We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Regulation of DNA Synthesis and Replication Checkpoint Activation During *C. elegans* Development

Suzan Ruijtenberg,
Sander van den Heuvel and Inge The
Developmental Biology, Utrecht University
The Netherlands

1. Introduction

Replication of the DNA during the synthesis (S) phase of the cell cycle is one of the most critical aspects of cell division. DNA replication must be highly accurate and tightly controlled to maintain genomic integrity over many rounds of cell division. This is particularly important during animal development, since genetic instability can lead to cell death, birth defects, developmental abnormalities and diseases such as cancer. The developmental context also adds specific constraints to S-phase regulation. For instance, variations in DNA replication control are needed to accommodate the rapid embryonic divisions in early embryos, the production of haploid germ cells, and the generation of polyploid tissues. A comprehensive understanding of DNA replication requires insight in these developmental aspects of S phase control. Here, we review the initiation of DNA replication in the genetic animal model *Caenorhabditis elegans* (*C. elegans*), with a focus on developmental-stage and tissue-specific regulation.

2. *Caenorhabditis elegans*

Caenorhabditis elegans (*C. elegans*) was introduced as a model organism in the 1960s by Sydney Brenner and became, in a relative short time, one of the leading model organisms in biological research (Ankeny 2001). One of the appealing aspects of this nematode is its rapid and reproducible development from the one-cell embryo to the adult stage (Sulston & Horvitz 1977). The invariance, combined with the fact that the animals are transparent and contain a relatively low number of cells (adult hermaphrodites contain only 959 somatic cell nuclei), has made it possible to record the entire somatic cell lineage of *C. elegans* (Horvitz & Sulston 1980; Sulston & Horvitz 1977; Sulston & Horvitz 1981). Knowing when each cell normally divides is a major benefit for studies of the cell cycle. Efficient genetics has allowed identification of mutations that alter the normal cell lineage (*lin* mutants), some of which affect DNA replication or DNA content (Horvitz & Sulston 1980; Sulston & Horvitz 1981). As an additional advantage, many cell cycle regulators that exist in gene families in higher eukaryotes are represented by single genes in *C. elegans*,

which helps identification of gene function and determination of the hierarchy of gene functions in regulatory pathways.

While these aspects make *C. elegans* suitable for cell cycle studies, there are additional reasons for adding this animal to the repertoire of cell cycle models. Studies of DNA replication in the context of a developing organism may identify regulatory mechanisms that are not important for single cell eukaryotes and cells in tissue culture. The developmental context adds an extra layer of S-phase regulation. For instance, in meiosis, two rounds of chromosome segregation follow each other without intervening S phase, while in endoreplication cycles, rounds of DNA replication continue in the absence of M phases. In addition, a broad range of models also increases the potential for uncovering important aspects of DNA replication control. For example, studies in *C. elegans* identified a CUL-4/DDB-1 E3 ubiquitin ligase complex as an important inhibitor of DNA re-replication, which is functionally conserved in mammals (Arias & Walter 2007; Kim & Kipreos 2007a; Zhong, et al. 2003). In addition, defects in DNA synthesis were found to cause lineage-specific delays in cell division in *C. elegans*, through a checkpoint mechanism that also contributes to the difference in timing of founder cell division in the early embryo (Brauchle, et al. 2003; Encalada, et al. 2000). Furthermore, our recent results support tissue specific contributions of a conserved general regulator of DNA replication, MCM-4 (Korzeliuss, et al. 2011). Below, we describe the currently known factors that control DNA replication in *C. elegans*, as well as their functions in particular stages of development and specific cell types. Several techniques used for analysis of DNA replication in *C. elegans* are summarized in BOX 1.

3. The factors that regulate DNA replication

The regulation of DNA replication in eukaryotes involves two discrete steps. First, pre-replication complexes assemble at sites of replication initiation ("origin licensing"), and subsequently, the actual initiation of DNA synthesis can be triggered ("origin firing"). Comprehensive studies aimed at identifying all components involved in DNA replication have not been reported for *C. elegans*. However, functional annotations by the *C. elegans* genome sequence consortium have revealed orthologs of many DNA replication components (www.wormbase.org). In addition, some DNA replication genes have been identified through mutations, and genome-wide RNA interference (RNAi) has confirmed that most putative replication components exert critical functions (Encalada, et al. 2000; Korzeliuss, et al. 2011; Sonnichsen, et al. 2005). Despite their clear conservation, certain well-known replication genes currently appear to lack *C. elegans* counterparts (see Table 1). For instance, in eukaryotes ranging from yeast to human, the origin recognition complex (ORC) has been found to consists of 6 subunits, ORC1 to ORC6. At present, ORC-2 is the only ORC protein identified in *C. elegans*, and its function has not been characterized in detail.

Recruitment of the ORC is normally the first step in pre-replication complex assembly, which is followed by association of the CDC6 and CDT1 proteins. *C. elegans* does contain legitimate CDC-6 and CDT-1 orthologs, which are essential for DNA replication and required for embryonic as well as larval viability (Kim, et al. 2007; Kim & Kipreos 2008; Kim & Kipreos 2007a; Kim & Kipreos 2007b). Simultaneous overactivation of CDC-6 and CDT-1 leads to extensive re-replication, which underscores the role of CDC-6 and CDT-1 as critical regulators of origin licensing.

BOX1: *C. elegans* DNA replication analysis

One of the advantages of the use of *C. elegans* as a model system is that the animal is fully transparent, which allows the use of Differential Interference Contrast (DIC, also known as Nomarski) microscopy for live observations of cell division. Moreover, expression and localization of the green fluorescent protein (GFP) and other fluorophores can be followed by time-lapse microscopy. Introduction of transgenes with tissue or cell type-specific promoters that drive expression of GFP or GFP-tagged fusion proteins is a routine procedure in *C. elegans* (Mello & Fire 1995). However, transgenes are usually silenced in the germline and in early embryos, which can be avoided by integrating a single copy transgene through DNA particle bombardment or the MosSCI technique (Frokjaer-Jensen, et al. 2008; Praitis, et al. 2001). We have recently applied the MosSCI strategy for integration of a single copy transgene expressing an MCM-4::mCherry protein fusion, which rescues *mcm-4* null mutants and shows a similar expression pattern and subcellular localizations as the endogenous MCM-4 protein (Korzeliuss, et al. 2011, and our unpublished results).

