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One ring to rule them all? Another cellular responsibility for PCNA

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Abstract

In order to prevent duplication or loss of genomic regions during DNA replication it is essential that the entire genome is copied precisely once every S phase. Cells achieve this by mutually exclusive regulation of origin firing and licensing. A crucial protein involved in origin licensing is Cdt1, and therefore activity of this protein must be strictly controlled. Four recent papers demonstrate that PCNA, an essential sliding clamp used in replication and DNA repair, plays a crucial role in this process by mediating the proteasomal degradation of Cdt1.

Regulation of replication origin firing

Control of DNA replication is vital to maintenance of genome stability as too little replication during S phase would result in loss of genetic information whereas too much would result in duplication of some loci. Thus, cells have robust mechanisms to ensure DNA is replicated exactly once per cell cycle. Crucial for this is the process of origin licensing. During G1 phase of the cell cycle, before DNA synthesis begins, each origin is licensed by the formation of a pre-RC. When a cell enters S phase the licensed origins fire, recruiting the replication machinery, and the ability to form such pre-RCs is turned off. Hence only origins that were pre-licensed, and not those that have just been replicated, can become active. Once all replication has finished, cells inactivate their replication initiation activity and re-activate the licensing machinery, ensuring an oscillation between licensing and replicating states [1].

This oscillation is under the overall control of the various CDKs that regulate the cell cycle. In G1 phase, when CDK activity is low, licensing proteins such as Cdc6 and Cdt1 are active and initiation proteins such as Cdc7/Dbf4 are inactive. In S phase and G2 when CDK activity is high the opposite scenario is enacted (Figure 1). This temporal regulation of activity/inactivity is brought about by a number of cellular events controlled directly or indirectly by the CDKs. These vary between species but include protein activation or inactivation by phosphorylation, sequestration of nuclear-acting proteins in the cytoplasm, protein inhibition (by the binding of inhibitory partners) and specific protein degradation (Figure 2)[2].

The licensing protein Cdt1

Cdt1 is essential for licensing in yeasts and higher eukaryotes. Cellular control of Cdt1 activity is complex, involving association with a regulatory partner (geminin) in metazoans and nuclear exclusion in yeast [3]. Furthermore, Cdt1 is also degraded by the proteasome both in S and G2 phases as well as after DNA damage induced by UV and gamma irradiation, and this degradation requires signals in the N-terminus of the protein. Both the SCF-Skp2 and the Cul4-DDB1 E3 ubiquitin ligases have been implicated in this degradation [4-7]. Recent studies provide evidence that PCNA is a crucial component of this destruction pathway [8-11].

The replication protein PCNA

PCNA has roles in many fundamental cellular processes. This homotrimeric ring encircles duplex DNA and slides along it, tethering PCNA-interacting proteins to DNA. The most crucial role of PCNA is during replication where it is an essential co-factor for DNA polymerases. After origin firing, when the two parental DNA strands have been separated by the replicative helicase, PCNA is loaded onto both leading and lagging strands. Many of the key steps in replication, including DNA polymerisation, Okazaki fragment processing and ligation, DNA methylation, mismatch repair and chromatin assembly are mediated by proteins that interact with PCNA at the replication forks (polδ, polε, Fen1, Lig1, DNMT1, MSH2, CAF-1 to name but a few) [12]. PCNA is also implicated in DNA repair pathways that operate outside of S phase such as nucleotide and base excision repair [12]. Many proteins that interact with PCNA do so via a conserved PCNA-interaction protein (PIP) motif [13].

Connecting Cdt1 and PCNA

A recent study using a *Xenopus laevis* cell-free replication system showed that the S phase-dependent ubiquitination and degradation of Cdt1 both required replication and took place on the chromatin [14]. In an extension of this work this has now been shown to require an interaction between Cdt1 and PCNA mediated by a PIP motif in the N terminus of Cdt1 [8]. This PIP motif was necessary and sufficient for Cdt1-PCNA binding. Mutation of this motif, or PCNA depletion, or prevention of the Cdt1-PCNA interaction using a competitor peptide, prevented the replication-dependent ubiquitination and subsequent degradation of Cdt1. Importantly addition of excess Cdt1 with a mutated PIP motif was sufficient to induce re-replication in this system, highlighting the importance of this degradation for genome stability. The Cul4-DDB1 ubiquitin ligase was responsible for this ubiquitination as depletion of DDB1 prevented Cdt1 degradation. Interestingly, DDB1 and Cdt1 co-immunoprecipitated even in non-replicating extracts but the E3 ligase activity of the complex became activated upon Cdt1 binding to chromatin-associated PCNA [8]. Hence it seems that in *Xenopus* eggs, replication-dependent degradation of Cdt1 is largely controlled by a PCNA and Cul4-DDB1 mediated ubiquitination. This fits with data from *C. elegans* showing that Cul4 removal induces re-replication [7].

