cdk-7 is required for mRNA transcription and cell cycle progression in *Caenorhabditis elegans* embryos

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CDK7 is a cyclin-dependent kinase proposed to function in two essential cellular processes: transcription and cell cycle regulation. CDK7 is the kinase subunit of the general transcription factor TFIIH that phosphorylates the C-terminal domain (CTD) of RNA polymerase II, and has been shown to be broadly required for transcription in *Saccharomyces cerevisiae*. CDK7 can also phosphorylate CDKs that promote cell cycle progression, and has been shown to function as a CDK-activating kinase (CAK) in *Schizosaccharomyces pombe* and *Drosophila melanogaster*. That CDK7 performs both functions in metazoans has been difficult to prove because transcription is essential for cell cycle progression in most cells. We have isolated a temperature-sensitive mutation in *Caenorhabditis elegans cdk-7* and have used it to analyze the role of *cdk-7* in embryonic blastomeres, where cell cycle progression is independent of transcription. Partial loss of *cdk-7* activity leads to a general decrease in CTD phosphorylation and embryonic transcription, and severe loss of *cdk-7* activity blocks all cell divisions. Our results support a dual role for metazoan CDK7 as a broadly required CTD kinase, and as a CAK essential for cell cycle progression.

Cyclin-dependent kinases (CDKs) are a family of highly related serine/threonine kinases that require binding to cyclin partners to become active. CDKs were first identified as essential regulators of cell cycle progression. More recently, other CDKs have been implicated in the regulation of mRNA transcription (reviewed in ref. 1). Interestingly, one CDK, CDK7, has been proposed to have essential roles in both of these processes.

CDK7 was originally purified from *Xenopus* extracts as part of a biochemical activity called CAK (CDK-activating kinase) that can phosphorylate a conserved residue in the T loop of CDKs required for full kinase activity (2). Subsequently, CDK7 was also found to be a component of the general transcription factor TFIIH (3–6). As part of TFIIH, CDK7 phosphorylates the C-terminal domain (CTD) of RNA polymerase II (RNAPII) (3, 5–7). The *in vitro* CDK- and CTD-kinase activities of CDK7 appear to be widely conserved, and have been demonstrated in systems ranging from *Schizosaccharomyas pombe* (8, 9) to rice (10) and mammals (11). One exception is Kin28, the *Saccharomyces cerevisiae* homologue of CDK7, which has CTD-kinase activity but lacks CAK activity (12, 13). Instead, a distantly related protein, Cak1/Civ1, has been identified as the sole essential CAK in this system (14, 15).

Although the *in vitro* activities of CDK7 are well established, direct demonstration that CDK7 performs both functions *in vivo* has proven more challenging. As phosphorylation of the CTD of RNAPII is required for promoter clearance during transcription, lack of CTD-kinase activity is expected to affect transcription of most genes. Consistent with this prediction, Kin28 was shown by microarray analysis to be required for virtually all mRNA transcription (16). Evidence for a broad transcriptional role in metazoans, however, has been less direct. In *Drosophila*, expression of a dominant negative CDK7 was found to delay the onset of embryonic transcription (17), but another study that used a loss-of-function mutation in CDK7 did not detect transcriptional defects, even though CAK activity and cell division were clearly defective (18). Similarly in mice, knockout of MAT1, an essential cofactor of CDK7, was found to block cell cycle progression, but, although defects in CTD phosphorylation were observed, defects in transcription were not (19).

The *Caenorhabditis elegans* embryo offers a unique opportunity to study the transcriptional and cell cycle roles of CDK7. Embryonic development begins with a series of highly reproducible cell divisions (20). mRNA transcription starts early 3-cell stage (21), but becomes required for cell cycling only after the 100-cell stage (22). Embryos in which RNAPII activity has been eliminated by RNA-mediated interference [*ama-1(RNAi)* embryos] or by treatment with α-amanatin undergo the normal pattern of cell divisions until gastrulation (23). This property makes *C. elegans* embryos an ideal system in which to study the effect of removing essential transcription factors without the complication of inducing cell lethality. Taking advantage of this system, Walker and colleagues (24) were able to distinguish general transcription factors (GTFs) required for essentially all mRNA transcription (such as TFIIB encoded by *ttb-1*) from GTFs with more gene-specific functions (such as the TAF_{II}s *taf-10* and *taf-11*). If CDK7 is required broadly for transcription in *C. elegans*, as it is in *S. cerevisiae*, we would expect *cdk-7* mutants to exhibit transcriptional phenotypes similar to those observed in *ttb-1(RNAi)* and *ama-1(RNAi)* embryos. Additionally, if CDK7 also functions as a CAK, we would expect *cdk-7* mutants to exhibit cell cycle defects before the 100-cell stage.

