Both Chromosome Decondensation and Condensation Are Dependent on DNA Replication in C. elegans Embryos

Graphical Abstract

Highlights

- Replication initiation triggers the rapid decondensation of chromatids
- Replication initiation counteracts condensins, inactive MCM-2–7, and MEL-28
- DNA replication promotes condensin II accumulation and chromosome condensation

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In Brief
Chromatin organization changes dramatically within a cell cycle. Sonneville et al. find that in rapidly dividing C. elegans embryos, DNA replication initiation triggers rapid decondensation of chromatids. In addition, DNA replication promotes chromosome condensation in prophase. Therefore, the chromosome-condensation cycle and DNA replication are functionally linked.
Both Chromosome Decondensation and Condensation Are Dependent on DNA Replication in C. elegans Embryos

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INTRODUCTION

Cell-cycle progression requires the ordered succession of cell-cycle stages, and checkpoints ensure that critical cell-cycle events such as DNA replication or chromosome alignment are completed before subsequent stages can occur. Changes in cyclin-dependent kinase (CDK) kinase activity and differential cyclin association drive major transitions such as the initiation of S phase, mitosis, and the subsequent segregation of chromatids. Faithful chromosome segregation requires the structural reorganization of chromosomes into condensed metaphase chromosomes, which is needed for the separation of chromatids during anaphase. Conversely, chromosome decondensation facilitates transcription and DNA replication. In rapidly dividing embryos, S phase and mitosis alternate without apparent G1 or G2 phases. Thus, decondensation, DNA replication, and re-condensation occur in a short period and could potentially overlap. Indeed, we know little about how DNA replication and chromosome condensation and decondensation are coordinated.

Condensation is mediated by condensin complexes, pentameric ring-shaped structures composed of two structural maintenance of chromosomes (SMC) subunits that exhibit ATPase activity and that are related to cohesin subunits, plus three regulatory units known as chromosome associated proteins (CAPs). Most organisms contain two condensin complexes, condensin I and II, which share the same SMC units (MIX-1/SMC2 and SMC-4 in the worm) but differ in their regulatory subunits, termed CAPG-1, DPY-26, and DPY-28 for C. elegans condensin I and CAPG-2, KLE-2, and HCP-6 for C. elegans condensin II (Csankovszki et al., 2009; Hirano, 2012; Piazza et al., 2013; Thadani et al., 2012). In vertebrates, condensin I is cytoplasmic during interphase and appears to stabilize chromosome rigidity after nuclear envelope breakdown (Hirota et al., 2004; Ono et al., 2004). Condensin II is nuclear, is required for sister chromatid resolution during S phase, and promotes chromosomal axis formation during prophase (Cuveir and Hirano, 2003; Ono et al., 2013). In C. elegans embryos, condensin II is required for condensation during prophase and is concentrated on chromosomal axes (Csankovszki et al., 2009; Hagstrom et al., 2002; Kaitna et al., 2002). Worm condensin I is cytoplasmic, localizes to chromosomes after nuclear envelope breakdown, and appears to be required for chromosome segregation (Csankovszki et al., 2009). In C. elegans, a third condensin complex functions in dosage compensation for sex chromosomes (Meyer, 2010). Recent evidence suggests that condensin rings encircle DNA (Cuylen et al., 2011, 2013), and chromosome compaction might involve the entrapment of more than one DNA molecule or the interaction of condensin rings (for review, see Thadani et al., 2012).

Eukaryotic DNA replication is divided into two non-overlapping phases (Blow and Dutta, 2005; DePamphilis et al., 2006). In late mitosis and early G1, replication origins are licensed for replication by loading Mcm2–7 double hexamers, which require the loading factors ORC, Cdc6, and Cdt1. During S phase, CDKs and Dbf4-dependent kinases activate the Mcm2–7 helicase by promoting its interaction with Cdc45 and the GINS
complex (Gambus et al., 2006; Ilves et al., 2010; Moyer et al., 2006). This CMG (Cdc45-MCM-GINS) helicase unwinds the template DNA, allowing for RPA (single-strand binding protein) binding and DNA synthesis by DNA polymerases. In C. elegans embryos, chromatin is licensed at the end of M phase; when nuclei form and chromatin decondenses, licensing factors are then exported from nuclei, thereby ending the licensing phase and preventing re-replication (Sonneville et al., 2012). The rapidly dividing early embryo has relatively weak cell-cycle checkpoints, which allow continued cycling even when essential processes, such as DNA replication, are defective (Brauchle et al., 2003; Budirahardja and Gόnczy, 2009; Encalada et al., 2000). Cell-cycle analysis is further facilitated by the rapid turnover of cytosolic proteins in the gonad (Oegema and Hyman, 2006) and the extended period of time needed for progressing from the pre-meiotic S phase through the extended meiotic prophase (Jaramillo-Lambert et al., 2007), which allows depletion of replication genes before the first embryonic S phase without affecting the previous, pre-meiotic S phase (see below).