In contrast, in human cells although Cul4-DDB1 ligase has been implicated in the degradation of Cdt1 after DNA damage [4, 15] (see below), the control of its degradation in S phase has remained mysterious. Cdt1 contains a cyclin E/A interaction domain which recruits cyclin-CDK and results in CDK-dependent phosphorylation of threonine 29 of Cdt1 [16-18]. This modification allows the recruitment of the E3 ubiquitin ligase SCF-Skp2. This might reasonably be expected to result in Cdt1 degradation, but abrogation of this pathway is not sufficient to completely prevent Cdt1 destruction in human cells [6, 16-18]. Two recent studies [10, 11] provide an explanation for this observation that ties in perfectly with the data from *Xenopus*. As well as by the SCF-Skp2 pathway, human Cdt1 was found to be degraded by Cul4-DDB1 in a PCNA-dependent manner [10, 11]. Thus, redundancy between the SCF-Skp2 and the Cul4-DDB1-dependent ubiquitination pathways explains why inhibition of either separately is insufficient to prevent Cdt1 destruction,

and indeed, co-inhibition of both these pathways by siRNA is sufficient to stabilise Cdt1 in S-phase [10].

Cdt1 degradation after DNA damage

The degradation of Cdt1 in response to DNA damage has been proposed to act as a checkpoint preventing continued origin licensing, and thus replication initiation, until repair is complete [15]. This is mediated by Cul4-DDB1 [4, 15]. As with the S-phase degradation, this ubiquitination and subsequent destruction is now also shown to require an interaction between Cdt1 and PCNA, both in human cells and in *S. pombe* [9-11]. PCNA is essential for DNA synthesis during the repair of UV damage and becomes associated with chromatin during this process, so the biochemical mechanism of these degradation events is likely to be similar.

Concluding remarks

Although these studies are in general complementary there are some discrepancies between them that may be the result of species differences or experimental set-ups, but which need to be resolved. For example the studies in human cells suggests that Cdt1 and PCNA constitutively interact [9-11], at least when Cdt1 is over expressed, a conclusion which is not supported by the *Xenopus* data [8]. Importantly however, the Arias and Walter paper demonstrated that only chromatin bound PCNA is capable of triggering the Cdt1 destruction [8]. This is essential to ensure that the licensing activity is destroyed only at those origins that have fired, and that the degradation of Cdt1 is spatially limited within the cell. Verification that the same holds in human cells, as well as identification of the mechanism preventing activation of Cdt1 destruction by soluble PCNA remain crucial aims for the future.

Over expression of Cdt1 is found in some non-small cell lung carcinomas and cancer cell lines [19, 20], and it has oncogenic potential [21, 22]. Hence it is likely that in some tumour types the balance between origin licensing and firing is perturbed. While this may contribute to genomic instability that can be a driving force for tumour development, it could also be seen as an “Achilles’ heel” for these cells. The multiple redundant methods evolved by cells to ensure licensing control suggests that it is crucial for the cell to properly regulate licensing activity. It may be possible to develop strategies that target the remaining licensing control mechanisms in these Cdt1-dysregulated tumours. While the normal cells of the patient would be resistant to such treatment due to their unperturbed control of Cdt1, the tumours would then have lost all control of licensing activity, presumably a lethal event. This hypothesis could be initially tested in tissue culture systems using siRNA technology and, if successful, efforts to identify small molecules that interfere with, for example, Cdc6 activity or regulation, would be justified.