In addition to gene expression studies, DNA replication itself can be visualized in multiple different ways. The most quantitative method makes use of determination of the DNA content. The DNA content of a cell correlates with the cell cycle phase: cells in G1 have a ploidy of $2n$; S phase cells between $2n$ and $4n$; and cells in the G2 and M phases $4n$. To measure the DNA content, animals are fixed and stained with a dye that fluoresces when bound to DNA, such as propidium iodide, Hoechst 33258, or DAPI (4',6'-diamidino-2-phenylindole dihydrochloride). The most accurate method, but also the most time consuming, for *in situ* quantification is analysis of the fluorescence signal in confocal serial sections of propidium iodide-stained nuclei (Boxem, et al. 1999; Feng, et al. 1999; Zhong, et al. 2003). The accuracy of this method makes it ideal for experiments in which small differences in DNA content must be distinguished, e.g. when comparing cells in G1 vs. S phase.

In order to investigate if cells go through the process of DNA replication, or whether DNA replication takes place at specific times of development, incorporation of the thymidine analogues 5-bromo-2'-deoxyuridine (BrdU) or 5-ethynyl-2'-deoxyuridine (EdU) can be used. BrdU incorporation can be detected by immunostaining with specific anti-BrdU antibodies. EdU detection is based on a copper (Cu^{1+}) catalyzed covalent "click" reaction between an azide attached to a fluorescent dye and the alkyne group of EdU (Salic & Mitchison 2008). While BrdU detection in *C. elegans* has been possible for some time (Boxem, et al. 1999), the EdU method is new and has been applied only in a few recent studies (Fig.1) (Cinquin, et al. 2010; Korzeliuss, et al. 2011). The EdU method has a major advantage over BrdU staining: while BrdU detection requires DNA denaturation, this step is not needed in the EdU procedure. As a result, EdU incorporation can be combined with immunostaining with antibodies, which can be a great help in visualizing cells of interest.

Flow cytometry is commonly used for DNA quantification in other systems. Although this technique is not widespread, flow cytometry has been used to produce accurate measurements of DNA content for freshly dissociated *C. elegans* cells (Bennett, et al. 2003). The dissociated *C. elegans* cells represented multiple cell types, which reduces the utility of the DNA distribution information. This limitation can be avoided by using strains in which cells of interest are marked with transgenes that express GFP (or other fluorescent tags). GFP expression can be used to gate cells of interest in the flow cytometry analysis so that the DNA distribution of only the GFP expressing cells is analyzed. In future studies, this coupling of selective GFP expression with propidium iodide staining will probably be applied more broadly in the analysis of the DNA distribution of specific tissues and cells of interest.

Fig. 1. EdU incorporation and staining visualizes DNA replication in *C. elegans* larvae. EdU incorporation in cells of the ventral nerve cord in a first stage larva (A, B, and C) and nuclei in the intestine of an early L4 larva (A', B', and C') are indicated by arrows. Panels show DNA staining by DAPI (A and A'), EdU staining (B and B') and merged images (C and C'). Note that cells that completed S phase prior to EdU addition stain with DAPI but do not incorporate EdU, such as the neurons indicated by arrowheads. One arm of the developing gonad is visible at the right (A', B', C').

Studies in other systems have shown that CDC-6 and CDT-1 are needed to load the minichromosome maintenance (MCM) protein complex onto the replication origins. The MCM complex consists of 6 proteins, MCM2 to MCM7, which is thought to act as the helicase that unwinds the DNA at the replication origins. *C. elegans* contains orthologs of all six MCM genes, which are known as *mcm-2* to *mcm-7* and cause similar embryonic lethal phenotypes when inactivated by RNAi (Sonnichsen, et al. 2005). MCM-4 was initially identified through a mutation in the *lin-6* gene, and is the only *C. elegans* MCM protein studied in detail (Korzelius, et al. 2011). MCM-4 is expressed in all dividing cells during embryonic and postembryonic development. It is strongly induced just prior to the G1/S transition in somatic cells and disappears when cells exit the cell cycle. MCM-4 localizes to the cell nucleus in interphase, while in mitosis MCM-4 localization becomes diffuse throughout the cell upon nuclear envelope breakdown. In late anaphase, MCM-4 starts to colocalize with the DNA, presumably licensing the DNA for the next round of S-phase (Fig. 2).

Protein Name	<i>S. cerevisiae</i>	<i>S. pombe</i>	<i>Drosophila melanogaster</i>	Mammals	<i>C. elegans</i>
Prereplication complex					
Orc1-6	Orc1-6	Orc1-6	Orc1-6	Orc1-6	ORC-2
Cdc6	Cdc6	Cdc18	Cdc6	Cdc6	CDC-6
Cdt1	Cdt1/Tah11/Sid2	Cdt1	Dup	Cdt1	CDT-1
Mcm2	Mcm2	Mcm2/Cdc19/Nda1	Mcm2	Mcm2	MCM-2
Mcm3	Mcm3	Mcm3	Mcm3	Mcm3	MCM-3
Mcm4	Cdc54/Mcm4	Cdc21	Dpa	Mcm4	MCM-4
Mcm5	Cdc46/Mcm5	Mcm5/Nda4	Mcm5	Mcm5	MCM-5
Mcm6	Mcm6	Mcm6/Mis5	Mcm6	Mcm6	MCM-6
Mcm7	Cdc47/Mcm7	Mcm7	Mcm7	Mcm7	MCM-7
Preinitiation complex					
Mcm10	Mcm10/Dna43	Cdc23	Mcm10	Mcm10	Y47D3a.28
Cdc45	Cdc45/Sld4	Sna41/Cdc45	Cdc45	Cdc45	F34D10.2
Sld3	Sld3	Sld3	-	-	-
Dbp11	Dbp11	Cut5/Rad4	Mus101	TopBP1	MUS-101
Sld2	Sld2/Drc1	Drc1	-	-	-
Sld5	Sld5	Sld5	Sld5	Sld5	Y113G7B.24
Psf1	Psf1	Psf1	Psf1	Psf1	R53.6*
Psf2	Psf2	Psf2	Psf2	Psf2	F31C3.5*
Psf3	Psf3	Psf3	Psf3	Psf3	-
Kinases					
Cdc7	Cdc7	Hsk1	Cdc7	Cdc7	C34G6.5
Dbf4	Dbf4	Dfp1	Chiffon	Dbf4/ Ask/ Drf1	-

Table 1. Homologues of DNA replication components. *Based on homology searches only.