A recent study identified all CDK/CAK-related genes in *C. elegans* and classified them according to their similarity to family members in yeast and metazoans. This study revealed no *C. elegans* orthologue of yeast Cak1/Civ1, but identified a single CDK7 orthologue, *cdk-7* (25). We have isolated a temperature-sensitive mutation in *cdk-7*, and have used it to study *cdk-7* function in the early embryo. Our analysis supports the view that metazoan CDK7 performs two functions in cells: one essential for mRNA transcription, and one essential for cell cycle progression.

Materials and Methods

Strains and Alleles. The following alleles and genetic rearrangements were used during the course of this study: *axEx1092* (*hsp16–1:his-24:GFP*) (GFP = green fluorescent protein), *wIs28* (*end-1*:GFP) (26), *wIs84* (*elt-2*:GFP) (27), *wIs93* (*med-1*:GFP) (28) LGI: *bli-3(e767), dpy-5(e61), lin-17(n677), unc-13(e450), tDf3, szT1*; LGIII: *ruIs32 (pie-1:GFP:histone H2B* (29); LGX: *axIs36 (pes-10:GFP*; ref. 21). For all experiments involving the

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Abbreviations: CDK, cyclin-dependent kinase; CAK, CDK-activating kinase; RNAPII, RNA polymerase II; CTD, C-terminal domain; RNAi, RNA-mediated interference.

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ax224 allele, mutant hermaphrodites were shifted from 15°C to the nonpermissive temperature of 25°C for 16–20 h before examination of embryos.

Antibodies. The following antibodies were used in this study: mAbH5 (1:10 dilution), mAbH14 (1:10), and mAb8WG16 (1:10; refs. 30 and 31), and mAbE7 (tubulin, 1:1000, Developmental Studies Hybridoma Bank).

Mapping and Cloning. *ax224* was outcrossed two times and mapped using 3-factor mapping and deficiency complementation. *ax224/bli-3(e767) lin-17(n677)* segregated 4/13 Lin non Bli recombinants that carried *ax224*. Complementation tests showed that *tDf3* uncovers *ax224. cdk-7(ax224) dpy-5/+* males were crossed to *tDf3 dpy-5(e61)/szT1* hermaphrodites. Dpy cross-progeny exhibited nonconditional larval lethality and adult sterility. This result placed *ax224* between *mab-20* and *lin-17*, a region covered by YAC Y39G10. Genomic DNA from yeast containing YAC Y39G10 was injected (100 μg/ml) with pRF4 (100 ng/μl) into *ax224* mutant mothers. In 2 of 2 stable lines obtained, weak rescue was observed; at 25°C, transgenic mothers produced a few viable progeny (<10), whereas nontransgenic *ax224* mothers gave 100% dead embryos.

RNA Interference. RNA interference was performed using the feeding method (32). *cdk-1* and *cdk-7* cDNAs were cloned into the feeding vector L4440 to create plasmids pMW1.06 and pMW1.17, respectively. pL4440+ama-1 (33) was used for *ama-1(RNAi). Escherichia coli* HT115 transformants were grown on New Nematode Growth Media plates containing 60 μg/ml ampicillin and 80 μg/ml isopropyl β-**d**-thiogalactoside. L4 hermaphrodites were allowed to feed for 24 h before examination of embryos. For double feeding experiments, one drop of each type of bacteria was seeded onto plates. In control experiments, injection (34) of *cdk-7* double-stranded RNA gave similar results as feeding, and both gave phenotypes similar to that of *cdk-7(ax224)* embryos.

Lineage Analysis. Four-dimensional (4D) movies were made using either a DAGE video camera with Scion LG3 frame grabber, or a Photometrics CoolSnap FX digital camera attached to a Zeiss Axioplan 2 equipped with motorized focus and Ludl shutters. Acquisition scripts were written with the IPLAB software package, and acquired images were processed into QUICKTIME movies by using the 4D turnaround software (Laboratory of Optical and Computational Imaging, Univ. of Wisconsin, Madison). For each embryo, 15 focal planes were imaged, with each focal plane $\approx$1 μm apart. A stack of images was acquired about every 60 s. Cell cycle times were measured relative to completion of cytokinesis.

For interphase and M phase determination, we filmed embryos containing the transgene *ruIs32* (maternally expressed histone H2B:GFP fusion). Single focal plane images were acquired every 20 s using a 0.1-s exposure time and $\approx$15% UV mercury lamp levels by using an AttoArc2 power supply. Under these conditions, embryos could be imaged for at least 2 h without any disruption of cell cycle timing (data not shown). Statistical analysis was performed using a one-tailed Student's *t* test.

***In Situ* Hybridization.** *In situ* hybridization was done as described (35), using the following probes: *pes-10, gfp, cey-2* (21) and rRNA ITS1 (36). ITS1 is a probe to an internal spacer element from the ribosomal DNA locus on LG I. This probe only recognizes transcripts at the site of transcription because the spacer element is rapidly degraded after transcription.

Western Blotting and Immunohistochemistry. mAbH5, mAbH14, and mAb8WG16 immunostaining of embryos was performed as described (36). For Western blotting with mAbH5 and mAbH14, embryonic protein extracts were prepared as described (37), and extracts were run on SDS/10% PAGE.