Figure legends

Figure 1 – A simplified view of the control of origin licensing and origin firing throughout the cell cycle.
Cells are either in a licensing state, characterised by the association of inactive MCM2-7 proteins with the origin recognition complexes (ORC) at origins, or a firing state, characterised by active replications forks in which MCM2-7 has moved away

from the origin and DNA synthesis can occur. The mutually exclusive nature of these states is ultimately regulated by the CDK activities, which control key licensing and firing components such as Cdc6 and Cdt1 or Cdc7 and Dbf4, respectively, by a variety of mechanisms – see text and Figure 2 for details.

Figure 2 – Multiple mechanisms to prevent origin licensing activity during origin firing stages.
Cdc6 and Cdt1 are two crucial regulators of origin licensing as they enable the binding of the helicase MCM2-7 to ORC at origins. In order to prevent re-replication licensing activity and origin firing activity must be tightly regulated. This is achieved by a variety of mechanisms. Four recent papers have demonstrated that PCNA is essential to mediate one of these (the proteasomal degradation of Cdt1) – see text for details.

References

1. Blow, J.J. and A. Dutta, *Preventing re-replication of chromosomal DNA.* Nat Rev Mol Cell Biol, 2005. **6**(6): p. 476-486.
2. Diffley, J.F., *Regulation of early events in chromosome replication.* Curr Biol, 2004. **14**(18): p. R778-786.
3. Saxena, S. and A. Dutta, *Geminin-Cdt1 balance is critical for genetic stability.* Mutat Res, 2005. **569**(1-2): p. 111-121.
4. Hu, J., et al., *Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage.* Nat Cell Biol, 2004. **6**(10): p. 1003-1009.
5. Kondo, T., et al., *Rapid degradation of Cdt1 upon UV-induced DNA damage is mediated by SCFSkp2 complex.* J Biol Chem, 2004. **279**(26): p. 27315-27319.
6. Li, X., et al., *The SCF(Skp2) ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1 degradation.* J Biol Chem, 2003. **278**(33): p. 30854-30858.
7. Zhong, W., et al., *CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing.* Nature, 2003. **423**(6942): p. 885-889.
8. Arias, E.E. and J.C. Walter, *PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication.* Nat Cell Biol, 2006. **8**(1): p. 84-90.
9. Hu, J. and Y. Xiong, *An evolutionarily conserved function of proliferating cell nuclear antigen for Cdt1 degradation by the Cul4-Ddb1 ubiquitin ligase in response to DNA damage.* J Biol Chem, 2006. **281**(7): p. 3753-3756.
10. Nishitani, H., et al., *Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis.* Embo J, 2006. **25**(5): p. 1126-1136.
11. Senga, T., et al., *PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination.* J Biol Chem, 2006. **281**(10): p. 6246-6252.
12. Maga, G. and U. Hubscher, *Proliferating cell nuclear antigen (PCNA): a dancer with many partners.* J Cell Sci, 2003. **116**(Pt 15): p. 3051-3060.
13. Warbrick, E., *The puzzle of PCNA's many partners.* Bioessays, 2000. **22**(11): p. 997-1006.
14. Arias, E.E. and J.C. Walter, *Replication-dependent destruction of Cdt1 limits DNA replication to a single round per cell cycle in Xenopus egg extracts.* Genes Dev, 2005. **19**(1): p. 114-126.

15. Higa, L.A., et al., *Radiation-mediated proteolysis of CDT1 by CUL4-ROC1 and CSN complexes constitutes a new checkpoint.* Nat Cell Biol, 2003. **5**(11): p. 1008-1015.
16. Liu, E., et al., *Cyclin-dependent kinases phosphorylate human Cdt1 and induce its degradation.* J Biol Chem, 2004. **279**(17): p. 17283-17288.
17. Sugimoto, N., et al., *Cdt1 phosphorylation by cyclin A-dependent kinases negatively regulates its function without affecting geminin binding.* J Biol Chem, 2004. **279**(19): p. 19691-19697.
18. Takeda, D.Y., J.D. Parvin, and A. Dutta, *Degradation of Cdt1 during S phase is Skp2-independent and is required for efficient progression of mammalian cells through S phase.* J Biol Chem, 2005. **280**(24): p. 23416-23423.
19. Xouri, G., et al., *Cdt1 and geminin are down-regulated upon cell cycle exit and are over-expressed in cancer-derived cell lines.* Eur J Biochem, 2004. **271**(16): p. 3368-3378.
20. Karakaidos, P., et al., *Overexpression of the replication licensing regulators hCdt1 and hCdc6 characterizes a subset of non-small-cell lung carcinomas: synergistic effect with mutant p53 on tumor growth and chromosomal instability--evidence of E2F-1 transcriptional control over hCdt1.* Am J Pathol, 2004. **165**(4): p. 1351-1365.
21. Seo, J., et al., *Cdt1 transgenic mice develop lymphoblastic lymphoma in the absence of p53.* Oncogene, 2005. **24**(55): p. 8176-8186.
22. Arentson, E., et al., *Oncogenic potential of the DNA replication licensing protein CDT1.* Oncogene, 2002. **21**(8): p. 1150-1158.