Here, we investigate the functional relationship between DNA replication and the chromosome-condensation cycle by combining RNAi and in vivo imaging in C. elegans embryos. We show that replication commences concomitant with decondensation and that replication initiation, but not elongation, promotes decondensation. We provide evidence that replication initiation is needed for the dissociation of the MEL-28 (ELYS) nucleoporin from chromatin during chromosome decondensation and that MEL-28 depletion rescues the decondensation defect associated with blocking replication initiation. Finally, we show that genome duplication is required for condensin II concentration on chromosomal axes and for proper chromatin condensation in prophase. Our results reveal that DNA replication and the chromosome-condensation cycles are tightly coupled.

RESULTS

We used RNAi and in vivo imaging of C. elegans embryos to uncover the links between DNA replication and the chromosome-condensation cycle. We visualized chromosomal DNA by employing mCherry fused to histone 2B (H2B) (mCherry-H2B) and the nuclear envelope by using the nucleoporin NPP-9 fused to GFP. Figure 1A and Movie S1, where the entire embryo is displayed, show the sequence of events occurring...
from meiotic anaphase II to the end of the first embryonic cell cycle. Shortly after fertilization, oocyte-derived chromosomes, which we will refer to as female chromosomes, complete the two meiotic divisions (meiosis I and II) leading to the extrusion of the two polar bodies and the formation of a haploid female nucleus. At the end of anaphase II, a ring of GFP-NP-9 forms around the female chromosomes, followed by rapid decondensation of the chromatin. The female and male haploid nuclei grow in size at opposite poles of the embryo concomitant with the bulk of DNA replication (Edgar and McGhee, 1988). These nuclei then migrate toward each other and meet at the posterior half of the cell and then move to the center of the cell. Concomitant with nuclear migration, chromatin patches indicative of condensation form and distinct chromosomes become progressively apparent. Upon nuclear disassembly, indicated by the disappearance of nucleoporins, chromosomes congress on the metaphase plate and anaphase ensues (Figure 1A; Movie S1).

**Chromosome Condensation Occurs Abnormally if DNA Replication Is Inhibited**

Within the gonad of an adult worm, the differentiation of a mature oocyte from a mitotic germ cell takes >24 hr (Jaramillo-Lambert et al., 2007), a period of time sufficient to inactivate genes by RNAi in the embryo without affecting the premeiotic S phase. As a first step to assess the relationship between DNA replication and chromosome condensation, we used RNAi to deplete the licensing factor MCM-7. The assembly of hexameric MCM-2–7 onto chromosomes during anaphase requires the presence of all six MCM-2–7 subunits. We therefore used GFP-MCM-3 as a marker for MCM-2–7 chromatin loading. When MCM-7 was depleted, GFP-MCM-3 failed to load onto anaphase chromosomes or accumulate in nuclei during S phase, consistent with the inactivation of the MCM-2–7 complex (Sonneville et al., 2012) (Figure 1B). When mcm-7 was knocked down by RNAi, chromosome condensation was compromised during prophase of the first embryonic cell cycle and massive chromatin bridges were observed during anaphase (“cut phenotype”) (Figure 1B; Movie S2). In addition, we observed a delay in chromosome decondensation at the end of meiotic anaphase II (Figure 1B, S phase; Movie S2), a phenotype we will examine in more detail below. Such a cut phenotype has been associated with replication defects in fission yeast and human cells (Hirano et al., 1986; Samejima et al., 1993; Steigemann et al., 2009) and is thought to be a consequence of cells passing through mitosis with unreplicated DNA. As an aside, in MCM-7-depleted embryos, no chromatin bridges were observed during the meiotic anaphases (Figure 1B, anaphase II), suggesting that the pre-miotic S phase was not affected. In order to show directly that MCM-7 depletion inhibits replication, we adapted 5-ethyl-2'-deoxyuridine (EdU) labeling procedures to measure replication in the entire first embryonic cell cycle (see Experimental Procedures). Permeabilized embryos incubated with EdU throughout the first embryonic cell cycle showed a ~90% reduction of EdU incorporation after one cell cycle when MCM-7 was depleted (Figures 1C and 1D). In agreement with compromised EdU incorporation, GFP-histone H2B intensity during the first mitosis of mcm-7 RNAi embryos was approximately half of wild-type, consistent with an almost complete block in replication during the first embryonic S phase (Figures 2B and 2H). Therefore, during the first embryonic cell cycle, MCM-2–7 are required for replication, condensation, and segregation of the chromatin.