Results

Identification of *ax224*, an Allele of *C. elegans cdk-7*. To identify genes required for mRNA transcription in embryos, we conducted a genome-wide screen for temperature-sensitive embryonic lethal mutants that fail to express a *pes-10:GFP* transgene (see *Materials and Methods* and ref. 38). Among 1,197 temperature-sensitive lethals, we identified 11 mutants that failed to express *pes-10:GFP* (data not shown). Ten of the 11 mutants arrested with a terminal phenotype similar to that seen in *ama-1(RNAi)* embryos (arrest at the 100-cell stage with no tissue differentiation; ref. 23; Fig. 1*A*). In contrast, one mutant, *ax224*, showed an earlier arrest ($\approx$50-cell stage, Fig. 1*B*). Genetic mapping (see *Materials and Methods*) positioned *ax224* between *mab-20* and *lin-17* on chromosome I. One YAC in this region, Y39G10, could rescue the embryonic lethality of *ax224*. This YAC was shown previously to contain *C. elegans cdk-7* (25). Two lines of evidence confirmed that *ax224* is an allele of *cdk-7*. First, RNA-mediated interference of *cdk-7* resulted in an embryonic lethal phenotype similar to that of *ax224* (Fig. 1*C*; Amy Walker, personal communication). Second, sequencing of *ax224* revealed a missense mutation (C280T) at a conserved cysteine in the last helix of the kinase domain of CDK-7. Based on these findings, we conclude that *ax224* is a temperature-sensitive allele of *cdk-7*.

***cdk-7* Is Required for mRNA Transcription and CTD Phosphorylation.** *cdk-7(ax224)* was identified based on its inability to express a *pes-10:GFP* transgene (Fig. 1 *D* and *E*). We found that *ax224* mutants also fail to express GFP fusions driven by other promoters, including the E lineage-specific *end-1*, *med-1*, and *elt-2* promoters (26–28), and the heat-shock-inducible promoter *hsp-16–2* (Fig. 1 *F–I*; ref. 39 and data not shown). In the latter case, low expression of the *hsp-16–2:GFP* transgene was occasionally seen in a minority of cells of *ax224* mutant embryos. This residual expression may be caused by the fact that *ax224* is not a null allele (see below and *Discussion*).

We used *in situ* hybridization, and confirmed that both GFP transcripts from the *pes-10:*GFP transgene and transcripts from the endogenous *pes-10* gene were absent in *ax224* embryos (Fig. 1 *J* and *K*, and data not shown). In contrast to these embryonically transcribed mRNAs, we were able to detect maternal RNAs in *ax224* embryos (Fig. 1 *L* and *M*). These results indicate that *cdk-7(ax224)* mutants are defective in mRNA synthesis rather than mRNA maintenance. We conclude that *cdk-7* is required for the transcription of many, if not most, mRNAs in *C. elegans* embryos.

CDK7 homologues in other organisms activate transcription by phosphorylating the CTD of RNAPII (3, 5–7). To test whether *C. elegans cdk-7* functions similarly, we examined the phosphorylation state of RNAPII in *cdk-7(ax224)* mutant embryos. The monoclonal antibodies mAbH5 and mAbH14 recognize phosphorylated serine 2 and serine 5, respectively, in each heptapeptide repeat of the CTD (31). In wild-type *C. elegans* embryos, mAb5 and mAbH14 stain all interphase somatic nuclei starting in the 4-cell stage (Fig. 2 *A* and *B* and data not shown; ref. 36). In contrast, in *cdk-7(ax224)* embryos, mAb5 and mAbH14 immunostaining was greatly reduced (Fig. 2 *C* and *D* and data not shown). We confirmed these results by Western analysis comparing protein extracts from wild-type and *ax224* embryos (Fig. 2*E*). H5 and H14 epitope levels were reduced significantly in *cdk-7(ax224)* mutant embryos. Reduced CTD phosphorylation in *ax224* embryos is unlikely to be an indirect consequence of the embryonic lethality of this mutant, because