The absence of DNA replication, as observed in *mcm-4* mutants, might be expected to trigger a checkpoint that delays mitotic entry. However, *mcm-4* mutants enter mitosis in the absence of DNA replication and, initially, with normal timing, suggesting that *mcm-4* is not only required for DNA replication but also activates a checkpoint that monitors completion of DNA replication (Korzelius, et al. 2011). This second function corresponds to the results obtained in studies with other organisms, which clarified the requirement of the MCM complex in activation of the DNA damage and replication checkpoints (Labib, et al. 2001; Zou & Elledge 2003). In addition to these well conserved functions, *mcm-4* also displays a tissue-specific requirement in *C. elegans*, which will be discussed below (Korzelius, et al. 2011).

Fig. 2. Time-lapse fluorescence microscopy shows expression and localization of MCM-4 in an early embryo. MCM-4 is fused to mCherry and expressed from the *mcm-4* promoter (A-E). Merged images of the DIC and fluorescence channels are shown in the bottom panels (A'-E'). The red MCM-4::mCherry fluorescence is visible in the anterior AB and posterior P1 cell in the two stage embryo (A and A'). Note that the AB cell enters mitosis before the P1 cell (B and B'). MCM-4 can be detected on the chromosomes in late anaphase (arrowhead in P1 cell, D and D').

Activation of the MCM2-7 complex is needed for opening the DNA helix and allowing the DNA polymerases to start DNA replication. This activation marks the end of origin licensing and the start of origin firing (Labib & Diffley 2001). Studies in several organisms have shown that the onset of S-phase requires CDK (cyclin dependent kinase) and DDK (Dbf-4 dependent Cdc7 kinase) activity to promote activation of the MCM2-7 helicase, while at the same time the recruitment of pre-replication complexes is inhibited (Bousset & Diffley 1998; Nguyen, et al. 2001; Remus, et al. 2005). CDKs and DDK4 are not only required for the activation of the MCM complex, they also trigger the assembly of additional factors. This results in the formation of a "preinitiation complex" that contains a large and still growing group of proteins, such as Cdc45, Mcm10, RPA and the DNA polymerases α and ϵ (Bell & Dutta 2002; McGarry & Kirschner 1998; Mechali 2010; van Leuken, et al. 2008). Most of these factors have not been identified or investigated in *C. elegans*, and the formation and function of the preinitiation complex in *C. elegans* therefore remains elusive (Table 1). In animal systems, Geminin acts as an inhibitor of CDT-1, which is degraded in mitosis in an APC/C-dependent fashion (McGarry & Kirschner 1998; van Leuken, et al. 2008). *C. elegans* Geminin GMN-1 also associates with CDT-1 and inhibits origin licensing when added to frog egg

extracts (Yanagi, et al. 2005). GMN-1 inhibition results in germline defects and intestinal abnormalities with chromatin bridges. Thus, Geminin may be an example of a metazoan-specific regulator of DNA replication initiation.

4. Preventing re-replication

When DNA replication is initiated, origin licensing should be prevented, as re-firing of only a single origin may lead to gene amplification and could have dramatic consequences. Hence, all eukaryotes use multiple levels of control to prevent more than one round of DNA synthesis within a single S-phase, although the exact players and mechanisms differ somewhat between species. In general, there are two mechanisms used to prevent re-replication: firstly, formation of the pre-replication complex (prior to S-phase) and the activation of the origins (during S phase) are temporally separated, and secondly, proteins required for the formation of the pre-replication complex are inactivated as soon as DNA replication starts (Arias & Walter 2007; Blow & Dutta 2005; Machida, et al. 2005). Surprisingly, despite the importance of a single round of DNA replication and the redundant levels of control, certain single gene mutations cause substantial re-replication. As an important example, *C. elegans cul-4* displays such a re-replication phenotype (Zhong, et al. 2003).

cul-4 encodes the core subunit of a cullin based E3 ubiquitin ligase that targets substrate proteins for ubiquitylation and degradation. Kipreos and coworkers studied the effects of *cul-4* inhibition by RNAi in the epithelial stem-cell like “seam” cells in the *C. elegans* skin. Interestingly, they observed that *cul-4* RNAi resulted in seam cells with up to a 100n DNA content and showed that this results from extensive re-replication rather than failed mitosis (Zhong, et al. 2003). As mentioned above, a key mechanism of preventing re-replication is inactivation of the components that form the pre-replication complex. Indeed, it was shown that *cul-4* is required for the degradation of one of these components. When *cul-4* is inhibited, CDT-1 levels do not drop at the end of G1 but remain constant throughout S-phase, indicating that CUL-4 is required for S-phase degradation of CDT-1. Subsequent studies in *C. elegans* and other systems demonstrated that CUL-4 in association with the DNA damage binding protein 1 (DDB-1) recognizes CDT-1 as a substrate (Arias & Walter 2007; Blow & Dutta 2005; Kim & Kipreos 2007a; Kim & Kipreos 2007b). However, degradation of CDT-1 by CUL-4 is not the whole story, since expression of stable CDT-1 alone does not cause noticeable re-replication. CUL-4 was also found to be responsible for the localization of CDC-6, another member of the pre-replication complex. CDC-6 normally accumulates in the nucleus during G1 phase, and is exported from the nucleus to the cytoplasm during S-phase. The activity of CUL-4 turned out to be needed for nuclear export of CDC-6. Thus, CUL-4 inactivation deregulates two essential factors of the pre-replication complex. High nuclear levels of both CDT-1 and CDC-6 in S-phase allow continued origin licensing and promote re-replication (Kim, et al. 2007; Kim & Kipreos 2007a).