We next wished to test if other replication genes are required for chromatin condensation. The majority of *C. elegans* genes have been systematically depleted by RNAi, and DIC (differential interference contrast) recordings of the first embryonic cell cycles have been deposited into “phenobank” (Sönnichsen et al., 2005). We screened phenobank to identify replication genes whose inactivation led to a cut phenotype akin to MCM depletion. Examining knockout phenotypes of 40 genes expected to be involved in DNA replication, we found that 14 were associated with a first-cycle cut phenotype (Table S1). The absence of a cut phenotype in other replication mutants is consistent with carryover of maternal protein, partial RNAi depletion, or genetic redundancy. To further analyze regulatory connections between DNA replication and the chromosome condensation cycle, we focused on replication factors involved in various stages of replication whose depletion gave a first-cycle cut phenotype. We thus depleted these replication factors: the CDT-1 licensing factor, MCM-7, the CDC-45 initiation factor, the RPA-1 single-strand binding protein, the proliferating cell nuclear antigen (PCNA) ortholog PCN-1, and the RNR-1 ribonucleotide reductase required for dNTP supply. With the exception of *mtt-1*, each of these RNAi treatments caused a large reduction in EdU incorporation (Figures 2A and 2G). The EdU incorporation in embryos treated with *mtt-1* RNAi can be explained by the depletion of the cellular dNTP pools, which favors the incorporation of EdU (itself not requiring ribonucleotide reductase) during residual replication. In addition, with the exception of *rpa-1* and *pcn-1*, each of these RNAi treatments halved the intensity of chromatin bound GFP-H2B at first mitosis, consistent with a replication block (Figures 2B and 2H). The high GFP-H2B intensity in cells treated with *rpa-1* and *pcn-1* RNAi is surprising, but it may reflect an abnormal chromatin structure in cells lacking these factors. Recording of GFP-H2B show that all of these depletions led to defective chromosome condensation at first mitosis (Figure 2B; Movie S3). Analysis of Hoechst-stained chromosomes (Figure 2C, left panels) of late prophase embryos, as defined by the position of the nuclei and by staining for phosphorylated Ser10 of histone H3 (Figure 2C), indicated that condensation was not completely abolished; chromatin patches formed during prophase but did not congress into single chromosomes. Such patches were much less discernible when GFP-H2B was imaged, likely due to the background of nucleoplasmic GFP-H2B. Defective condensation was followed by massive anaphase bridging (Figure 2B; Movie S3). Such condensation and segregation defects resemble the effect of inactivating the condensin II complex (Csankovszki et al., 2009) (Figure 2B, smc-4; Movie S3; see below). We observed that nuclear accumulation of GFP-AIR-2 (the *C. elegans* Aurora B homolog) and the commencement of chromosome condensation occur at the same time in wild-type embryos (Figure S1A). We thus asked if those two events are linked. GFP-AIR-2 nuclear accumulation occurred upon depletion of replication genes (Figures S1A and S1B, red arrows). Thus, assuming that GFP-AIR-2 and phospho-H3S10 staining serve as prophase makers, our
data show that the condensation defect associated with the depletion of replication genes is not due to a lack of prophase (Figure S1A; Figure 2C).

We next investigated if S phase checkpoint activation is linked to chromosome-condensation defects. As in other organisms, the inhibition of replication fork elongation in *C. elegans* embryos leads to the activation of a cell-cycle checkpoint delaying entry into mitosis (Brauchle et al., 2003; Encalada et al., 2000; Korze-lius et al., 2011). Consistent with earlier reports, inactivation of *pcn-1* and *rnr-1* caused a delay in nuclear envelope breakdown (Figure 2B, 2C, and 2I). To substantiate further this interpretation, we employed a graded depletion of MCM-7 (Figure 3A). The maximum RNAi dose (100%) led to an undetectable level of MCM-3 loaded onto anaphase chromosomes (Figures 3A and 3B) and a failure to import GFP-MCM-3 into interphase nuclei. At this 100% RNAi dose, little or no delay in nuclear envelope breakdown was observed (Figure 3C, dark bars), indicating that there was no significant activation of the replication checkpoint. Consistent with our previous results, this high RNAi dose caused a failure of chromosome condensation and the formation of anaphase bridges (Figures 3A and 3C, light bars). Lower levels of mcm-7 RNAi allowed a graded loading of GFP-MCM-3 onto chromatin (Figure 3B). RNAi doses ranging from 50% to 25% led to strong reduction of GFP-MCM-3 loading, the formation of anaphase bridges, and a delay in nuclear envelope breakdown (NEB) from five embryos prepared as in (B). Scale bars represent 20 μm (A) and 5 μm (B–E). Error bars represent SD. See Movies S3 and S4 for embryos shown in (B) and in (D).

extent that the replication checkpoint, which requires the generation of RPA-coated single-stranded DNA, cannot be activated (Zou and Elledge, 2003). In line with the expected role of RPA-1 in checkpoint activation, the depletion of RPA-1 also failed to elicit a cell-cycle delay but nevertheless lead to a defect in chromosome condensation (Figures 2B, 2C, and 2I).
bridges, and the maximal level of checkpoint activation as
judged by lengthening of the cell cycle. This phenotype is ex-
pected if the number of chromatin-bound MCM-2–7 is insuffi-
cient to complete replication, while compromised replication
triggers checkpoint activation. Limiting RNAi doses from 17%
to 8% led to a less severe reduction of GFP-MCM-3 loading
without checkpoint activation and without strong chromo-
some-condensation defects but with occasional anaphase
bridges. This phenotype is consistent with the idea that an
excess of MCM complexes are loaded onto “dormant” replica-
tion origins, which are not normally needed for genome dupli-
cation (Ge et al., 2007; Newman et al., 2013; Woodward et al.,
2006).