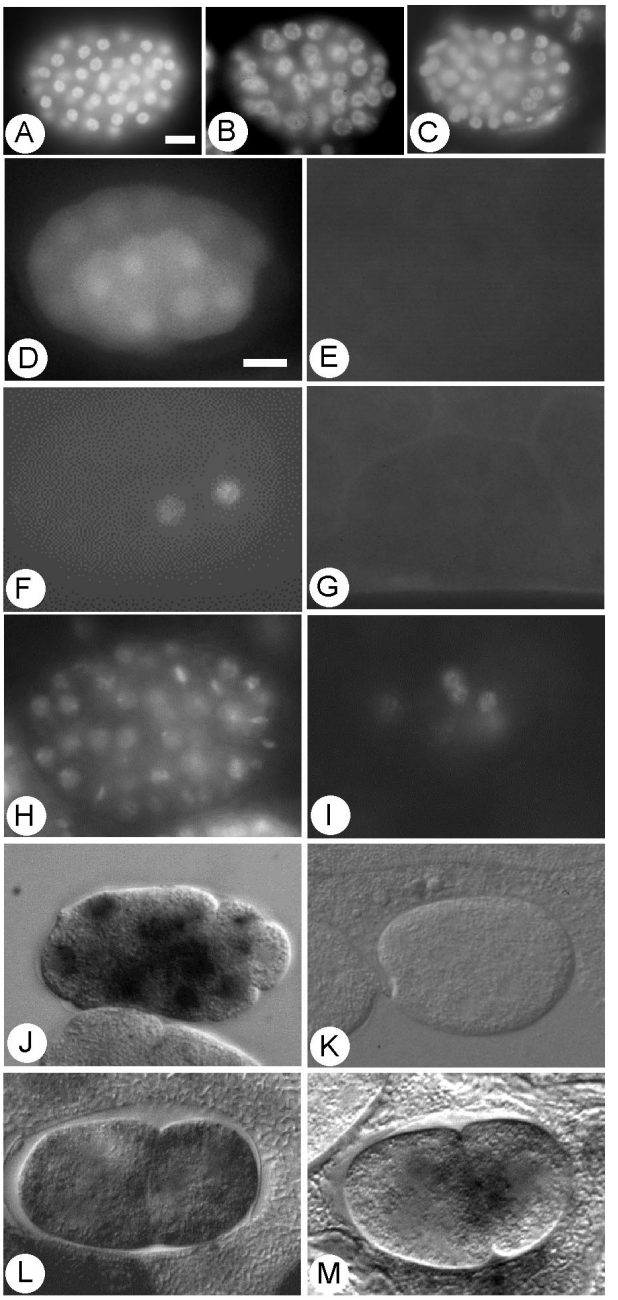

Fig. 1. *cdk-7(ax224)* embryos arrest in the 50-cell stage and do not express embryonically-transcribed RNAs. (*A–C*) DAPI staining of terminal stage *ama-1(RNAi)* (*A*), *cdk-7(ax224)* (*B*), and *cdk-7(RNAi)* (*C*) embryos. *ama-1(RNAi)* embryos arrest after the 100-cell stage, whereas *cdk-7(ax224)* and *cdk-7(RNAi)* embryos arrest around the 50-cell stage. (Scale bar in *A* is 10 μm.) (*D–I*) Expression of GFP transgenes in wild-type and *cdk-7(ax224)* embryos. Wild-type embryos express *pes-10:GFP* in all somatic cells (*D*); no expression is observed in *cdk-7(ax224)* embryos (*E*). Wild-type embryos express END-1:GFP in the two blastomeres of the gut lineage (Ea and Ep) (*F*, 10 of 10 embryos examined); no expression is observed in most *cdk-7(ax224)* embryos (*G*, 9 of 10 embryos examined; the exceptional embryo had weak expression). Wild-type embryos express heat shock promoter: histone:GFP strongly in all somatic nuclei 2 h after a 30-min heat shock at 34°C (*H*); variable expression is observed in some, but not all, nuclei of *cdk-7(ax224)* embryos after heat shock treatment (*I*). (*J–M*) RNA *in situ* hybridization to wild-type (*J* and *L*) and *cdk-7(ax224)* (*K* and *M*) embryos. *pes-10* RNA is expressed in wild-type (*J*), but not in *cdk-7(ax224)* mutant embryos (*K*). Maternal *cey-2* RNA is present in both wild-type (*L*) and *cdk-7(ax224)* (*M*) embryos (diffuse cytoplasmic staining). (Scale bar in *D*, 10 μm, applies to all subsequent embryo images.)