Glossary

Replication origin
: A genomic locus at which DNA replication can initiate. In *S. cerevisiae* replication origins have specific sequence requirements but to date the determinants of replication origins in higher eukaryotes remains unclear.

Pre-replication complex (Pre-RC)
: The protein-DNA complex that assembles on a replication origin and serves to mark the places where replication can commence.

Licensing
: The process of assembling pre-RCs at replication origins and priming them to a state competent to fire.

Re-replication
: The result of loss of control of licensing and firing activities that results in some or all of the genome being replicated multiple times in the same S phase.

Cyclin dependent kinases (CDKs)
: The various protein kinases that control progression through the cell cycle and are activated by association with specific cyclin partners.

Proteasome
: A multi protein proteolysis complex that recognises and degrades poly-ubiquitinated target proteins.

Ubiquitin ligase
: An enzyme that covalently attaches ubiquitin to substrate proteins, in the cases described here resulting in their recognition and degradation by the proteasome.

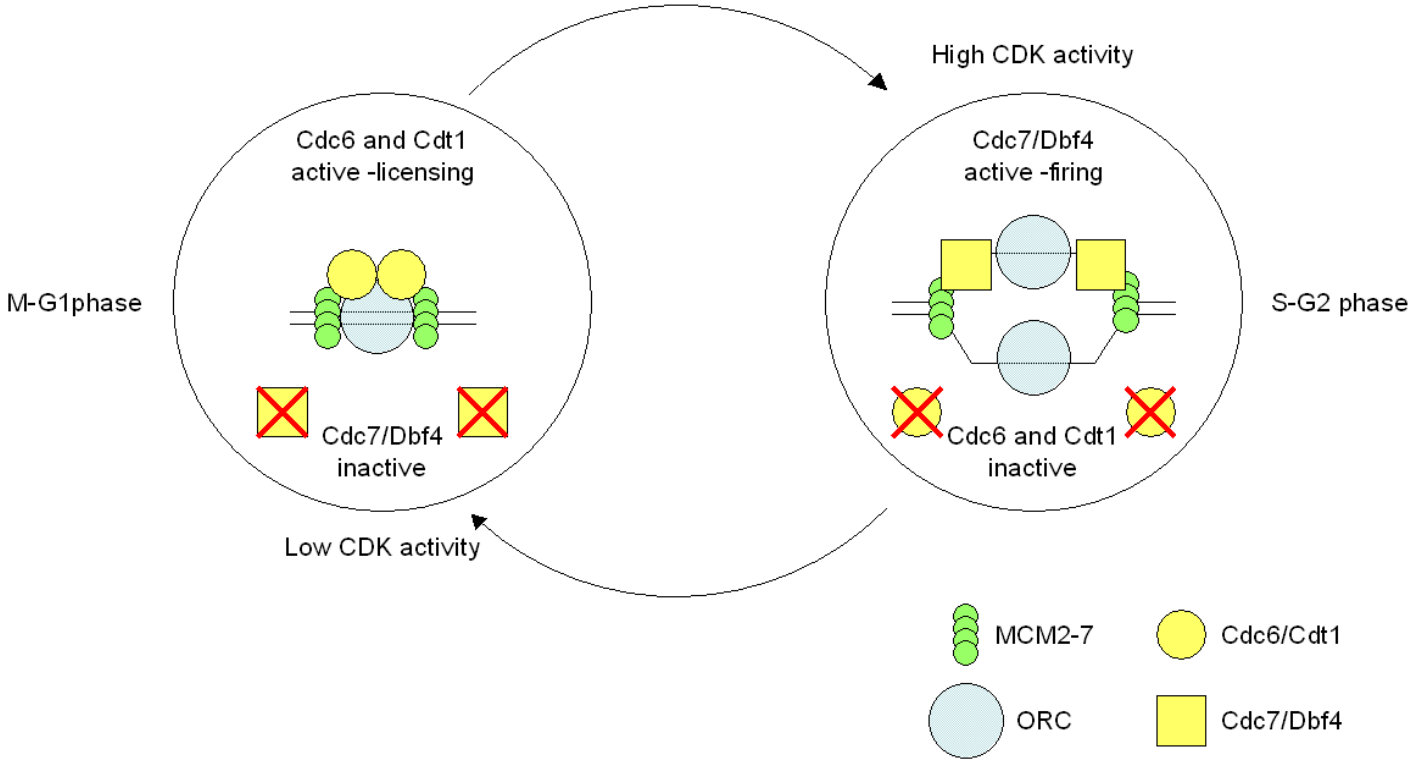
High CDK activity
Cdc6 and Cdt1
active -licensing
Cdc7/Dbf4
active -firing
M-G1phase
S-G2 phase
Cdc7/Dbf4
inactive
Cdc6 and Cdt1
inactive
Low CDK activity
MCM2-7
Cdc6/Cdt1
ORC
Cdc7/Dbf4

Figure 1

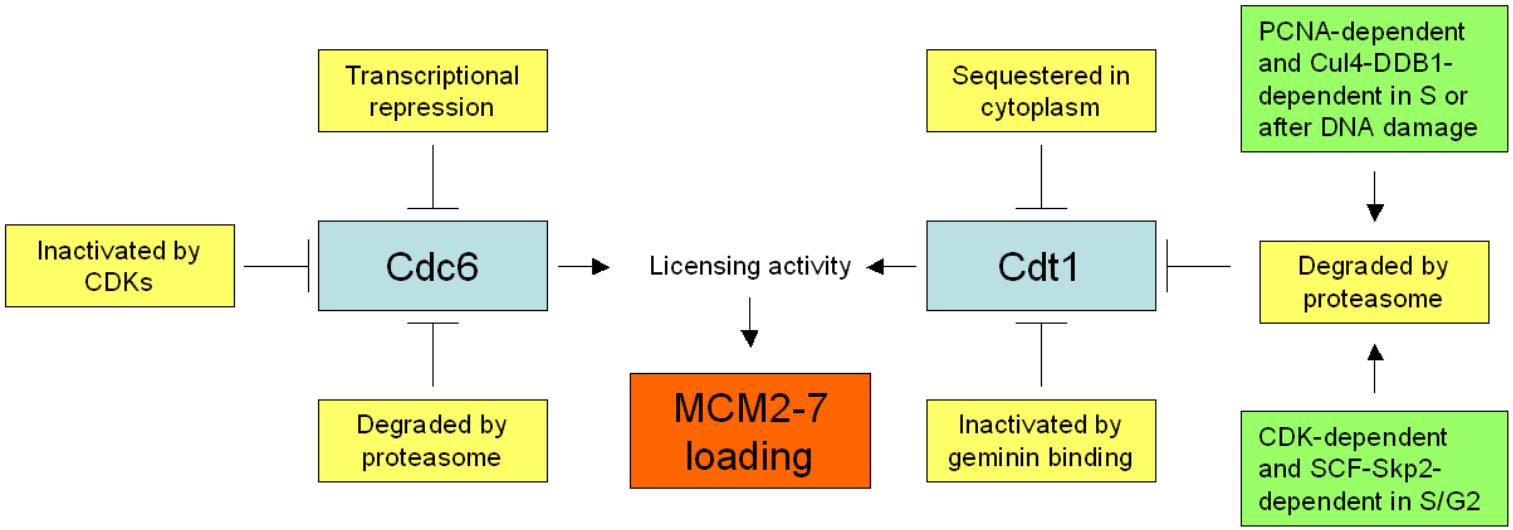
Transcriptional repression
Sequestered in cytoplasm
PCNA-dependent and Cul4-DDB1-dependent in S or after DNA damage
Inactivated by CDKs
Cdc6
Licensing activity
Cdt1
Degraded by proteasome
MCM2-7 loading
Degraded by proteasome
Inactivated by geminin binding
CDK-dependent and SCF-Skp2-dependent in S/G2

Figure 2