It is established that condensin II is required for chromosone
condensation during prophase ( Hirano, 2012; Piazza et al., 2013;
Thadani et al., 2012 ). We therefore generated worms expressing
the GFP-KLE-2 condensin II subunit. As expected from previous
studies, GFP-KLE-2 was diffused throughout the nucleoplasm
during S phase and concentrated on chromosomal axes during
prophase, each of the chromatid axes being resolved during
metaphase ( Figure 2D; Movie S4 ). Similarly, embryos stained
for another condensin II subunit, HCP-6, showed chromatin-
bound HCP-6 during prophase and metaphase (Figures 2 E
and 2 F). In contrast, inactivation of mcm-7, cdc-45, or rpa-1 pre-
vented KLE-2 or HCP-6 foci formation during prophase (Figures
2D and 2E; Movie S4). Controls showed that KLE-2 nuclear localization required
HCP-6 and that KLE-2 chromatin loading was dependent on the SMC-4 and HCP-6
condensin subunits ( Figure S1D ), while KLE-2 and HCP-6 were chromatin bound
during mitosis when replication was blocked (Figures 2 D and 2 F; Movie S4).
Taken together, our results suggest that condensin II concentration on chromo-
somal axes and chromosome condensa-
tion are both dependent on DNA having been replicated.

Conversely, we found that blocking chromosome condensation
by inactivating smc-4 did not inhibit chromosome duplication,
as measured by EdU incorporation (Figures 2 A and 2 G), GFP-
CDC-45 binding to chromatin (data not shown), or duplication
of chromatin-bound histones ( Figure 2 H).

Activation of the CMG Helicase Promotes Chromatin
Decondensation
Having shown that DNA replication is required for chromosome
decondensation during prophase, we next wanted to address the
converse question, namely, whether DNA replication is required
to decondense the chromatin on exit from M phase. In cell types
with a significant G1 phase, chromosome decondensation oc-
curs well before DNA replication, but in certain embryonic cells
(such as Xenopus, Drosophila, and C. elegans early embryos),
which have either short G1 phases or lack them entirely, it is
possible that chromosome decondensation and the initiation of
DNA replication occur at the same time. In order to examine
precisely when S phase starts relative to chromosome decon-
densation, we used EdU incorporation to label C. elegans em-
bryos during early stages of S phase in the first cell cycle after
fertilization. EdU staining in the female haploid nucleus was first
observed in embryos with partially condensed DNA, indicating
that some DNA replication occurs during decondensation

Figure 3. Graded Depletion of Chromatin
Bound MCM-2–7
(A) Images taken from time-lapse sequences upon
mcm-7 RNAi titration in embryos expressing GFP-
MCM-3 (upper images) and mCherry-H2B (lower
images) during prophase ( upper panel ) and
anaphase of mitosis (lower panel). The percentage
of mcm-7 RNAi expressing bacteria relative to
bacteria containing an empty vector is shown.
(B) Quantification of average chromatin bound
GFP-MCM-3 1 min after anaphase onset (n = 3).
(C) Average time elapsing between the meeting
of nuclei and NEB (black bars) and the number
of mitoses with anaphase bridges (white bars) (n = 3).
Stronger staining of the mCherry-H2B reporter in
the female chromatin as compared to the male
chromatin, allowed to separately score anaphase
bridges on female and male chromosomes. Embryos
with anaphase bridges on female and male chro-
matin scored 1, while embryos with anaphase
bridges only on male chromatin scored 0.5.
Scale bars are 5 μm. Error bars represent SD.
As an aside, EdU staining at this stage was always stronger in the male nucleus, indicating that replication initiates earlier or occurs faster in the male nucleus (Figure 4A, yellow arrow). To corroborate that replication commences concomitant with decondensation, we investigated GFP-CDC-45 chromatin recruitment, which requires MCM-2–7 (Figures 4C and 4F) and serves as a marker for ongoing DNA replication (Sonneville et al., 2012). We found that GFP-CDC-45 localized to condensed chromosomes 3–4 min after the onset of anaphase II, coinciding with nuclear envelope assembly. GFP-CDC-45 accumulated on chromatin as chromosomes decondensed (Figure 4B). To test if this also applies to other cell cycles, embryos passing though the second cell cycle were treated with a short (5 min) pulse of EdU, and we observed incorporation during decondensation, but not during anaphase (Figure 4D). GFP-CDC-45 also localized to chromatin during decondensation at this stage (Figure 4E), and its nuclear enrichment was largely dependent on MCM-2–7 (Figure 4F). Therefore, both EdU labeling and GFP-CDC-45 localization indicate that DNA replication starts on condensed chromosomes and continues during chromosome decondensation in at least two S phases following fertilization. The nucleoporin NPP-8/NUP155 is essential for both nuclear pore formation and chromatin decondensation (Galy et al., 2003; Sonneville et al., 2012). In embryos treated with RNAi against npp-8, the chromatin remained condensed and GFP-CDC-45 was reduced on chromatin, suggesting that DNA replication did not initiate properly (Figure 4G). This is consistent with the idea that nuclear assembly precedes chromatin decondensation and is required for the initiation of DNA synthesis in C. elegans embryos, as it is in Xenopus embryos (Blow and Sleeman, 1990; Blow and Watson, 1987; Newport, 1987).