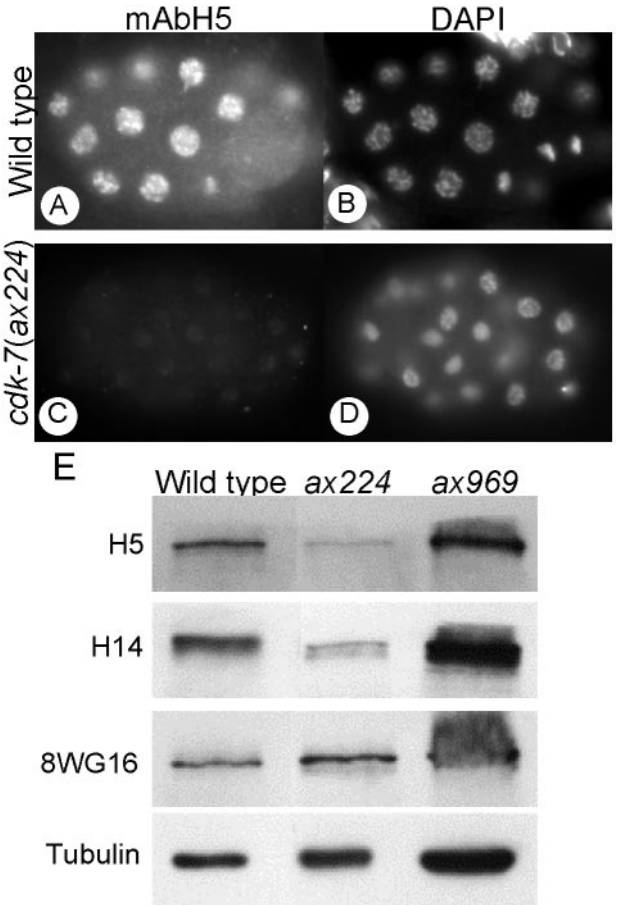

Fig. 2. Phosphorylation of the CTD of RNA polymerase II is reduced in *cdk-7(ax224)* embryos. (*A–D*) Embryonic expression of the CTD phospho-epitope H5 (*A* and *C*) detected by immunofluorescence, with corresponding DAPI staining (*B* and *D*). In wild-type embryos (*A*), H5 staining is observed in all interphase somatic nuclei. In contrast, H5 epitope staining is greatly reduced in *cdk-7(ax224)* mutant embryos (*C*). (*E*) Western blotting of protein extracts from wild-type, *cdk-7(ax224)*, and *ax969* mutant embryos. mAb8WG16 recognizes predominantly unphosphorylated CTD (30), and is included here to demonstrate that RNAPII levels are normal in the mutants. *ax969* is an embryonic lethal mutant with no obvious transcription defects. Tubulin is maternally contributed in embryos (21), and is included here as a protein loading control. Western data shown is from a single gel.

this phenotype was not observed in *ax969*, another lethal mutant recovered in our screen (Fig. 2*E*).

***cdk-7* Is Required for Cell Cycle Progression.** The early (50-cell stage) arrest of *ax224* embryos suggested that *cdk-7* might be required for cell cycle progression in addition to transcription. To explore this possibility, we compared cell division timings between *cdk-7(ax224)*, *ama-1(RNAi)*, and wild-type embryos (Fig. 3*A*). As reported previously (23), we found no significant differences in cell cycle timing between *ama-1(RNAi)* and wild-type embryos, with the exception of the blastomeres Ea and Ep, which divide precociously in *ama-1(RNAi)* embryos (data not shown; the uniquely long cell cycle time of these cells depends on zygotic gene expression, ref. 23). In contrast, *cdk-7(ax224)* mutant embryos consistently exhibited extended cell cycle timings in almost all of the examined lineages. On average, cell cycle times were increased by 52% compared with wild type, with late blastomeres generally more affected than early blastomeres.

To determine whether the increase in cell cycle time was caused by an increase in time spent in interphase or M phase, we

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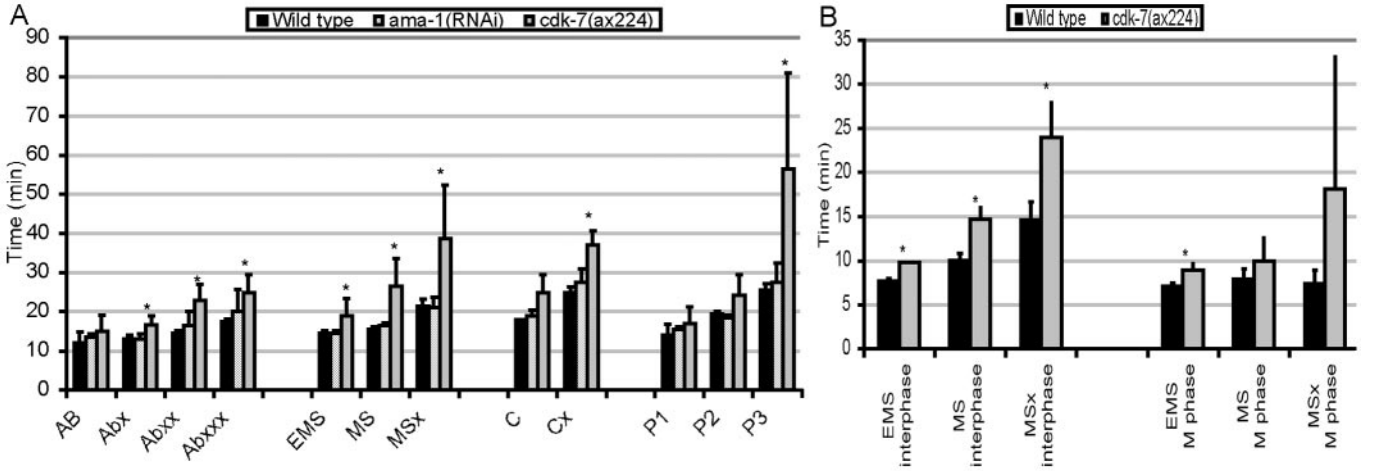