Although intriguing, the mechanism by which CUL-4 regulates nuclear export of CDC-6 in S-phase was not immediately apparent. However, two clues were available: CDC6 nuclear export is regulated by Cyclin-CDKs in other systems, and, similar to the human homolog, the amino terminus of *C. elegans* CDC-6 contains multiple nuclear localization signals flanked by potential CDK phosphorylation sites (Kim, et al. 2007; Kim & Kipreos 2007b; Kim, et al. 2008). Phosphorylation at these sites coincides with nuclear export, as demonstrated by phosphospecific-antibody staining, and mutation of all six CDK sites

In each of these models, a cullin RING ubiquitin E3 ligase (CRL) has been identified that contains CUL-4, DDB-1 and a substrate recognition unit CDT-2. This CRL4^{Cdt2} complex recognizes its substrates in an unusual manner. CKI-1, p21^{Cip1} and CDT-1 all contain a PCNA interacting protein (PIP) motif in the N-terminus (Havens & Walter 2009). PCNA is an auxiliary factor of DNA polymerases, which forms a ring around the DNA and acts as a sliding clamp. Because interaction with PCNA is a prerequisite for CRL4^{Cdt2} substrate ubiquitylation, degradation of the CKI and CDT-1 substrates is coupled to DNA replication. In summary, upon association with PCNA, the CDK-inhibitor CKI-1 is recognized by CRL4^{Cdt2} and targeted for degradation. This allows S-phase Cyclin-CDKs to phosphorylate CDC-6, which triggers CDC-6 export from the nucleus. In addition to CKI-1, CRL4^{Cdt2} also targets PCNA-bound CDT-1 for ubiquitin-dependent proteolysis. In *C. elegans*, CDC-6 nuclear export and CDT-1 degradation are two redundant mechanisms that prevent re-replication (Fig. 3) (Kim & Kipreos 2007b; Korzelius & van den Heuvel 2007). Because *C. elegans* does not show redundancy for the CRL4^{Cdt2} E3 ligase in CDT-1 degradation, the function of this complex has been more obvious in *C. elegans*.

5. Activation of the DNA replication checkpoint in early embryos

Incomplete DNA replication activates an S-phase checkpoint, which delays progression through the cell cycle to create time for repair (Branzei & Foiani 2010). Central in this checkpoint is the ATR-Chk1 protein kinase pathway, which is activated by lesions created by stalled replication forks. Active Chk1 phosphorylates downstream cell cycle regulators such as the CDC25 phosphatase that controls the activity of CDK1. This S-phase checkpoint is generally not functional in early embryos. For example, inhibition of DNA replication with a low concentration of hydroxyurea (HU) does not affect cell cycle progression in embryos of *Drosophila*, *Xenopus* or Zebrafish (Hartwell & Weinert 1989). However, the situation is quite different in early *C. elegans* embryos, which not only contain an active S-phase checkpoint, but also activate the ATR-1/Chk-1 pathway as part of normal development (Brauchle, et al. 2003; Encalada, et al. 2000).

The first division of the *C. elegans* zygote is unequal and generates a larger anterior blastomere, AB, and smaller posterior blastomere, P1. These cells give rise to different daughter cell lineages. For instance, P1 continues an additional three asymmetric divisions to produce the germline precursor P4 (Sulston, et al. 1983). In addition to the different fates, cell division in the AB and P1 lineages also occurs with a different timing, with the AB cell dividing approximately 2 minutes earlier than the P1 cell (visible in Fig. 2). Interestingly, *atl-1* ATR and *chk-1* function contributes to this asynchrony of cell division in normal embryos (Brauchle, et al. 2003). Double inactivation of *atl-1* and *chk-1* reduced the time between mitotic entry (nuclear envelope breakdown) of AB and P1 from 125 sec in the wild-type to 75 sec after *atl-1/chk-1* RNAi. Thus, somehow the P1 blastomere might preferentially and highly reproducibly activate the S phase checkpoint. Asymmetric division of the zygote is needed for this distinction between AB and P1 (Brauchle, et al. 2003).

Preferred checkpoint activation in P1 is also visible in mutants with defects in DNA replication, or embryos treated with HU, which inhibits ribonucleotide reductase (Brauchle, et al. 2003; Encalada, et al. 2000; Encalada, et al. 2005; Korzelius, et al. 2011). Both the zygote (P0) and P1 daughter are able to delay mitosis by about 12 minutes when replication is compromised, while the AB daughter halts for only a few minutes. Inactivation of *atl-1* and/or *chk-1* prevents these delays, indicating that this is a legitimate, though limited, S-

phase checkpoint response. The different response of the P1 versus AB lineage has been interpreted as protection of the germline against replication errors. Surprisingly, however, the checkpoint response to DNA damage (rather than replication arrest) appears actively repressed in the P1 lineage (Holway, et al. 2006). Bypassing the checkpoint could serve to maintain the relative timing of blastomere divisions, which is an essential part of development.

6. The MCM helicase is needed for activation of the replication checkpoint

Defects in some replication components trigger a checkpoint arrest, while others do not. For instance, partial loss of function of *div-1*, which encodes a DNA polymerase α -subunit, gives rise to substantial cell cycle delays (Encalada, et al. 2000). The same is true for inhibition of ribonucleotide reductase by HU treatment or *rnr-1* RNAi (Brauchle, et al. 2003). However, *mcm-4* inactivation interferes with DNA synthesis without the induction of a checkpoint response (Korzeliuss, et al. 2011). Cells in *mcm-4*(RNAi) embryos and *mcm-4* mutant larvae enter mitosis at the appropriate time and continue chromosome segregation as well as cell division. Moreover, RNAi of *mcm-4* suppressed the checkpoint delay induced by *rnr-1* inhibition. These data indicate that MCM-4 is not only required for DNA replication but also for activation of the S phase checkpoint. Genome fragmentation has also been reported for *cdt-1*(RNAi) and *cdc-6*(RNAi) embryos. Thus, the assembly of a pre-replication complex appears to be needed to trigger the S-phase checkpoint.

Studies in other organisms support these observations and have demonstrated that activation of the DNA damage and replication checkpoints requires MCM helicase activity. Recruitment of Replication Protein A (RPA) to single-stranded DNA is probably the actual checkpoint trigger (Zou & Elledge 2003). The helicase activity of MCM proteins generates ssDNA, through unwinding the DNA at the replication fork. Stalling of replication forks, e.g. after HU treatment, causes uncoupling of the MCM helicase from DNA polymerase activity (Byun, et al. 2005). Consequently, fork stalling leads to an accumulation of ssDNA, which recruits additional RPA and causes activation of the checkpoint kinases ATR and Chk1. The formation of replication forks and the generation of ssDNA both require MCM function. This explains why *C. elegans mcm-4* loss of function prevents DNA synthesis without activation of the replication checkpoint.

7. Endoreplication: polyploidy required for growth

Endoreplication cycles bypass mitosis while DNA replication continues, which results in a doubling of the ploidy during each endocycle. Endoreplication commonly occurs in specific cell types during metazoan development. In *C. elegans*, only two tissues become polyploid as a result of endoreplication: the intestine and the epidermis (formally known as hypodermis). Intestinal cells endoreplicate during each larval stage, increasing the ploidy to $4n$ at the transition from first to second larval stage and leading to intestinal nuclei with $32n$ DNA in adult animals (Hedgecock & White 1985).