We next explored whether DNA replication is required for chromosome decondensation. In cdc-45 RNAi embryos,
MCM-2–7 was loaded normally onto chromatin (Figure 4H, anaphase), but loading of GFP-RPA-1 was abolished at the least in the early stage of S phase (Figures S2A and S2B, 5 and 5.5 min, arrow), consistent with the expected role of CDC-45 in DNA unwinding. Strikingly, chromatids remained condensed at the nuclear periphery under such conditions, while nuclei increased in size (Figure 4H). The nuclear envelope was functional in cdc-45 RNAi embryos, as nuclear accumulation of GFP-MCM-3 and GFP-RPA-1 occurred normally (Figure 4H, Figures S2A and S2D). When origin licensing was inhibited using RNAi against *mcm-7*, a mild phenotype was observed: chromosome decondensation was delayed relative to wild-type but occurred faster than in cdc-45 RNAi embryos (Figures 5A and 5B). The phenotype of *cdt-1* depletion varied between embryos ranging from mild (three out of five embryos) to strong (two out of five) decondensation defects (Figure 5A; Movie S5; data not shown). In all cases, there was no change in the timing of GFP-NPP-9 recruitment. However, the initial NPP-9 distribution at times appeared uneven in cdc-45 RNAi embryos (green arrowhead). Under all these conditions, early S phase chromatin had similar levels of phospho-H3S10 as compared to the wild-type (Figure 5B). In contrast, decondensation was normal when replication elongation was blocked by RNAi against *rpa-1* (Figures 5A and 5B), *mr-1* or *pcn-1* (R.S., unpublished data). These findings indicate that chromosome decondensation requires activation of the CMG helicase during the initiation of chromosome replication but is independent of ongoing DNA synthesis during elongation.

To explore the mechanism by which replication initiation drives chromatin decondensation, we first analyzed the decondensation defects correlate with persistent chromatin binding of condensin I.

To test whether condensin is required to maintain chromatin condensation when the initiation of replication is blocked, we performed double depletions of *mcm-7* and *smc-4*, or cdc-45 and *smc-4*, in embryos expressing GFP-CAPG-1 and mCherry-H2B. Quantitation of mCherry-H2B intensity after one cell cycle showed that the level of H2B was halved upon both double depletions, as compared to the single *smc-4* inactivation, consistent with a complete lack of replication following depletion of *mcm-7* or *cdc-45* (Figure 6C). Moreover, the chromatin binding of GFP-CAPG-1 during metaphase was strongly reduced in all embryos treated with *smc-4* RNAi (Figures 6B and 6D). As predicted by the removal of condensin from chromatin, decondensation was restored in embryos treated with *mcm-7* RNAi (Figures 6B; Movie S6). Interestingly, however, double depletion of cdc-45 and *smc-4* only led to the partial restoration of chromatin decondensation upon entry into S phase. Chromatin was more diffuse in early interphase nuclei of cdc-45 smc-4 embryos, in embryos expressing GFP-CAPG-1 and mCherry-H2B. Quantitation of mCherry-H2B intensity after one cell cycle showed that the level of H2B was halved upon both double depletions, as compared to the single *smc-4* inactivation, consistent with a complete lack of replication following depletion of *mcm-7* or *cdc-45* (Figure 6C). Moreover, the chromatin binding of GFP-CAPG-1 during metaphase was strongly reduced in all embryos treated with *smc-4* RNAi (Figures 6B and 6D). As predicted by the removal of condensin from chromatin, decondensation was restored in embryos treated with *mcm-7* RNAi (Figures 6B; Movie S6). Interestingly, however, double depletion of cdc-45 and *smc-4* only led to the partial restoration of chromatin decondensation upon entry into S phase. Chromatin was more diffuse in early interphase nuclei of cdc-45 smc-4 embryos, compared to single cdc-45 RNAi embryos (Figure 6B, compare nuclei at 7 and 9 min after anaphase onset; Movie S6), but chromatin patches were still observed and they persisted throughout S phase. Therefore, although the decondensation defect of *mcm-7* RNAi embryos can be explained by the persistence of condensin on chromatin, these findings suggest that an additional condensin-independent mechanism impairs decondensation in cdc-45 RNAi embryos.

Double-hexameric rings of MCM-2–7 are loaded around origin DNA during the licensing reaction at the end of M phase. These

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**Figure 5. CMG Helicase Promotes the Rapid Chromatin Decondensation**

(A) Images taken from time-lapse sequences of wild-type, cdc-45, mcm-7, cdt-1, and rpa-1 RNAi embryos expressing GFP-NPP-9 (upper images) and mCherry-H2B (lower images) during the first embryonic S phase. (B) Images of wild-type, cdc-45, mcm-7, cdt-1, and rpa-1 RNAi embryos during early S phase. Embryos were stained for phospho-H3S10 (red), nuclear pore (mAb414, green), and DNA (blue). Magnifications of the female and male nuclei are shown on the left. Scale bars represent 5 μm (A) and 20 μm (B). See Movie S5 for embryos shown in (A).
inactive MCM-2–7 complexes disappear from chromatin during the course of S phase, due either to their conversion into active CMG helicase complexes during replication initiation (Blow and Dutta, 2005; Ilves et al., 2010; Moyer et al., 2006) or to their displacement by an active fork. Therefore, when CDC-45 is inactivated, MCM-2–7 double hexamers should remain on DNA throughout the cell cycle, potentially being involved in a condensin-independent mechanism that limits decondensation in cdc-45 RNAi embryos. When DNA replication was blocked by depletion of CDC-45, inactive MCM-2–7 complexes remained stably on chromatin throughout the cell cycle.