Fig. 3. Cell cycle times are progressively lengthened in *cdk-7(ax224)* mutant embryos. (*A*) Cell cycle times for blastomeres in wild-type, *ama-1(RNAi)*, and *cdk-7(ax224)* embryos. Blastomere names are indicated on the *x* axis, where x indicates daughter blastomeres that have equivalent cell cycle timing (e.g., ABx indicates the 2 daughters of AB, and ABxx indicates the 4 granddaughters). Each bar represents an average of blastomere times measured from 2–4 embryos, with error bars representing standard deviations. *, Times determined to be statistically greater than wild type ($P < 0.05$). (*B*) Comparison of interphase (*Left*) and M phase (*Right*) times for MS and its daughters in wild-type and *cdk-7(ax224)* mutant embryos expressing a H2B:GFP transgene. H2B:GFP expression was used to distinguish interphase (decondensed chromatin) from mitosis (condensed chromatin).

examined by time-lapse microscopy *cdk-7(ax224)* embryos that express a histone:GFP fusion (Fig. 3*B*). This maternally expressed fusion localizes to chromosomes, and can be used to determine cell cycle phase by monitoring chromosome condensation (29, 40). We observed increased times for both interphase and M phase, suggesting that multiple aspects of cell cycle regulation are affected by loss of *cdk-7*. We conclude that, in addition to its role in transcription, *cdk7* is also required for cell cycle progression.

***cdk-7* Is Required to Maintain Wild-Type Ploidy.** To determine whether DNA content is affected by loss of *cdk-7*, we determined the ploidy of *ax224* embryos by *in situ* hybridization to the ribosomal DNA locus on chromosome I (*Materials and Methods*). Nuclei in wild-type and *ama-1(RNAi)* embryos generally exhibit two spots of hybridization, consistent with a 2n content before S phase, and a 4n content with paired sister chromatids during or after S phase (Fig. 4 *A* and *B*; ref. 36). In contrast, 67% of *cdk-7(ax224)* embryos contained nuclei with more than two spots ($n = 43$; Fig. 4*C*), and 16% of embryos had nuclei with 5 spots or more (data not shown). These observations suggest that *cdk-7* is required to maintain wild-type ploidy, perhaps by preventing cells from reentering S phase before completing mitosis or by preventing missegregation of chromosomes during mitosis.

***cdk-7* Is Essential for Meiosis.** Although *cdk-7(ax224)* embryos exhibit cell cycle defects, they are still capable of undergoing several rounds of cell division, suggesting that CAK activity is not completely eliminated in these mutants. Two hypotheses could account for this observation; (*i*) *cdk-7(ax224)* is not a null allele, or (*ii*) another CAK exists besides CDK-7. Consistent with the first possibility, we found that inactivation of *cdk-7* by RNAi significantly worsens the *ax224* phenotype; hermaphrodites homozygous for the *ax224* mutation and fed *cdk-7* double-stranded RNA [*cdk-7(ax224, RNAi)*] produce embryos that arrest in the 1-cell stage (Fig. 5*D*), in contrast to *cdk-7(ax224)* and *cdk-7(RNAi)* embryos, which arrest in the 50-cell stage (Fig. 5 *B* and *C*). This result indicates that the cell divisions observed in *ax224* embryos depend on residual *cdk-7* activity.

To characterize the 1-cell arrest phenotype of *cdk-7*(*ax224*,*RNAi*) embryos, we stained these embryos with 4′,6-diamidino-2-phenylindole (DAPI). After fertilization, wild-type embryos complete meiosis and extrude two polar bodies as identified by DAPI staining. We found that *cdk-7*(*ax224*,*RNAi*) embryos do not form polar bodies, indicating a failure to undergo meiotic divisions (Fig. 5*E*). Mitotic figures were also not observed in these embryos. Most embryos (60%, $n = 183$) arrested with condensed chromosomes, as are seen in *cdk-1(RNAi)*, which never exit meiotic prophase (Fig. 5*F*; ref. 41).

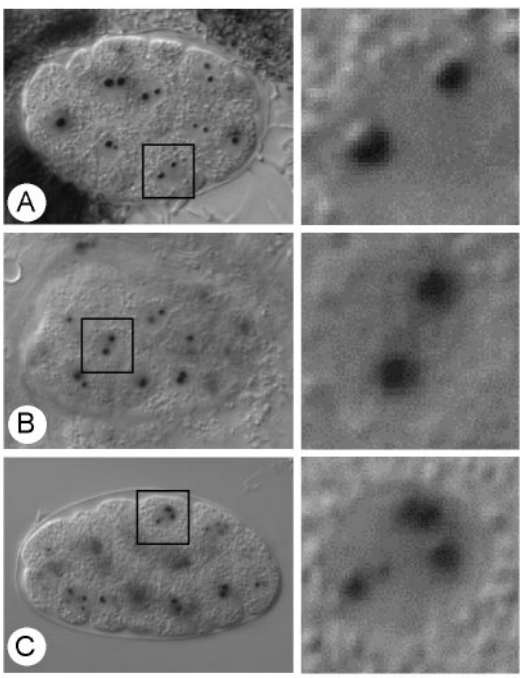

Fig. 4. Abnormal ploidy in *cdk-7(ax224)*. The ribosomal RNA locus on chromosome I was visualized by *in situ* hybridization. In wild-type (*A*) and *ama-1(RNAi)* mutant (*B*) embryos, 2 spots of hybridization were observed in most nuclei (*Right*). 0% of wild type embryos ($n = 13$) and 8% of *ama-1(RNAi)* embryos ($n = 13$) had nuclei with 3 or more spots. In contrast, 67% ($n = 43$) of *cdk-7* mutant embryos had nuclei with 3 or more spots (*C*).

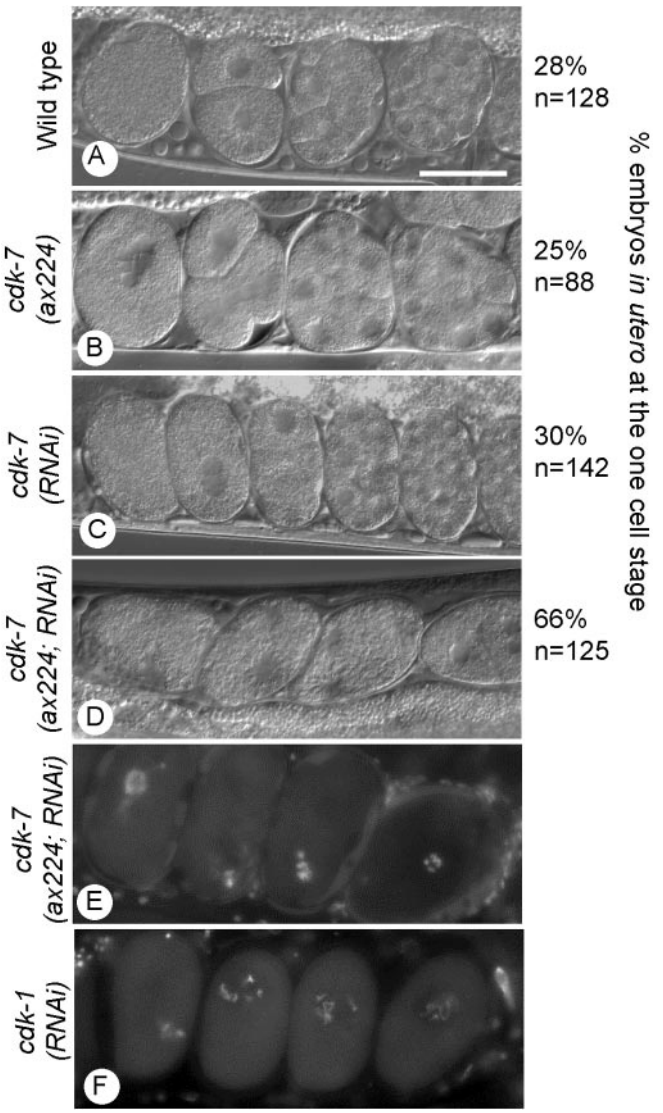

Fig. 5. Severe loss of *cdk-7* activity leads to arrest in the 1-cell stage. (*A–D*) Nomarski images of recently fertilized embryos *in utero*. An embryo is fertilized approximately every 40 min and rapidly begins dividing, such that in wild type (*A*), *cdk-7(ax224)* (*B*), and *cdk-7(RNAi)* (*C*), a variety of developmental stages are present with only a minority of embryos at the 1-cell stage. In contrast, the majority of embryos are arrested in the 1-cell stage in *cdk-7(ax224; RNAi)* (*D*). (*E* and *F*) DAPI staining of 1-cell embryos. *cdk-7(ax224; RNAi)* (*E*) and *cdk-1(RNAi)* (*F*) embryos do not produce polar bodies and arrest with condensed chromatin. (Scale bar for *A–F*, 50 μm.)

The remaining 40% of embryos contained chromatin in varying stages of decondensation. The frequency of this class decreased significantly when *cdk-1* was also inactivated [only 15% of *cdk-7(ax224;RNAi) cdk-1(RNAi)* showed decondensed chromatin, $n = 110$], suggesting that this class may caused by incomplete penetrance of the RNAi and residual *cdk-7* and *cdk-1* activities in the *cdk-7*(*ax224*,*RNAi*) embryos. We conclude that *cdk-7* is essential for meiosis, and that complete loss of *cdk-7* activity blocks all cell divisions.

Discussion

We have isolated and characterized a hypomorphic allele of *C. elegans cdk-7*. This mutant fails to activate mRNA transcription and exhibits reduced CTD phosphorylation, indicating a broad requirement for CDK-7 in transcriptional activation. *cdk-7(ax224)* mutants also have lengthened cell cycle times, defects that cannot be accounted by lack of transcription alone. Moreover, we show that complete or near complete inhibition of *cdk-7* activity by RNAi blocks cell divisions entirely. We conclude that *cdk-7* performs two roles in embryonic blastomeres: one essential for transcription and one essential for cell cycle progression.

***cdk-7*, the Transcriptional Regulator.** We used GFP expression and, in one case, *in situ* hybridization, to show that *cdk-7(ax224)* mutants fail to activate 4 different RNAPII-dependent promoters, including one promoter active in all somatic lineages (*pes-10*) and three promoters active in the E lineage (*end-1*, *elt-2*, and *med-1*). *cdk-7(ax224)* embryos also exhibit reduced levels of phosphorylation on the CTD of RNAPII. Reduced CTD phosphorylation has also been observed in embryos depleted for *ttb-1*, the *C. elegans* TFIIB homolog, and is consistent with a general defect in RNAPII transcription (24). *In vitro*, CDK7 possesses robust CTD-kinase activity specific for phosphorylation at Ser-5 of the CTD consensus repeat (42–45). Interestingly, using the monoclonal antibodies H5 and H14 (30, 31), we found that phosphorylation at both Ser-2 and Ser-5 is reduced in *cdk-7* mutants. As phosphorylation at Ser-5 is found primarily at the promoter regions, whereas phosphorylation at Ser-2 is thought to occur during elongation (46), it may be that *in vivo* phosphorylation at Ser-2 cannot occur in the absence of Ser-5-directed kinase activity. Alternatively, *C. elegans* CDK-7 may be involved directly in phosphorylation at Ser-2 and Ser-5, as suggested by a recent report for mammalian CDK7 (47). In either case, we conclude that, as is true in yeast, metazoan CDK7 is broadly required for RNAPII-dependent gene expression.

Unlike other promoters, expression from the heat shock promoter *hsp16–2* was reduced but not completely eliminated in *cdk-7(ax224)* mutants. Expression of heat-shock genes can occur in the absence of *kin-28* in yeast (48, 49), and phosphorylation of the CTD at heat shock promoters has been observed in the absence of CDK7 in *Chironomus tentans* (50). Our finding that *hsp16–2* is partially resistant to a reduction in *cdk-7* activity is consistent with the idea that CDK7 is not required equally at all promoters. *hsp16–2* expression, however, was clearly reduced in the *cdk-7(ax224)* mutants, suggesting that CDK7 does contribute to heat-shock gene expression.

***cdk-7*, the Cell Cycle Regulator.** Among the 11 mutants isolated in our screen that fail to activate transcription, only one, *cdk-7(ax224),* arrested earlier than *ama-1(RNAi)* embryos. A trivial explanation for this observation is that transcription is more completely blocked in *cdk-7(ax224)* embryos than in *ama-1(RNAi)* embryos, and that a low-level embryonic transcription is actually required for early cell divisions. Three lines of evidence argue against this possibility. First, RNA polymerase II is undetectable in *ama-1(RNAi)* embryos (24). Second, treatment with the potent and specific inhibitor of RNAPII, α-amanitin, leads to an arrest in the 100-cell stage as in seen in *ama-1(RNAi)* embryos (23). Third, removal of essential general transcription factors including *ttb-1* (TFIIB homologue) and *taf-5* ($hTAF_{II}130$ homologue) also arrests embryos in the 100-cell stage with no effect on the early divisions (24). Our observation that *cdk-7(ax224)* embryos arrest at an earlier stage is therefore most consistent with *cdk-7* having an additional function besides transcription. Lineage analysis of *cdk-7(ax224)* embryos showed that cell divisions slow down gradually before all blastomeres stop dividing in the 50-cell stage. The progressive phenotype of *cdk-7(ax224)* may be caused by perdurance of CDK-7 synthesized before the temperature shift and/or to residual activity associated with *cdk-7(ax224*). We found that both interphase and mitosis were slowed down in *cdk-7(ax224*) embryos, suggesting that CDK-7 regulates several cell cycle transitions. Consistent with this possibility, CDK7 can phosphor-

ylate multiple CDKs *in vitro*, including CDK4 and CDK6, which are required for commitment to cell division during G_1, CDK2, which regulates DNA synthesis and the G_2–M transition, and CDK1, which is required for progression through M phase (2, 51–53).

Further evidence that CDK7 has a cell cycle function came from our analysis of *cdk-7(ax224)* animals treated with double-stranded RNA against *cdk-7*. This treatment, which presumably removes residual activity remaining in the *cdk-7* mutant, blocked all cell divisions, causing embryos to arrest in the 1-cell stage. The arrested embryos did not form polar bodies, indicating that *cdk-7* is required for the meiotic divisions that are normally completed after fertilization. These data indicate that *cdk-7* is essential for both mitosis and meiosis in *C. elegans*.

In conclusion, our findings support the view that CDK7 functions both as a CAK and a CTD kinase in metazoans. This work establishes the early *C. elegans* embryo as an excellent system in which to study other outstanding questions of CDK7 function and regulation. Evidence is mounting that the kinase activity of CDK7 may be regulated differentially with respect to its different substrates (47, 54). How the two functions of CDK7 are regulated and serve to connect transcription and the cell cycle promises to be an exciting area for future investigations.

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