The situation in the epidermis is more complex. Epidermal nuclei reside in syncytia, sharing a common cytoplasm without separating membranes. The largest epidermal syncytium is hyp7, which covers most of the body except for regions of the head and tail (Hedgecock & White 1985). In each larval stage, stem-cell like precursors in the epidermis, known as “seam cells”, divide to create novel seam cells and daughter cells that fuse with the hyp7

syncytium (Sulston & Horvitz 1977). Ultimately, this creates a syncytium with 133 nuclei. The newly created epidermal cells duplicate their genomic DNA prior to fusion, so that they enter the syncytium as $4n$ nuclei (Hedgecock & White 1985). Endoreplication has been reported to occur in adult stage *hyp7* nuclei, although the level varies between nuclei, with an average ploidy of $10n$ to $12n$ in older adults (Fig. 4) (Flemming, et al. 2000; Morita, et al. 2002; Nystrom, et al. 2002).

Fig. 4. DNA endoreplication in the epidermis. A. Propidium iodide staining of a young *C. elegans* adult is shown, arrowhead indicate polyploid nuclei of the epidermis. B. Quantification of DNA content based on propidium iodide staining. Nuclei of the body wall muscles are used as a reference for $2n$ DNA content. The epidermal nuclei show increased ploidy with up to $8n$ DNA content. The DNA content of epidermal nuclei further increases in concert with growth of late stage adults. Each dot represents a single nucleus.

Why these two cell types, the skin and intestine, undergo endoreplication is not fully understood. It has been speculated that endoreplication is used to maintain the integrity of these tissues, while allowing increased genome ploidy to support increases in cell volume and metabolic activity (Kipreos 2005). In many organisms however, endoreplication has been correlated with growth. Indeed, the *C. elegans* epidermis and intestine grow extensively during larval development, and endoreplication in the epidermis has been correlated with the size of the entire animal.

Several observations support this conclusion. Hydroxyurea (HU) treatment of adult animals, in which somatic cell proliferation has been completed, prevents endoreplication in the epidermis as well as growth of the animals. (Lozano, et al. 2006). In contrast, tetraploid

animals are 40% larger in volume than wild type worms, which closely corresponds to the increase in epidermal polyploidy (from average 11.2n to 16.7n, in adults at 148 hrs.). Furthermore, the first generation homozygous *cye-1* cyclin E mutants survive till adulthood because of maternal CYE-1 supplies and these animals show reduced endoreplication in the epidermis and a corresponding reduction in body size (Lozano, et al. 2006). Finally, *mcm-4* mutants fail DNA replication and are severely growth retarded and larval lethal. Specific expression of MCM-4 in the epidermis of such mutants is sufficient to rescue larval growth and lethality (Korzeliuss, et al. 2011). Thus, the polyploidy of the epidermis contributes to the body size of the adult animal.

8. Tissue-specific regulation of DNA replication

Interestingly, endoreplication in the epidermis is regulated by a TGF- β signal transduction pathway. *C. elegans* uses several different TGF- β pathways to control a variety of developmental processes, including growth. Mutations in components of this pathway lead to smaller and thinner adult animals (small phenotype: Sma). The ligand for the growth pathway is DBL-1, which is homologous to DPP/BMP-4 (Morita, et al. 2002; Suzuki, et al. 1999). DBL-1 signals through the Type I and II TGF- β serine/threonine kinase receptors SMA-6 and DAF-4, respectively, to the downstream SMAD transcriptional regulators SMA-2, SMA-3 and SMA-4 (Savage-Dunn 2005). Notably, *daf-4* and *sma-2* mutants are not only small and thin, but also show reduced ploidy of epidermal nuclei (Flemming, et al. 2000; Nystrom, et al. 2002).

A critical downstream target of the DBL-1 pathway has also been identified: *lon-1* (Maduzia, et al. 2002; Morita, et al. 2002). Homozygous *lon-1* mutant animals are longer than normal (Lon phenotype), while overexpression of *lon-1* leads to a small phenotype. Several observations indicate that *lon-1* acts downstream of the SMA-6 TGF- β type I receptor: double *sma-6; lon-1* mutants are still somewhat long, and *lon-1* mRNA levels are increased in *sma-6* mutants. Surprisingly, *lon-1* encodes a putative transmembrane protein, related to the plant pathogenesis-related protein 1 (PR-1) and human glioma-pathogenesis related protein (GliPR-1). This LON-1 protein is expressed and required in the epidermis, and anti-LON-1 antibodies showed localization to apical junctions (Morita, et al. 2002). LON-1 is claimed to repress endoreplication, based on the increased epidermal ploidy in *lon-1(e185)* and *lon-1(RNAi)* adults. However, two other *lon-1* mutations show somewhat reduced ploidy compared to wild-type (Morita, et al. 2002). Thus, although further research is needed, there is strong evidence that the DBL-1 ligand, produced in a set of neurons, activates a TGF- β /SMA pathway, which inhibits *lon-1* expression in the epidermis, and thereby allows endoreplication and growth of the adults.

The TGF- β /SMA/LON-1 pathway should somehow connect to the cell cycle in order to regulate endoreplication. Based on the Sma phenotype of *cye-1* mutants, it has been proposed that Cyclin E is the key regulator (Lozano, et al. 2006). Cyclin E mutant mice also show defects in trophoblast endoreplication (Parisi, et al. 2003), and the fluctuating activity of Cyclin E with its kinase partner CDK-2 drives endoreplication in *Drosophila* (Claycomb & Orr-Weaver 2005; Lilly & Duronio 2005). However, TGF- β /SMA/LON-1 signaling could also act more upstream of *cye-1*, e.g., in the regulation of *cyd-1* Cyclin D. *cyd-1* mutants are also small, and *C. elegans* Cyclin D is needed for endoreplication, at least in the intestine (Boxem & van den Heuvel 2001). At least in *C. elegans*, Cyclin D is essential for G1/S

progression and induction of S phase genes such as MCM proteins (Boxem & van den Heuvel 2001; Boxem & van den Heuvel 2002; Korzelius, et al. 2011).