To confirm that the MCM-2–7 on metaphase chromatin in cdc-45 RNAi was due to persistence of MCM-2–7 complexes on chromatin throughout interphase and was not due to premature loading of MCM-2–7 in early mitosis, we photobleached GFP-MCM-3 during early S phase in female nuclei and determined the recovery in the following mitosis (Figure 7B, red arrows). Male DNA served as a positive control (Figure 7B, yellow arrows). As nuclei increased in size, unbleached GFP-MCM-3 was imported into the photobleached female nucleus (Figure 7B, S phase). After nuclear envelope breakdown at the end of the cell cycle, a GFP-MCM-3 signal was not recovered on bleached chromatin, indicating that GFP-MCM-3 is not loaded prematurely onto metaphase chromatin in cdc-45 RNAi embryos (Figure 7A) but instead had persisted on chromatin throughout interphase in the absence of replication (Figure 7B; Movie S8).

These experiments raised the possibility that when the initiation of replication has been blocked by cdc-45 RNAi, the persistence of inactive MCM-2–7 double-hexamers around DNA might support a condensin-independent mechanism that inhibits chromatin decondensation. We therefore simultaneously inactivated cdc-45 and mcm-7 to test this hypothesis. As a control for the double RNAi knockdown, we combined cdc-45 and par-2 RNAi, the latter gene being required for asymmetric cell division. As anticipated, cdc-45 par-2 double RNAi resulted both in the severe decondensation defects typical of cdc-45 RNAi and in the symmetric cell divisions expected for par-2 RNAi (Figure 7C; Movie S9), confirming the efficiency of the double RNAi approach. We then performed cdc-45 and mcm-7 double RNAi and observed the milder decondensation defect that is typical of mcm-7 RNAi, rather than the severe defect that is...
normally associated with \( cdc-45 \) inactivation (Figure 7C: Movie S9). This result is consistent with the idea that the severe decondensation defects associated with \( cdc-45 \) RNAi are produced not only by persistence of condensin on chromatin but also by the presence of inactive MCM-2–7 complexes that are loaded tightly around the DNA.

Our results suggest that there are proteins involved in maintaining the condensed state of metaphase chromosomes at the nuclear periphery that are removed, inactivated, or relocalized as a consequence of replication initiation. One possible candidate is ELYS/MEL-28, a nucleoporin that mediates the initial assembly of nuclear pores onto chromosomal DNA (Franz et al., 2007; Gillespie et al., 2007; Rasala et al., 2006) and is also localized to mitotic chromosomes, where it has a function in organizing kinetochores (Fernandez and Piano, 2006; Galy et al., 2006). Importantly, Xenopus MEL-28 has been shown to associate with chromatin-bound MCM-2–7 prior to replication initiation (Davuluri et al., 2008; Gillespie et al., 2007). We therefore investigated the idea that removal of MEL-28 from chromatin might be dependent on CDC-45 and the initiation of replication. Consistent with previous reports (Fernandez and Piano, 2006; Franz et al., 2007; Galy et al., 2006), MEL-28 was localized to the nuclear envelope and the nucleoplasm during S phase (Figure 7D). In contrast, when cells were depleted of CDC-45, MEL-28 remained associated with the condensed chromatin (Figure 7D). This is in line with the idea that activation of the CMG helicase is required to release MEL-28 from chromatin as replication initiates.
RNAsi against mel-28 still allowed chromosome decondensation, assembly of the nuclear envelope, and recruitment of the nucleoporins recognized by the mAb414 antibody. To determine whether loss of MEL-28 rescued the chromosome-condensation defect seen upon CDC-45 depletion, cells were treated with RNAsi against both mel-28 and cdc-45. Embryo staining (Figure 7D) and live imaging (Figure 7E; Movie S10) showed that co-depletion of MEL-28 and CDC-45 restored the decondensation of the chromatin. Thus, our results suggest that MEL-28 chromatin relocalization contributes to chromosome decondensation and is driven by the initiation of chromosome replication. Consistent with MEL-28 relocalization being stimulated by the loss of MCM-2–7 from chromatin, we found that MEL-28 redistribution occurred similarly after mcm-7 single and cdc-45 mcm-7 double RNAs (Figure S3). Our combined data are consistent with the idea that MCM-2–7 complexes dissociate from MEL-28 when MCM-2–7 is either activated or displaced from chromatin, as a consequence of the initiation of DNA replication.

