In cks-1(RNAi) embryos, microtubules remain associated with the maternal DNA as the centrosomes, nucleate the mitotic spindle. (o,p) The mitotic spindle has rotated onto the A–P axis, and the chromosomes appear to be in prometaphase or early anaphase. Notice the extra microtubule mass still associated with the maternal DNA in an over-sized polar body (arrow). (q,r) Two spindles have formed in a common cytoplasm. Each spindle is associated with multiple pieces of DNA. (s,t) Later-stage embryo containing many spindle poles. Little DNA is associated with the spindles.
occurred (Figure 2s,t). Our studies indicate that CKS-1-depleted embryos fail to exit mitosis, although their ability to continue duplicating MTOCs reveals that not all aspects of the cell cycle are arrested.

Proposed mitotic functions for Cks can be separated into four classes. First, activation of MPF and entry into M phase [3,11]. Cks may promote interaction of the mitotic CDK with its activating phosphatase CDC25. Interference with this step should prevent a cell from entering M phase. Second, interactions between MPF and early mitotic substrates [4]. If Cks facilitates these interactions, then interference with Cks function should cause defects in the early stages of M phase. Third, MPF activation of the ubiquitin-mediated degradation pathway and initiation of anaphase. Cks may promote an interaction of MPF with the anaphase-promoting complex (APC) or with the proteasome itself [5,6]. Disruption of this pathway should cause a metaphase-arrest phenotype due to the stabilization of chromosome ‘cohesion’ proteins. Fourth, MPF inactivation by the ubiquitin pathway and exit from M phase. Cks may facilitate an interaction between ubiquitin pathway components and cyclin B, their target in the MPF complex. Specific interference with cyclin degradation should cause cells to arrest in anaphase or telophase of mitosis [12,13]. Our results place the function of C. elegans CKS-1 in the last class, promoting an interaction between the ubiquitin pathway and MPF, which leads to degradation of cyclin B.

In Xenopus interphase extracts, depletion of Xe-p9 prevents activation of MPF by CDC25 [11]. The ability of CKS-1-depleted embryos to enter M phase suggests that CKS-1 is not required for activation of MPF. This may reflect a situation similar to that in Drosophila embryos; during the initial rapid cell cycles, there is an excess of the normally rate-limiting CDC25 protein [14]. This excess may alleviate the need for CKS-1 to promote an MPF–CDC25 interaction. Indeed, RNAi depletion of the three CDC25 homologs or of the MPF cyclin-dependent kinase (NCC-1) in C. elegans results in failure to progress from prophase to metaphase of meiosis I ([15] and N. Ashcroft, personal communication).

Biochemical studies in Xenopus suggest that Xe-p9 is required for activating the APC during mitosis, while results in budding yeast suggest that Cks1 function affects the activity of the proteasome during this phase [5,6]. Disrupting the function of either the APC or proteasome

Figure 4

Chromosome dynamics in embryos containing GFP–histone. The first cell cycle is shown for (a–g) a wild-type and (h–n) a cks-1(RNAi) embryo. The embryos were produced in a strain expressing a GFP-tagged version of histone H2B (S.S., J. Powers, M. Dunn, K. Reese, G. Seydoux and W. Saxton, in preparation). The expression of this fusion protein results in GFP-labeled chromosomes, as well as diffuse nuclear staining when the nuclear envelope is intact. Expression of GFP–histone and illumination of embryos does not impair cell division or embryo development. (a–c) In wild type, the pronuclei meet and migrate to the center of the embryo. Distinct chromosomes are visible inside the pronuclei. Two compact polar bodies are seen at the anterior end. (d) The disappearance of the diffuse nuclear staining signals nuclear envelope breakdown. (e,f) The chromosomes are in (e) metaphase and (f) anaphase. (g) Two-cell embryo. Chromosomes have decondensed and nuclear envelopes have reformed. (h,j) cks-1(RNAi) embryos form multiple maternal micronuclei, of which only a subset migrate to the paternal pronucleus. (k,l) The nuclear envelopes break down and the centrally located chromosomes assume a metaphase configuration. (m) Chromosomes have separated, but appear more ragged than in wild type. (n) Nuclear envelope reformation and chromosome decondensation do not follow anaphase separation.
Results

Our results reveal an essential role for C. elegans CKS-1 in exit from M phase, and support a model in which CKS-1 promotes an interaction between MPF and ubiquitin-pathway components that leads to degradation of the B-type cyclins and inactivation of MPF. As we cannot be certain that cks-1(RNAi) embryos are truly devoid of CKS-1, we do not claim that CKS-1 is not required for MPF and/or APC activity in C. elegans. In fact, the ‘ragged’ appearance of DNA during metaphase/anaphase in cks-1(RNAi) embryos may reflect an aberrant pattern of ubiquitin-mediated degradation of chromosome cohesion proteins. Regardless, our approach of depleting CKS-1 by RNAi has allowed us to characterize in detail an essential function of CKS-1 in early C. elegans embryogenesis. If other functions of CKS-1 do exist, our results suggest that a role in exit from M phase is the most sensitive to a reduction in CKS-1 levels.

Materials and methods

C. elegans N2 variety Bristol was the wild-type parent of all strains used. Sense and antisense strands of RNA were synthesized using the MEGAscript in vitro transcription kit (AMBION) and annealed before injection at approximately 0.5 mg/ml. Live embryos were observed and recorded by Nomarski differential interference contrast microscopy using a Zeiss Axioplan microscope equipped with a 100× Plan Neofluor objective, a Hamamatsu C2400 series video camera, and an Argus-10 image processor (Hamamatsu Photonics). To visualize protein distributions in embryos, adult hermaphrodites were cut, fixed and stained as described by Strome and Wood [16]. GFP-tagged histone distributions were examined using a BioRad MRC-600 laser scanning confocal microscope. A Z-series was collected at approximately 1 min intervals. Each time point represents an extended focal image, composed of frames collected at 1 μm steps through the embryo using Kalman averaging (7–20 frames per Z-series). Approximate numbers of cks-1(RNAi) embryos observed in each experiment were: Nomarski (17), fixed embryos (> 100), DAPI permeability (> 50) and GFP–histone (5). RNAi embryos consistently displayed the defects described in Results and discussion.

Supplementary material

Supplementary material including a comparison of CKS-1 and CKS-2 with Cks/Suc1 homologs from other organisms and discussion of the evidence that meiotic defects do not themselves lead to mitotic defects is available at http://current-biology.com/supmat/supmatin.htm.

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