The *cyd-1* and *mcm-4* mutants show an interesting phenotypic difference. Homozygous mutants of either *cyd-1* or *mcm-4* complete embryogenesis, because of maternal supplies, fail DNA replication from the first larval stage onward, and show severe growth retardation. However, only *mcm-4* mutants show larval lethality, which is fully suppressed by expression of *mcm-4* from an epidermis specific promoter (Korzelius, et al. 2011). The ability to arrest the cell cycle probably underlies the difference between *cyd-1* and *mcm-4* mutants: post-embryonic blast cells in *cyd-1* mutants arrest prior to S phase entry, while they continue abnormal mitosis in *mcm-4* mutants. As a result, the structural integrity of the epidermis is lost only in *mcm-4* mutants, which often causes larval death. Thus, not absence of DNA replication but lack of an S-phase checkpoint response may lead to death of the animal.

9. In conclusion

Studies of DNA replication in *C. elegans* have thus far been limited. Given the variation of well-established models for replication studies, which include budding and fission yeast, *Xenopus* egg extracts, cells in culture, *in vitro* systems and even flies, one could question the need for studying S phase in the worm. However, several important mechanistic insights have been obtained from observations of DNA replication-defective phenotypes in the worm. Moreover, such analyses have emphasized the variation in regulatory mechanisms between different developmental stages and in different cell types, underscoring the need for studies of replication control in a developmental context. The combination of its large embryonic cells, strong cell biology, genetic tractability and highly reproducible lineage now allows for a detailed analysis of the assembly of pre-replication and replication initiation complexes in real time in *C. elegans*. High-throughput studies have already defined RNAi phenotypes for many known DNA replication components, and have identified currently uncharacterized genes with similar phenotypes. Thus, *C. elegans* increasingly adds an attractive developmental animal system for gene discovery, functional characterizations *in vivo*, and live imaging of replication component localizations.

10. References

- Ankeny, R.A. (2001). The natural history of *Caenorhabditis elegans* research. *Nature reviews.Genetics*, Vol.2, No.6, pp. 474-479, ISSN 1471-0056
- Arias, E.E.&Walter, J.C. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes & development*, Vol.21, No.5, pp. 497-518, , ISSN 0890-9369
- Bell, S.P.&Dutta, A. (2002). DNA replication in eukaryotic cells. *Annual Review of Biochemistry*, Vol.71, pp. 333-374, ISSN 0066-4154
- Bennett, M.D.; Leitch, I.J.; Price, H.J. & Johnston, J.S. (2003). Comparisons with *Caenorhabditis* (approximately 100 Mb) and *Drosophila* (approximately 175 Mb) using flow cytometry show genome size in *Arabidopsis* to be approximately 157 Mb and thus approximately 25% larger than the *Arabidopsis* genome initiative

- estimate of approximately 125 Mb. *Annals of botany*, Vol.91, No.5, pp. 547-557, ISSN 0305-7364
- Blow, J.J.&Dutta, A. (2005). Preventing re-replication of chromosomal DNA. *Nature reviews.Molecular cell biology*, Vol.6, No.6, pp. 476-486, ISSN 1471-0072
- Bondar, T.; Kalinina, A.; Khair, L.; Kopanja, D.; Nag, A.; Bagchi, S. & Raychaudhuri, P. (2006). Cul4A and DDB1 associate with Skp2 to target p27Kip1 for proteolysis involving the COP9 signalosome. *Molecular and cellular biology*, Vol.26, No.7, pp. 2531-2539, ISSN 0270-7306
- Bousset, K.&Diffley, J.F. (1998). The Cdc7 protein kinase is required for origin firing during S phase. *Genes & development*, Vol.12, No.4, pp. 480-490, ISSN 0890-9369
- Boxem, M.; Srinivasan, D.G. & van den Heuvel, S. (1999). The *Caenorhabditis elegans* gene *ncc-1* encodes a cdc2-related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase. *Development (Cambridge, England)*, Vol.126, No.10, pp. 2227-2239, ISSN 0950-1991
- Boxem, M.&van den Heuvel, S. (2002). *C. elegans* class B synthetic multivulva genes act in G(1) regulation. *Current biology : CB*, Vol.12, No.11, pp. 906-911, ISSN 0960-9822
- Boxem, M.&van den Heuvel, S. (2001). *lin-35* Rb and *cki-1* Cip/Kip cooperate in developmental regulation of G1 progression in *C. elegans*. *Development (Cambridge, England)*, Vol.128, No.21, pp. 4349-4359, ISSN 0950-1991
- Branzei, D.&Foiani, M. (2010). Maintaining genome stability at the replication fork. *Nature reviews. Molecular cell biology*, Vol.11, No.3, pp. 208-219, ISSN 1471-0080
- Brauchle, M.; Baumer, K. & Gonczy, P. (2003). Differential activation of the DNA replication checkpoint contributes to asynchrony of cell division in *C. elegans* embryos. *Current biology : CB*, Vol.13, No.10, pp. 819-827, ISSN 0960-9822
- Byun, T.S.; Pacek, M.; Yee, M.C.; Walter, J.C. & Cimprich, K.A. (2005). Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes & development*, Vol.19, No.9, pp. 1040-1052, ISSN 0890-9369
- Cinquin, O.; Crittenden, S.L.; Morgan, D.E. & Kimble, J. (2010). Progression from a stem cell-like state to early differentiation in the *C. elegans* germ line. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.107, No.5, pp. 2048-2053, ISSN 1091-6490
- Claycomb, J.M.&Orr-Weaver, T.L. (2005). Developmental gene amplification: insights into DNA replication and gene expression. *Trends in genetics : TIG*, Vol.21, No.3, pp. 149-162, ISSN 0168-9525
- Encalada, S.E.; Martin, P.R.; Phillips, J.B.; Lyczak, R.; Hamill, D.R.; Swan, K.A. & Bowerman, B. (2000). DNA replication defects delay cell division and disrupt cell polarity in early *Caenorhabditis elegans* embryos. *Developmental biology*, Vol.228, No.2, pp. 225-238, ISSN 0012-1606
- Encalada, S.E.; Willis, J.; Lyczak, R. & Bowerman, B. (2005). A spindle checkpoint functions during mitosis in the early *Caenorhabditis elegans* embryo. *Molecular biology of the cell*, Vol.16, No.3, pp. 1056-1070, ISSN 1059-1524
- Feng, H.; Zhong, W.; Punkosdy, G.; Gu, S.; Zhou, L.; Seabolt, E.K. & Kipreos, E.T. (1999). CUL-2 is required for the G1-to-S-phase transition and mitotic chromosome