DISCUSSION

Dependency of Chromosome Condensation on Prior DNA Replication

We show that if any aspect of DNA replication is inhibited, whether by depleting licensed origins or initiation factors or by inhibiting replication fork elongation, chromosome condensation is defective. Therefore, the condensation defects we observe are not a consequence of the persistence of active replication forks or MCM-2–7 hexamers on the DNA, because condensation defects occur even if MCM-2–7 chromatin loading is inhibited. This phenotype resembles condensin II depletion in C. elegans embryos (Csankovszki et al., 2008; Hagstrom et al., 2002; Kaitna et al., 2002; this article), and prompted us to examine condensin localization. The diffuse nuclear localization of the condensin II subunits KLE-2 and HCP-6 prevented us from determining if condensin is loaded onto prophase chromatin when replication is blocked. However, when cells replicate normally, condensin II progressively focuses on chromosomal axes during prophase, and this focusing fails to happen if replication is blocked. Therefore, our results indicate that DNA replication may modify the chromatin such that condensin II promotes chromosome condensation during prophase. This model would be in line with previous reports. In HeLa cells, condensin II gradually accumulates on chromatin during S phase and promotes the separation of replicated DNA (Ono et al., 2013). In Xenopus egg extracts, topoisomerase II, which is required for chromosome condensation, associates more tightly with replicated DNA (Cuvelier and Hirano, 2003).

Although replication is required for chromosome condensation during prophase, unreplicated chromatin partially condenses during mitosis, leading to an abnormal metaphase plate (Figure 2B; Movie S3). Such a “metaphase condensation” process was previously observed after condensin depletion (Kaitna et al., 2002) and therefore requires neither condensin nor replication.

The replication and condensation defects we observe lead to excessive chromatin bridge formation and a cut phenotype when chromatin is separated during cytokinesis. Such cut phenotypes have been described in fission yeast when mutations affecting replication licensing were first described (Hirano et al., 1986; Hofmann and Beach, 1994; Kelly et al., 1993; Miyake et al., 1993). However, to the best of our knowledge, defects in chromosome condensation have not been described in fission yeast, possibly because the level of DNA condensation could not be assessed easily by DAPI staining of the small chromosomes. In budding yeast, the deletion of genes required for replication initiation leads to a “reductional cell division” occurring with wild-type kinetics and producing two distinct but linked masses of unreplicated DNA in the daughter cells (Platti et al., 1995; Tercero et al., 2000; Toyn et al., 1995). These phenotypes can be explained by the almost complete absence of replication forks in these mutants, which means that no S phase checkpoint can be generated and, as a consequence, cells enter mitosis on schedule but with unreplicated DNA. Our graded depletion of MCM-7 provides strong support for this interpretation: high levels of depletion led to a cut phenotype with little or no cell-cycle delay, but at lower levels of depletion, presumably associated with partial genome duplication where replication forks are present and capable of activating cell-cycle checkpoints, a pronounced delay of entry into mitosis was observed. However, when these cells ultimately enter mitosis with unreplicated DNA, they nevertheless display a cut phenotype associated with partially condensed DNA.

Dependency of Chromosome Decondensation on the Initiation of DNA Replication

The rapid cell cycles of embryonic cells have a short or non-existent G1 phase, which means that the initiation of DNA replication happens at about the same time or shortly after decondensation of mitotic chromosomes. In Xenopus egg extracts, the initiation of DNA replication occurs only after chromosomes have been incorporated into a functional interphase nucleus (Blow and Sleeman, 1990; Blow and Watson, 1987; Newport, 1987). Chromosome decondensation is largely unaffected when the initiation of DNA replication is inhibited in these extracts (Blow, 1993; Gillespie et al., 2007; Strausfeld et al., 1994). We were therefore surprised to find that in the C. elegans embryo, replication commences early, before full chromosome decondensation. However, in vivo studies of the early Xenopus embryo also show a similar effect, with DNA replication initiating on only partially decondensed chromosomes (Leibovici et al., 1992; Lemaitre et al., 1998). It appears surprising that DNA replication can occur when chromosomes are in a compacted state. However, DNA synthesis can occur, though at a reduced rate, when DNA is induced to take up a “metaphase-like” condensed form (Gotoh, 2007 and references therein).

We postulate that focusing replication initiation on highly condensed chromatin might aid rapid and synchronous replication. Linking CMG complexes to condensed chromatin and MEL-28 could focus replication closer to the nuclear pore, where imported replication proteins are most accessible (Figure 7F). At the same time, mindful that only a minority of licensed MCM-2–7 double hexamers are activated in any one S phase, initiation on condensed chromatin could also be part of a mechanism facilitating the equal distribution of activated CMG helicases. For example, among the licensed MCM-2–7 located within a condensation loop, the MCM-2–7 complex located closest to
the nuclear pore could be preferably activated (Figure 7F). Such a mechanism could ensure that helicase activation is equally distributed along the DNA, thus ensuring the rapid replication of the entire genome.

Our findings also indicate that replication initiation, but not elongation, promotes the rapid decondensation of chromatin. We postulate that the active replicative helicase may induce the release of chromatin-bound complexes, which otherwise maintain condensation. Consistent with this idea, condensin inactivation indeed completely rescued that decondensation defects of MCM-7 depletion. Only a partial rescue occurred when CDC-45 and condensin were co-depleted, in line with defects of MCM-7 depletion. This CMG helicase could displace condensins from DNA and allow decondensation. A second explanation is that DNA unwinding by the CMG helicase could displace condensins from DNA and allow decondensation.

It has been previously shown in Xenopus that ELYS/MEL-28 interacts on chromatin with MCM-2–7 (Davuluri et al., 2008; Gillespie et al., 2007). In the Xenopus early embryo, ELYS/MEL-28 dependent nuclear assembly shuts down the replication licensing system (Hodgson et al., 2002; Kisielewska and Blow, 2012), to create a feed-forward loop that accelerates nuclear assembly and S phase entry once origin licensing is complete. There is also evidence in the Xenopus system that DNA replication promotes decondensation of mitotically condensed chromatin (Prokhorova et al., 2003). If a similar interaction also occurs in the C. elegans embryo (Figure 7B, telophase), this would explain why depletion of MCM-7 rescues the decondensation defect that occurs in the absence of CDC-45. Under this interpretation, the initiation of DNA replication, which is dependent on both nuclear assembly and CDC-45, would promote dissociation of MCMS from MEL-28, thereby allowing the DNA to rapidly decondense once replication has initiated (Figure 7F, S phase). Consistent with this interpretation, we find that MEL-28 remained associated with condensed chromatin when CDC-45 was depleted. The possible CDC-45-dependent extraction of MEL-28 from chromatin would therefore be part of a mechanism that links replication licensing to nuclear assembly and chromosome decondensation.

With the sole exception of the spindle assembly checkpoint, the cell-cycle engine of somatic cells, powered by CDK activity, is not strongly influenced by feedback from cell-cycle events occurring normally (i.e., in the absence of contingencies such as DNA damage). We show here that in the short cell cycles of C. elegans embryonic cells, chromosome decondensation, replication licensing, DNA replication, and chromosome recondensation all occur in a tightly coordinated manner. This coupling helps ensure the correct order of cell-cycle events and the faithful propagation of chromosomal DNA, which is particularly important for embryonic cells as they ultimately contribute to the germ line and DNA of future generations.

**EXPERIMENTAL PROCEDURES**

**C. elegans Maintenance and RNAi**

C. elegans were maintained according to standard procedures (Brenner, 1974). RNAi was performed by feeding worms with bacteria expressing double-stranded RNA (Timmons and Fire, 1998). Details about worms and RNAi can be found in Supplemental Experimental Procedures.

**Microscopy**

Different procedures were used to record embryos from the first embryonic cell cycle or from meiotic divisions, and embryos were imaged using confocal or widefield fluorescent microscopy (see Supplemental Experimental Procedures). For immunostaining, embryos were fixed in methanol at −20°C and stained using standard procedures with rabbit antibodies for phospho-H3S10 (1:1,200; Upstate), MEL-28 (1:500) (Galy et al., 2006), and HCP-6 (1:500) (Hargitai et al., 2009) and with mouse monoclonal antibodies mAb414 (1:200; Covance). Secondary antibodies were donkey anti-rabbit conjugated to Alexa Fluor 546 (Invitrogen) and donkey anti-mouse conjugated with Alexa 488 (Invitrogen). DNA was visualized with Hoechst 33258. Embryos were imaged using a confocal laser-scanning microscope (SP2; Leica) using a 63×1.40 plan-apochromat oil-immersion lens (Leica), except for EdU staining, in which a DeltaVision Core microscope was used (see details above).

**EdU Incorporation**

In Figure 1, embryos at meiosis II, permeabilized by perm-1 (diluted 1/6) RNAi (Carvalho et al., 2011), were incubated for 25–35 min in isotonic buffer (see Supplemental Experimental Procedures) + 20 μM EdU and incubated for 25–35 min. Embryos at first embryonic mitosis were fixed in 3.6% formaldehyde; permeabilized in PBS, 0.5% Triton X-100 for 20 min; and washed in PBS. EdU incorporation was revealed with Click-iT EdU Alexa 488 Imaging Kit (Invitrogen). DNA was stained with Hoechst 33342, and embryos were mounted on polylysine-coated slides. In Figures 2 and 4, embryos were dissected from ten worms and incubated in isotonic buffer supplemented with 20 μM EdU for 30 min (Figure 2), 10 min (Figure 4A), or 5 min (Figure 4D) and then treated as described above.

**Image Quantification**

ImageJ was used for all quantifications. GFP enrichment on mitotic DNA was determined using middle plan images during metaphase and calculated by subtracting the integrated density of an area containing cytoplasm from the integrated density of a similar area containing the chromatin. Values were normalized to wild-type levels. The same procedure was applied for the quantification of EdU staining, except that the image was the intensity sum of 15 z stacks acquired every 0.5 μm.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, three figures, one table, and ten movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.06.046.

**AUTHOR CONTRIBUTIONS**

R.S. was responsible for designing and conducting all the experiments and supervising G.C., R.S., A.G., K.L., and J.J.B. planned the research strategy, analyzed the data, and wrote the manuscript.

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