- condensation in *Caenorhabditis elegans*. *Nature cell biology*, Vol.1, No.8, pp. 486-492, ISSN 1465-7392
- Flemming, A.J.; Shen, Z.Z.; Cunha, A.; Emmons, S.W. & Leroi, A.M. (2000). Somatic polyploidization and cellular proliferation drive body size evolution in nematodes. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.97, No.10, pp. 5285-5290, ISSN 0027-8424
- Frokjaer-Jensen, C.; Davis, M.W.; Hopkins, C.E.; Newman, B.J.; Thummel, J.M.; Olesen, S.P.; Grunnet, M. & Jorgensen, E.M. (2008). Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nature genetics*, Vol.40, No.11, pp. 1375-1383, ISSN 1546-1718
- Hartwell, L.H. & Weinert, T.A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science (New York, N.Y.)*, Vol.246, No.4930, pp. 629-634, ISSN 0036-8075
- Havens, C.G. & Walter, J.C. (2009). Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. *Molecular cell*, Vol.35, No.1, pp. 93-104, ISSN 1097-4164
- Hedgecock, E.M. & White, J.G. (1985). Polyploid tissues in the nematode *Caenorhabditis elegans*. *Developmental biology*, Vol.107, No.1, pp. 128-133, ISSN 0012-1606
- Higa, L.A.; Yang, X.; Zheng, J.; Banks, D.; Wu, M.; Ghosh, P.; Sun, H. & Zhang, H. (2006). Involvement of CUL4 ubiquitin E3 ligases in regulating CDK inhibitors Dacapo/p27Kip1 and cyclin E degradation. *Cell cycle (Georgetown, Tex.)*, Vol.5, No.1, pp. 71-77, ISSN 1551-4005
- Holway, A.H.; Kim, S.H.; La Volpe, A. & Michael, W.M. (2006). Checkpoint silencing during the DNA damage response in *Caenorhabditis elegans* embryos. *The Journal of cell biology*, Vol.172, No.7, pp. 999-1008, ISSN 0021-9525
- Horvitz, H.R. & Sulston, J.E. (1980). Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. *Genetics*, Vol.96, No.2, pp. 435-454, ISSN 0016-6731
- Kim, J.; Feng, H. & Kipreos, E.T. (2007). *C. elegans* CUL-4 prevents rereplication by promoting the nuclear export of CDC-6 via a CKI-1-dependent pathway. *Current biology : CB*, Vol.17, No.11, pp. 966-972, ISSN 0960-9822
- Kim, J. & Kipreos, E.T. (2008). Control of the Cdc6 replication licensing factor in metazoa: the role of nuclear export and the CUL4 ubiquitin ligase. *Cell cycle (Georgetown, Tex.)*, Vol.7, No.2, pp. 146-150, ISSN 1551-4005
- Kim, Y. & Kipreos, E.T. (2007a). The *Caenorhabditis elegans* replication licensing factor CDT-1 is targeted for degradation by the CUL-4/DDB-1 complex. *Molecular and cellular biology*, Vol.27, No.4, pp. 1394-1406, ISSN 0270-7306
- Kim, Y. & Kipreos, E.T. (2007b). Cdt1 degradation to prevent DNA re-replication: conserved and non-conserved pathways. *Cell division*, Vol.2, pp. 18, ISSN 1747-1028
- Kim, Y.; Starostina, N.G. & Kipreos, E.T. (2008). The CRL4Cdt2 ubiquitin ligase targets the degradation of p21Cip1 to control replication licensing. *Genes & development*, Vol.22, No.18, pp. 2507-2519, ISSN 0890-9369
- Kipreos, E.T. (2005). *C. elegans* cell cycles: invariance and stem cell divisions. *Nature reviews. Molecular cell biology*, Vol.6, No.10, pp. 766-776, ISSN 1471-0072

- Korzeliuss, J.; The, I.; Ruijtenberg, S.; Portegijs, V.; Xu, H.; Horvitz, H.R. & van den Heuvel, S. (2011). *C. elegans* MCM-4 is a general DNA replication and checkpoint component with an epidermis-specific requirement for growth and viability. *Developmental biology*, Vol.350, No.2, pp. 358-369, ISSN 1095-564X
- Korzeliuss, J.&van den Heuvel, S. (2007). Replication licensing: oops! ... I did it again. *Current biology : CB*, Vol.17, No.16, pp. R630-2, ISSN 0960-9822
- Labib, K.&Diffley, J.F. (2001). Is the MCM2-7 complex the eukaryotic DNA replication fork helicase? *Current opinion in genetics & development*, Vol.11, No.1, pp. 64-70, ISSN 0959-437X
- Labib, K.; Kearsey, S.E. & Diffley, J.F. (2001). MCM2-7 proteins are essential components of prereplicative complexes that accumulate cooperatively in the nucleus during G1-phase and are required to establish, but not maintain, the S-phase checkpoint. *Molecular biology of the cell*, Vol.12, No.11, pp. 3658-3667, ISSN 1059-1524
- Lilly, M.A.&Duronio, R.J. (2005). New insights into cell cycle control from the *Drosophila* endocycle. *Oncogene*, Vol.24, No.17, pp. 2765-2775, ISSN 0950-9232
- Lozano, E.; Saez, A.G.; Flemming, A.J.; Cunha, A. & Leroi, A.M. (2006). Regulation of growth by ploidy in *Caenorhabditis elegans*. *Current biology : CB*, Vol.16, No.5, pp. 493-498, ISSN 0960-9822
- Machida, Y.J.; Hamlin, J.L. & Dutta, A. (2005). Right place, right time, and only once: replication initiation in metazoans. *Cell*, Vol.123, No.1, pp. 13-24, ISSN 0092-8674
- Maduzia, L.L.; Gumienny, T.L.; Zimmerman, C.M.; Wang, H.; Shetgiri, P.; Krishna, S.; Roberts, A.F. & Padgett, R.W. (2002). *lon-1* regulates *Caenorhabditis elegans* body size downstream of the *dbl-1* TGF beta signaling pathway. *Developmental biology*, Vol.246, No.2, pp. 418-428, ISSN 0012-1606
- McGarry, T.J.&Kirschner, M.W. (1998). Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell*, Vol.93, No.6, pp. 1043-1053, ISSN 0092-8674
- Mechali, M. (2010). Eukaryotic DNA replication origins: many choices for appropriate answers. *Nature reviews.Molecular cell biology*, Vol.11, No.10, pp. 728-738, ISSN 1471-0080
- Mello, C.&Fire, A. (1995). DNA transformation. *Methods in cell biology*, Vol.48, pp. 451-482, ISSN 0091-679X
- Morita, K.; Flemming, A.J.; Sugihara, Y.; Mochii, M.; Suzuki, Y.; Yoshida, S.; Wood, W.B.; Kohara, Y.; Leroi, A.M. & Ueno, N. (2002). A *Caenorhabditis elegans* TGF-beta, DBL-1, controls the expression of LON-1, a PR-related protein, that regulates polyploidization and body length. *The EMBO journal*, Vol.21, No.5, pp. 1063-1073, ISSN 0261-4189
- Nguyen, V.Q.; Co, C. & Li, J.J. (2001). Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature*, Vol.411, No.6841, pp. 1068-1073, ISSN 0028-0836
- Nystrom, J.; Shen, Z.Z.; Aili, M.; Flemming, A.J.; Leroi, A. & Tuck, S. (2002). Increased or decreased levels of *Caenorhabditis elegans lon-3*, a gene encoding a collagen, cause reciprocal changes in body length. *Genetics*, Vol.161, No.1, pp. 83-97, ISSN 0016-6731

- Parisi, T.; Beck, A.R.; Rougier, N.; McNeil, T.; Lucian, L.; Werb, Z. & Amati, B. (2003). Cyclins E1 and E2 are required for endoreplication in placental trophoblast giant cells. *The EMBO journal*, Vol.22, No.18, pp. 4794-4803, ISSN 0261-4189
- Praitis, V.; Casey, E.; Collar, D. & Austin, J. (2001). Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. *Genetics*, Vol.157, No.3, pp. 1217-1226, ISSN 0016-6731
- Remus, D.; Blanchette, M.; Rio, D.C. & Botchan, M.R. (2005). CDK phosphorylation inhibits the DNA-binding and ATP-hydrolysis activities of the *Drosophila* origin recognition complex. *The Journal of biological chemistry*, Vol.280, No.48, pp. 39740-39751, ISSN 0021-9258
- Salic, A. & Mitchison, T.J. (2008). A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.105, No.7, pp. 2415-2420, ISSN 1091-6490
- Savage-Dunn, C. (2005). TGF-beta signaling, In: *Wormbook*, The *C. elegans* Research Community. Retrieved from <<http://www.wormbook.org>>
- Sonnichsen, B.; Koski, L.B.; Walsh, A.; Marschall, P.; Neumann, B.; Brehm, M.; Alleaume, A.M.; Artelt, J.; Bettencourt, P.; Cassin, E.; Hewitson, M.; Holz, C.; Khan, M.; Lazik, S.; Martin, C.; Nitzsche, B.; Ruer, M.; Stamford, J.; Winzi, M.; Heinkel, R.; Roder, M.; Finell, J.; Hantsch, H.; Jones, S.J.; Jones, M.; Piano, F.; Gunsalus, K.C.; Oegema, K.; Gonczy, P.; Coulson, A.; Hyman, A.A. & Echeverri, C.J. (2005). Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature*, Vol.434, No.7032, pp. 462-469, ISSN 1476-4687
- Sulston, J.E. & Horvitz, H.R. (1981). Abnormal cell lineages in mutants of the nematode *Caenorhabditis elegans*. *Developmental biology*, Vol.82, No.1, pp. 41-55, ISSN 0012-1606
- Sulston, J.E. & Horvitz, H.R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Developmental biology*, Vol.56, No.1, pp. 110-156, 0012-1606; 0012-1606
- Sulston, J.E.; Schierenberg, E.; White, J.G. & Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental biology*, Vol.100, No.1, pp. 64-119, ISSN 0012-1606
- Suzuki, Y.; Yandell, M.D.; Roy, P.J.; Krishna, S.; Savage-Dunn, C.; Ross, R.M.; Padgett, R.W. & Wood, W.B. (1999). A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. *Development (Cambridge, England)*, Vol.126, No.2, pp. 241-250, ISSN 0950-1991
- van Leuken, R.; Clijsters, L. & Wolthuis, R. (2008). To cell cycle, swing the APC/C. *Biochimica et biophysica acta*, Vol.1786, No.1, pp. 49-59, ISSN 0006-3002
- Yanagi, K.; Mizuno, T.; Tsuyama, T.; Tada, S.; Iida, Y.; Sugimoto, A.; Eki, T.; Enomoto, T. & Hanaoka, F. (2005). *Caenorhabditis elegans* geminin homologue participates in cell cycle regulation and germ line development. *The Journal of biological chemistry*, Vol.280, No.20, pp. 19689-19694, ISSN 0021-9258

- Zhong, W.; Feng, H.; Santiago, F.E. & Kipreos, E.T. (2003). CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature*, Vol.423, No.6942, pp. 885-889, ISSN 0028-0836
- Zou, L. & Elledge, S.J. (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science (New York, N.Y.)*, Vol.300, No.5625, pp. 1542-1548, ISSN 1095-9203

IntechOpen

IntechOpen

DNA Replication and Related Cellular Processes

Edited by Dr. Jelena Kusic-Tisma

ISBN 978-953-307-775-8

Hard cover, 300 pages

Publisher InTech

Published online 26, September, 2011

Published in print edition September, 2011

Since the discovery of the DNA structure researchers have been highly interested in the molecular basis of genome inheritance. This book covers a wide range of aspects and issues related to the field of DNA replication. The association between genome replication, repair and recombination is also addressed, as well as summaries of recent work of the replication cycles of prokaryotic and eukaryotic viruses. The reader will gain an overview of our current understanding of DNA replication and related cellular processes, and useful resources for further reading.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Suzan Ruijtenberg, Sander van den Heuvel and Inge The (2011). Regulation of DNA Synthesis and Replication Checkpoint Activation During *C. elegans* Development, DNA Replication and Related Cellular Processes, Dr. Jelena Kusic-Tisma (Ed.), ISBN: 978-953-307-775-8, InTech, Available from: <http://www.intechopen.com/books/dna-replication-and-related-cellular-processes/regulation-of-dna-synthesis-and-replication-checkpoint-activation-during-c-elegans-development>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen