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Replication Licensing and Cancer - a Fatal Entanglement?

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Preface

Correct regulation of the replication licensing system ensures that chromosomal DNA is precisely duplicated in each cell division cycle. Licensing proteins are inappropriately expressed at an early stage of tumorigenesis in a wide variety of cancers. Here we discuss evidence that misregulation of replication licensing is a consequence of oncogene-induced cell proliferation. This misregulation can cause either under- or over-replication of chromosomal DNA, and could explain the genetic instability commonly seen in cancer cells.

Overview of the replication licensing system

To prevent the occurrence of potentially cancer-causing alterations to the genome, it is crucial that chromosomal DNA is precisely duplicated during S phase of the cell division cycle. Because of the large size of animal chromosomes, it is necessary for them to be replicated by thousands of replication forks initiated at replication origins scattered throughout the genome. The activity of these replication origins must be carefully regulated to ensure precise chromosome duplication. If too few replication origins are active, there is a danger that the chromosomal DNA will not be completely replicated during S phase, which can potentially lead to DNA strand breaks and gross chromosomal rearrangements in surviving daughter cells. It is equally important to ensure that no replication origin initiates more than once in each cell cycle, as this would lead to re-duplication (amplification) of the DNA in the vicinity of the over-firing origin and other consequent chromosomal rearrangements.

Correct regulation of the replication licensing system is responsible for ensuring the proper regulation of replication origins during cell cycle progression¹⁻³. Origin licensing, which occurs prior to S phase in late mitosis and early G1 (Figure 1) involves the stable loading of the Mcm2-7 replication proteins onto DNA at replication origins. Mcm2-7 are essential replication fork proteins which probably provide the helicase activity to unwind the template DNA ahead of the fork^{4,5}. Because of this behaviour, Mcm2-7 complexes move away from each origin as it initiates, thereby leaving the origin in an unlicensed state. In the absence of DNA-bound Mcm2-7, replication origins cannot initiate (Figure 1). Therefore to prevent replicated origins from being re-licensed (and hence re-replicated) after they have initiated, it is necessary for the replication licensing system to be shut down prior to entry into S phase.

Replication licensing requires at least 3 proteins in addition to Mcm2-7: the origin recognition complex (ORC), Cdc6 and Cdt1^{1-3,6}. ORC first binds to DNA at replication origins and recruits Cdc6 and Cdt1. These proteins then act together to load Mcm2-7 onto DNA, plausibly by opening up the ring-shaped Mcm2-7 complex and clamping it around the DNA⁷. Consistent with this model, once Mcm2-7 have been loaded onto the DNA, ORC, Cdc6 and Cdt1 are no longer required for Mcm2-7 to remain bound and for the origin to

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remain functionally licensed². The complex of ORC, Cdc6, Cdt1 and Mcm2-7 at replication origins is termed the 'pre-replicative complex' or pre-RC.

ORC, Cdc6 and Cdt1 are each subject to cell cycle regulation that could individually contribute to the inactivation of the licensing system on entry into S phase. In most animal cells, however, it appears that down-regulation of Cdt1 at the G1/S transition is the key event that prevents re-licensing of replicated origins. Cdt1 is regulated in at least two different ways. First it is subject to cell cycle dependent proteolysis during S phase and G2-M. Secondly, Cdt1 activity is inhibited by the tight binding of a small regulatory protein called geminin¹²⁻¹⁴. The levels and activity of geminin are cell cycle-regulated, so that geminin only binds to Cdt1 during S, G2 and early mitosis. In most cell types, it is sufficient to prevent Cdt1 degradation or geminin inhibition for significant re-licensing and re-replication of DNA to occur¹⁵⁻²¹.

In addition to this cell-cycle control, the licensing system is also down-regulated when cells exit from the cell division cycle²²⁻²⁶. Many of the cells in an adult human are not actively engaged in the cell division cycle, but have either withdrawn from it, either temporarily into G0 or permanently as a consequence of terminal differentiation or senescence. When this happens, replication origins are converted into the unlicensed state. In addition, Mcm2-7 and other pre-RC proteins are degraded (Figure 1). Because Mcm2-7 proteins are abundant throughout the cell division cycle, this makes them a unique marker of cells with proliferative capacity, providing great potential for histopathology²⁴⁻²⁷. Fig 2a shows a cross-section through normal cervical epithelium stained for Mcm5. The basal proliferating layers of the cervical squamous epithelium contain high levels of Mcm5 which are lost as cells differentiate and migrate towards the outer surface.

Expression of licensing proteins in cancer cells

Many studies have now shown that there is inappropriate expression of Mcm2-7 and other pre-RC proteins in a wide variety of pre-malignant dysplasias and cancers^{24-26,28,29}. This is typically associated with an increased number of cells expressing Mcm2-7 or cells expressing Mcm2-7 where they should not normally do so. For example, in low- and high-grade squamous intraepithelial lesions of the cervix, cells with proliferative potential are seen at increasing distances from the basal layer, and this is mirrored by immunostaining for Mcm5 (Figures 2b and c). Similar over-expression of Mcm2-7 and other licensing proteins have been seen in a wide variety of different cancers, including oral, laryngeal, oesophageal, pulmonary, mammary, ovarian, renal, prostatic, urothelial, colorectal and haematological cancers^{24,26}. This protein overexpression also appears to be reflected in overexpression of mRNA levels. Expression of Mcm2-7 has prognostic value and can help predict survival in patients with a range of different cancers²⁴⁻²⁶.

It is currently unclear why dysplastic and malignant cells express licensing proteins inappropriately. One possibility is that it may reflect the failure of cells to exit the cell cycle properly. During malignant transformation the cell cycle is disrupted, typically with increased activity of CDKs that drive cell cycle progression. This may result in fewer cells following their normal differentiation programme and exiting the cell cycle, with the consequence that an increased proportion of cells remain in-cycle and express licensing proteins. In this view, the increased expression of Mcm2-7 and other licensing proteins in cancers is a consequence of oncogene-induced stimulation of cell division that decreases the proportion of cells that are in a quiescent (out-of-cycle) state. As such, Mcm2-7 would not be directly involved in oncogenic progression, but would provide a powerful marker for in-cycle cells.

An alternative and intriguing possibility, supported by recent data, is that misregulation of the licensing system may play a causal role in the development of cancer. Not only do licensing proteins themselves show oncogenic activity, but genes that are commonly mutated in cancer such as Ras, cyclin D1 and cyclin E, can cause misregulation of the licensing system. Oncogenes can either induce the re-licensing of replicated DNA or can allow cells to enter S phase with an insufficient number of licensed replication origins. In either case, the integrity of chromosomal DNA is compromised. The possible ways that oncogenes might misregulate the licensing system will be explored in the remainder of this review.

Re-licensing in S and G2

If the licensing machinery is not completely shut down prior to entry into S phase, replication origins can be re-licensed after they have initiated a pair of forks, allowing the origin to fire a second time in S phase. This is distinct from endoreduplication, where a failure of mitosis leads to a second S phase in the absence of cell division²³. The main route by which the licensing system is down-regulated in S phase in animal cells is by proteolysis of Cdt1 and activation of the Cdt1 inhibitor geminin. Overexpression of Cdt1 or the loss of geminin is sufficient to misregulate the licensing system and to induce re-replication¹⁵⁻²¹. It is therefore essential that both Cdt1 and geminin activities are well controlled, especially during S and G2 phase, such that a balance between the two activities is maintained¹⁻³.

Cell cycle regulated proteolysis of Cdt1 is mediated by its ubiquitination, which is controlled by at least two distinct mechanisms. In the first mechanism, Cdt1 is a substrate for the Cul4-Ddb1-Cdt2 ubiquitin ligase which requires Cul4-Ddb1-Cdt2 to be recruited to the DNA polymerase processivity factor PCNA^{11,19,30-37}. This means Cdt1 ubiquitination is coupled to ongoing DNA synthesis, thus limiting the likelihood of inappropriate origin re-licensing during DNA replication or repair. In the second mechanism, the SCF-Skp2 complex promotes Cdt1 proteolysis during S and G2 phases in a manner dependent on the phosphorylation of Cdt1 by Cdk2-cyclin A^{9,38-40}. Since Cdk2-cyclin A is active during S phase and G2, and is involved in promoting the initiation of DNA replication, this provides a second mechanism by which Cdt1 destruction is functionally coupled to S phase progression. Loss of Ddb1, Cdt2 or Cul4, but not Skp2, is sufficient to induce re-replication^{10,32,33,36} suggesting that Cdt1 degradation mediated by Cul4-Ddb1-Cdt2 provides a major control mechanism for preventing re-replication. Cdt1 is also a substrate of the APC/C ubiquitin ligase, which is active from anaphase through to late G1^{20,41}. APC/C activity does not lead to a complete loss of Cdt1 in G1, but may serve to prevent excessive accumulation of active Cdt1 at this time, prior to S phase entry and geminin accumulation.

Geminin is a small protein that binds to Cdt1 and blocks its ability to load Mcm2-7 onto DNA¹²⁻¹⁴ and is active during S, G2 and M phases of the cell cycle^{3,12,42,43}. At the metaphase to anaphase transition geminin is ubiquitinated by the APC/C, which leads either to its proteolysis or inactivation. This establishes a period of licensing competence during late M phase and G1. Loss of geminin promotes re-licensing and re-replication in many, but not all, cell types^{15,17,20,21,44}. Misregulation of APC/C activity can therefore potentially lead to re-replication as a consequence of a failure to correctly regulate geminin. Loss of the APC/C regulator Emi1 promotes re-replication in this way⁴⁵⁻⁴⁸. Emi1 serves to inhibit APC/C activity in late G1 so that sufficient CDK activity can accumulate to permit S phase entry. Loss of Emi1 destabilises geminin during S phase as APC/C activity is maintained, and thus provides a licensing competent environment. Although cyclin A and cyclin B activity is reduced in the absence of Emi1, cyclin E activity is increased to a level that replication initiation can be supported^{45,49}.

Both ORC and Cdc6 activity are regulated in animal cell cycles, though in most cell types this does not appear to play a major role in preventing the re-licensing of replicated DNA^{2,3,50}. A proportion of Cdc6 is exported from the nucleus during S and G₂, but the extent of this appears to vary between cell types⁵¹⁻⁵³. There is some evidence for limited re-replication of DNA occurring as a consequence of deregulation of Cdc6 in some, but not all, cell types^{16,54-56}. Overexpression of Cdc6 does, however, significantly increase the degree of re-replication that occurs on de-regulation of Cdt1, suggesting that low levels of Cdc6 in the nucleus during S and G₂ play a supporting role in preventing re-replication^{16,54,57}. A potential role for ORC regulation in preventing re-replication has not been extensively studied.

Stabilisation of Cdt1 and Cdc6, or loss of either geminin, Emi1, Cdt2, Ddb1 or Cul4 can promote re-replication. Re-replication induced by misregulation of these factors can cause accumulation of double strand DNA breaks and activation of the DNA damage response DDR^{15-17,20,21,32,33,46,49,58,59}. The DDR activates cell cycle checkpoints, which delays progression through S phase and the onset on M phase or promotes apoptosis (Figure 3).

The DNA structures generated by re-replication may depend on the frequency of origin re-initiation³. Infrequent origin re-activation would generate local regions of DNA that had been re-replicated just once (Figure 4a). Forks from these origins will eventually stall or collapse (Figure 4d). Alternatively, frequent re-initiation would drive multiple rounds of re-replication occurring from a single origin (Figure 4b-c). Experiments in *Xenopus* suggest that consecutive forks travelling in the same direction from the same parental origin have a high probability of colliding⁵⁸. Such 'head-to-tail' fork collisions result in the accumulation of extruded linear strands DNA structures (Figure 4c).

It is currently unclear what ultimately happens to these aberrant DNA structures. It is likely that they will be recognised and processed by the DDR machinery, possibly becoming a substrate for homologous recombination. Likely outcomes are that either an extra chromosomal DNA fragment is generated (Figure 4e) or that the re-replicated fragment is recombined to generate a localised intrachromosomal duplication (Figure 4f). Since the re-replicated DNA contains an origin of replication this instability may be heritable. Heritable instability would be amplified if specific replication origins were more likely to support re-replication. Partial misregulation of the licensing system in *S. cerevisiae* results in detectable re-replication occurring preferentially at specific chromosomal loci⁶⁰. Propagation of this amplification over several generations could result in repeated duplication of this particular locus, causing significant genetic instability.

Consistent with these ideas, both Cdt1 and Cdc6 are oncogenes^{55,61-63}. Overexpression of Cdt1 in cells injected into nude mice results in tumour formation⁶¹ and mice specifically overexpressing Cdt1 in T-cells develop thymic lymphoblastic lymphoma in p53 null mice⁶³. It is important to note that although replication licensing is essential for cell division, there is no evidence to suggest that it is a rate-limiting step for progression through G₁. Therefore increasing the rate of loading Mcm2-7 onto DNA in G₁ is unlikely to increase the rate of cell division by itself. Instead it is more likely that the oncogenic activity of Cdt1 is a consequence of the genetic instability it induces. Consistent with this idea, tumour cells derived from cells overexpressing Cdt1 displayed severe chromosomal aberrations and genetic instability.

The role of Cdc6 in tumourigenesis is potentially more complex. In addition to facilitating re-replication, Cdc6 overexpression causes heterochromatinisation and repression of the INK4/ARF tumour suppressor locus⁶². Cdc6 has also been shown to play a role in

activating checkpoint kinases in response to replication inhibition⁶⁴⁻⁶⁷. Furthermore, Cdc6 may stimulate inappropriate recovery from a Cip1-mediated cell cycle arrest in response to DNA damage by releasing Cip1 from CDK68. Cdc6 overexpression may therefore exert a plethora of effects, each of which could lead to genetic instability⁶⁹. Nevertheless it is possible that the oncogenic activity of Cdc6 is in part mediated by its ability to promote re-replication.

Re-replication caused by mutation of components of the licensing system may therefore contribute to the genetic instability seen in cancer. However, there is no evidence that such mutations are commonly seen in cancer cells (see for example Supplementary Table S1). A lack of mutations in the licensing system might be a consequence of animal cells primarily using down-regulation of Cdt1 in S and G2 to prevent re-licensing of replicated DNA. Focussing all of the regulation on Cdt1 might make it harder for mutations to arise that lead to genetic instability, since loss of geminin or stabilisation of Cdt1 typically cause lethal levels of re-replication⁷⁰.

However, recent evidence suggests that activation of oncogenes more commonly involved in tumorigenesis can interfere with the mechanisms that normally shut down replication licensing in S phase and G2. This causes low levels of re-replication that are sufficient to cause genetic instability but are compatible with cell survival. One such case is cyclin D1, a potent oncogene that is frequently mutated in human tumours. Overexpression of a mutant form of cyclin D1 has recently been shown to cause origin re-licensing and re-replication in a single cell cycle ⁷¹. This cyclin D1 induced re-replication appears to be caused by loss of Cul4 expression and consequent stabilisation of Cdt1 in S phase.

A second case involves the Ras oncogene. Ectopic expression of Ras in primary cells induces them to undergo oncogene induced senescence⁷². Senescent cells arrest with partially replicated DNA and strong induction of the DDR. Fluorescence in situ hybridization showed that the senescent cells had more than the expected 2 copies of certain chromosomal loci, which suggests that DNA re-replication had occurred. In addition, there was evidence for a high rate of replication fork stalling and the activation of dormant replication origins. Similar DDR activation, fork stalling and induction of oncogene induced senescence were seen when the Mos oncogene or Cdc6 were ectopically expressed in primary cells⁷³. Suppression of the DDR after these oncogenes had induced senescence led to reactivation of proliferation and tumour formation, providing an appealing model for the early events of tumorigenesis⁷²⁻⁷⁴.

It is currently unclear how frequently re-replication is induced by oncogene activation in primary cells. One possibility is that oncogenes activating growth regulatory pathways upstream of Rb behave like cyclin D1 and Ras and prevent full inactivation of the licensing system in S phase and G2, thus leading to re-replication, DNA damage and DDR activation. This could well be the first part of the recent two step model of tumorigenesis⁷²⁻⁷⁴. Oncogene-induced re-replication could drive cells into oncogene-induced senescence, from which they would only emerge as a consequence of additional mutations in the DDR system, thereby providing an explanation for the observed phenotypes of cells early in tumorigenesis. It would be of great interest to test this idea and to determine which pathways controlling licensing activity are misregulated by oncogenes.

Insufficient origin licensing

An alternative consequence of misregulation of the licensing system might be to reduce the loading of Mcm2-7 onto chromatin prior to entry into S phase. Mcm2-7 are essential replication proteins so if their loading onto DNA is completely prevented, DNA replication cannot occur⁷⁵⁻⁸⁰. However, reducing (rather than abolishing) the quantity of Mcm2-7

loaded onto DNA can cause complicated consequences by reducing the number of replication origins that the cell can use.

Eukaryotic cells use a significantly larger number of replication origins than seems strictly necessary to complete replication in the time available for S phase. For example, typical mammalian somatic cells use replication origins spaced on average 30 - 150 kb apart, even though the forks initiated from a single origin could potentially replicate ~1.5 Mb of DNA over the entire period of S phase. There are probably several reasons for the excessive number of replication origins used, but one important reason is likely to be to add a degree of redundancy to the system to deal with problems that may occur during S phase. Forks encountering DNA damage or tightly associated DNA-protein complexes can irreversibly stall⁸¹, and if two converging forks stall it is difficult for the intervening DNA to be replicated (Figure 5a). If Mcm2-7 loading is reduced, fewer replication origins are used and this results in DNA strand breaks, checkpoint activation, genetic instability and cell death⁸²⁻⁹⁰, consistent with the idea that abundant origins are required to compensate for replication fork failures.

Not only is there an excess of replication origins over the minimum number required to complete S phase in a timely manner, but there are 10-20 times more Mcm2-7 molecules loaded onto DNA in G1 than there are active replication origins^{83,91-93}. Cells continue to synthesise DNA at normal rates when the level of Mcm2-7 is reduced⁹⁴⁻⁹⁶ and in *Xenopus* egg extracts normal replication rates are maintained when Mcm2-7 levels are reduced to only ~2 per origin^{66,92,93}.

Recent work has provided evidence that these excess Mcm2-7 are required for cells to properly cope with replicative stresses that might induce replication fork stalling⁹⁶⁻⁹⁸. When two converging replication forks irreversibly stall (Figure 5a) it is not possible to licence a new origin between the two stalled forks, because the licensing system needs to be inactivated prior to entry into S phase (Figure 1). If further licensing were allowed at this stage, there is no known mechanism that could direct the Mcm2-7 to the unreplicated portion of DNA rather than the replicated DNA, so there would be a high risk of DNA being re-replicated. It is possible that homologous recombination could be used to restart the stalled forks, but this creates the risk of DNA strand breakage or chromosome rearrangement. However, inhibition of replication forks promotes the activation of dormant origins that do not fire in unperturbed S phases⁹⁶⁻¹⁰². The activity of these dormant origins is dependent on the full complement of Mcm2-7 being loaded onto DNA^{96,97}. In the absence of replicative stress, these dormant origins are passively replicated by forks from neighbouring origins, so do not normally fire (Figure 5b). However, dormant origins become essential for complete replication and cell survival under conditions of replicative stress⁹⁶⁻⁹⁸ (Figure 5c).

Consistent with these ideas, mice heterozygous for an Mcm4 hypomorphic mutation which apparently destabilises Mcm4 protein (Mcm4^{Chaos3}) showed greatly increased rates of chromosome breakage in response to a replication inhibitor¹⁰³. Chaos3 mutant mice also showed increased levels of micronuclei, another sign of increased chromosome instability. Significantly, 80% of Chaos3 females died of mammary adenocarcinomas. In a separate study¹⁰⁴, cells from a mouse strain which produce lowered levels of transgenic Mcm2 (Mcm2^{IRES-CreERT2}) also showed increased micronuclei and increased γ -H2AX foci (a sign of double-strand DNA breaks). Mcm2^{IRES-CreERT2} cells proliferated at normal rates, though they appeared to have a severe stem cell deficiency in various tissues. Mcm2^{IRES-CreERT2} mouse died of cancer at an early age, but unlike the Chaos3 mice, this was predominantly T- and B-cell lymphomas.

These studies are consistent with the idea that insufficient origin licensing can promote the development of cancer. The virtually normal proliferative capacity of cells with mutant Mcm2-7 coupled with their increased sensitivity to replication inhibitors and increased levels of spontaneous DNA damage is consistent with the idea that they are unable to license a sufficient number of dormant origins and so cannot deal properly with sporadic replication defects. This would create genetic instability and thereby promote the development of cancer. As discussed above, misregulation of the licensing system appears to be an early event in the development of many cancers. To date this has been noted as an increased proportion of cells expressing Mcm2-7 and other licensing proteins. However, it would be important to know whether individual cells are expressing adequate levels of the licensing proteins and are being driven through S phase with an sufficient number of licensed origins.

When cyclin E is overexpressed in cells, they show a reduction in the amount of Mcm2-7 loaded onto DNA during late mitosis and G1⁷⁸. Consistent with this causing a reduction in the number of origins licensed, cyclin E overexpressing cells also show decreased rates of S phase progression, genetic instability and accelerated tumorigenesis^{78,105,106}. Like Ras overexpression⁷², cyclin E overexpression in primary cells caused DDR activation and oncogene induced senescence⁷³. It would be interesting to investigate how common it is for oncogene activation to cause insufficient Mcm2-7 loading in G1, and the mechanism by which cyclin E overexpression causes this.

The results discussed here and in the section above suggest that oncogenes can induce DNA damage and DDR activation both by re-licensing origins in S and G2 and by insufficient origin licensing in G1^{71-73,78}. Both ways of misregulating the licensing system lead to stalled replication forks that generate structures recognized by the DDR machinery that can potentially drive cells into oncogene-induced senescence⁷²⁻⁷⁴. Cells escaping from this by acquiring a secondary mutation in the DDR then have a lethal combination of high proliferative capacity due to oncogene activation and genetic instability due to oncogene-induced licensing defects and loss of adequate DDR responses.

The licensing checkpoint

Since it is critical that cells load sufficient Mcm2-7 onto DNA before they embark on S phase, it is plausible that they possess a feedback system that could delay progression into S phase until a sufficient number of origins are licensed. This idea has been addressed using a number of different approaches to reduce the quantity of functional Mcm2-7 loaded onto DNA^{86,88,89,107-109}. The response of cells to inhibition of licensing, either by forced expression of geminin or by RNAi against licensing proteins, depended on the cell type^{86,88}. Primary cell lines responded to licensing inhibition by delaying entry into S phase, thereby keeping cells at a cell cycle stage where further licensing could potentially occur. This suggests that primary cells possess some sort of licensing checkpoint that delays entry into S phase if insufficient Mcm2-7 has been loaded onto DNA. In contrast, when licensing was inhibited in a range of different cancer cell lines they did not delay progression into S phase, but instead entered an S phase that they were unable to complete, and which was therefore lethal. Some less-transformed cancer cells with active checkpoint mechanisms underwent a relatively rapid apoptosis, whilst more transformed cells survived longer but ultimately died at a later cell cycle stage with partially-replicated chromosomes^{86,89}. A similar difference between normal and cancer cells was seen when cells were treated with siRNA against the Cdc7 protein kinase¹⁰⁹. Since the essential function of Cdc7 is to phosphorylate and activate Mcm2-7, this treatment may have many similar effects to reducing the total amount of Mcm2-7. The observation that the licensing checkpoint appears defective in many cancer cell lines suggests that they frequently experience inefficient origin licensing.

The way that primary cells detect and respond to decreased Mcm2-7 levels is currently unclear. In *Xenopus* egg extracts, a feedback loop has been described that promotes entry into S phase only when Mcm2-7 have been loaded onto DNA¹¹⁰. This works by Mcm2-7 on chromatin stimulating the loading of an essential nuclear pore protein ELYS/MEL-28; since nuclear pore function is required both for progression into S phase and for the activation of geminin as a licensing inhibitor^{42,43} this creates a feedback loop. It is unlikely that this sort of mechanism will operate in somatic cells with a lengthy G1 phase. Instead, the licensing checkpoint appears to depend on down-regulating CDK activity in late G1 of somatic cells. Normal cells treated with the licensing inhibitor geminin or with siRNA against one of the ORC subunits caused cell cycle arrest in a G1-like state with low cyclin E Cdk2 activity and induction of the CDK inhibitor Cip^{186,89,108}. Activation of this 'licensing checkpoint' may involve a novel pathway that blocks activation of S-phase CDKs without involving the classical DDR pathway (Jean Cook, personal communication).

The licensing system as potential anti-cancer target

A large number of chemotherapeutic drugs target, either directly or indirectly, the process of DNA replication (Supplementary Table S2). The anti-metabolite class of drugs, for example, directly affect the supply of dNTPs to the replicative DNA polymerases, whereas many DNA damaging agents, such as alkylating agents, are primarily recognised by the DDR whilst the DNA is being replicated¹¹¹. The replication fork, which is the target of these chemotherapeutic drugs, appears to be essentially normal in cancer cells, but as discussed above, the replication licensing system may be frequently misregulated. An intriguing possibility is that the effectiveness of these chemotherapeutic drugs is due to misregulation of the licensing system in cancer cells. For example, cancer cells that license a reduced number of origins in G1 would be expected to be hypersensitive to a range of replication inhibitors^{96,97}.

Further, if there is a 'licensing checkpoint' that is defective in cancer cells, then small molecule inhibitors of the replication licensing system will specifically kill these cancer cells, whilst only delaying proliferation of normal cells. Normal cells finding their origins unlicensed would respond by activating the licensing checkpoint and arresting temporarily in a G1-like state. When the drug was removed or metabolised, re-licensing of origins and entry into S phase could then occur. Cancer cells lacking the licensing checkpoint would suffer a different fate, as they would pass into S phase with an insufficient number of licensed replication origins to complete replication. It is impossible for these cells to regain viability, because even if the inhibitor were subsequently removed or metabolised, no further origin licensing could take place once the cells had progressed into S phase. Since replication licensing is essential for cell proliferation, cancer cells could not become resistant to licensing inhibitors by using an alternative pathway. Licensing inhibitors might also be expected to synergise well with existing chemotherapeutic drugs. These considerations make replication licensing an attractive anti-cancer target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Nishitani H, Lygerou Z. DNA replication licensing. *Frontiers in Bioscience*. 2004; 9:2115–2132. [PubMed: 15353274]
2. Blow JJ, Dutta A. Preventing re-replication of chromosomal DNA. *Nat Rev Mol Cell Biol*. 2005; 6:476–86. [PubMed: 15928711]
3. Arias EE, Walter JC. Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes Dev*. 2007; 21:497–518. [PubMed: 17344412]
4. Labib K, Diffley JF. Is the MCM2-7 complex the eukaryotic DNA replication fork helicase? *Curr Opin Genet Dev*. 2001; 11:64–70. [PubMed: 11163153]
5. Moyer SE, Lewis PW, Botchan MR. Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc Natl Acad Sci USA*. 2006; 103:10236–41. [PubMed: 16798881]
6. Gillespie PJ, Li A, Blow JJ. Reconstitution of licensed replication origins on *Xenopus* sperm nuclei using purified proteins. *BMC Biochem*. 2001; 2:15. [PubMed: 11737877]
7. Perkins G, Diffley JF. Nucleotide-dependent prereplicative complex assembly by Cdc6p, a homolog of eukaryotic and prokaryotic clamp-loaders. *Mol Cell*. 1998; 2:23–32. [PubMed: 9702188]
8. Nishitani H, Taraviras S, Lygerou Z, Nishimoto T. The human licensing factor for DNA replication Cdt1 accumulates in G1 and is destabilized after initiation of S-phase. *J Biol Chem*. 2001; 276:44905–11. [PubMed: 11555648]
9. Li X, Zhao Q, Liao R, Sun P, Wu X. The SCF(Skp2) ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1 degradation. *J Biol Chem*. 2003; 278:30854–8. [PubMed: 12840033]
10. Zhong W, Feng H, Santiago FE, Kipreos ET. CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature*. 2003; 423:885–9. [PubMed: 12815436]
11. Arias EE, Walter JC. PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nat Cell Biol*. 2006; 8:84–90. [PubMed: 16362051]
12. McGarry TJ, Kirschner MW. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell*. 1998; 93:1043–1053. [PubMed: 9635433]
13. Tada S, Li A, Maiorano D, Mechali M, Blow JJ. Repression of origin assembly in metaphase depends on inhibition of RLF-B/Cdt1 by geminin. *Nature Cell Biol*. 2001; 3:107–113. [PubMed: 11175741]
14. Wohlschlegel JA, Dwyer BT, Dhar SK, Cvetic C, Walter JC, et al. Inhibition of eukaryotic replication by geminin binding to Cdt1. *Science*. 2000; 290:2309–2312. [PubMed: 11125146]
15. Mihaylov IS, Kondo T, Jones L, Ryzhikov S, Tanaka J, et al. Control of DNA replication and chromosome ploidy by geminin and cyclin A. *Mol Cell Biol*. 2002; 22:1868–1880. [PubMed: 11865064]
16. Vaziri C, Saxena S, Jeon Y, Lee C, Murata K, et al. A p53-dependent checkpoint pathway prevents rereplication. *Mol Cell*. 2003; 11:997–1008. [PubMed: 12718885]
17. Melixetian M, Ballabeni A, Masiero L, Gasparini P, Zamponi R, et al. Loss of Geminin induces rereplication in the presence of functional p53. *J Cell Biol*. 2004; 165:473–482. [PubMed: 15159417]
18. Thomer M, May NR, Aggarwal BD, Kwok G, Calvi BR. *Drosophila* double-parked is sufficient to induce re-replication during development and is regulated by cyclin E/CDK2. *Development*. 2004; 131:4807–18. [PubMed: 15342466]
19. Arias EE, Walter JC. Replication-dependent destruction of Cdt1 limits DNA replication to a single round per cell cycle in *Xenopus* egg extracts. *Genes Dev*. 2005; 19:114–26. [PubMed: 15598982]
20. Li A, Blow JJ. Cdt1 downregulation by proteolysis and geminin inhibition prevents DNA re-replication in *Xenopus*. *EMBO J*. 2005; 24:395–404. [PubMed: 15616577]
21. Zhu W, Chen Y, Dutta A. Rereplication by depletion of geminin is seen regardless of p53 status and activates a G2/M checkpoint. *Mol Cell Biol*. 2004; 24:7140–7150. [PubMed: 15282313]
22. Stoeber K, Tlsty TD, Happerfield L, Thomas GA, Romanov S, et al. DNA replication licensing and human cell proliferation. *J Cell Sci*. 2001; 114:2027–41. [PubMed: 11493639]

23. Blow JJ, Hodgson B. Replication licensing: defining the proliferative state? *Trends Cell Biol.* 2002; 12:72–78. [PubMed: 11849970]
24. Gonzalez MA, Tachibana KE, Laskey RA, Coleman N. Control of DNA replication and its potential clinical exploitation. *Nat Rev Cancer.* 2005; 5:135–41. [PubMed: 15660109]
25. Hook SS, Lin JJ, Dutta A. Mechanisms to control rereplication and implications for cancer. *Curr Opin Cell Biol.* 2007; 19:663–71. [PubMed: 18053699]
26. Williams GH, Stoeber K. Cell cycle markers in clinical oncology. *Curr Opin Cell Biol.* 2007; 19:672–9. [PubMed: 18032010]
27. Williams GH, Romanowski P, Morris L, Madine M, Mills AD, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci USA.* 1998; 95:14932–7. [PubMed: 9843993]
28. Xouri G, Lygerou Z, Nishitani H, Pachnis V, Nurse P, 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; 271:3368–78. [PubMed: 15291814]
29. Lau E, Tsuji T, Guo L, Lu SH, Jiang W. The role of pre-replicative complex (pre-RC) components in oncogenesis. *Faseb J.* 2007; 21:3786–94. [PubMed: 17690155]
30. Hu J, McCall CM, Ohta T, Xiong Y. Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage. *Nat Cell Biol.* 2004; 6:1003–9. [PubMed: 15448697]
31. Hu J, Xiong Y. 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; 281:3753–6. [PubMed: 16407242]
32. Jin J, Arias EE, Chen J, Harper JW, Walter JC. A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. *Mol Cell.* 2006; 23:709–21. [PubMed: 16949367]
33. Lovejoy CA, Lock K, Yenamandra A, Cortez D. DDB1 maintains genome integrity through regulation of Cdt1. *Mol Cell Biol.* 2006; 26:7977–90. [PubMed: 16940174]
34. Nishitani H, Sugimoto N, Roukos V, Nakanishi Y, Saijo M, et al. Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. *EMBO J.* 2006; 25:1126–36. [PubMed: 16482215]
35. Ralph E, Boye E, Kearsey SE. DNA damage induces Cdt1 proteolysis in fission yeast through a pathway dependent on Cdt2 and Ddb1. *EMBO Rep.* 2006; 7:1134–9. [PubMed: 17039252]
36. Sansam CL, Shepard JL, Lai K, Ianari A, Danielian PS, et al. DTL/CDT2 is essential for both CDT1 regulation and the early G2/M checkpoint. *Genes Dev.* 2006; 20:3117–29. [PubMed: 17085480]
37. Senga T, Sivaprasad U, Zhu W, Park JH, Arias EE, et al. PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. *J Biol Chem.* 2006; 281:6246–52. [PubMed: 16407252]
38. Liu E, Li X, Yan F, Zhao Q, Wu X. Cyclin-dependent kinases phosphorylate human Cdt1 and induce its degradation. *J Biol Chem.* 2004; 279:17283–17288. [PubMed: 15004027]
39. Sugimoto N, Tatsumi Y, Tsurumi T, Matsukage A, Kiyono T, et al. Cdt1 phosphorylation by cyclin A-dependent kinases negatively regulates its function without affecting geminin binding. *J Biol Chem.* 2004; 279:19691–19697. [PubMed: 14993212]
40. Takeda DY, Parvin JD, Dutta A. 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; 280:23416–23. [PubMed: 15855168]
41. Sugimoto N, Kitabayashi I, Osano S, Tatsumi Y, Yugawa T, et al. Identification of Novel Human Cdt1-binding Proteins by a Proteomics Approach: Proteolytic Regulation by APC/CCdh1. *Mol Biol Cell.* 2008; 19:1007–21. [PubMed: 18162579]
42. Hodgson B, Li A, Tada S, Blow JJ. Geminin becomes activated as an inhibitor of Cdt1/RLF-B following nuclear import. *Curr Biol.* 2002; 12:678–683. [PubMed: 11967157]
43. Li A, Blow JJ. Non-proteolytic inactivation of geminin requires CDK-dependent ubiquitination. *Nat Cell Biol.* 2004; 6:260–267. [PubMed: 14767479]

44. Quinn LM, Herr A, McGarry TJ, Richardson H. The Drosophila Geminin homolog: roles for Geminin in limiting DNA replication, in anaphase and in neurogenesis. *Genes Dev.* 2001; 15:2741–2754. [PubMed: 11641279]
45. Di Fiore B, Pines J. Emi1 is needed to couple DNA replication with mitosis but does not regulate activation of the mitotic APC/C. *J Cell Biol.* 2007; 177:425–37. [PubMed: 17485488]
46. Machida YJ, Dutta A. The APC/C inhibitor, Emi1, is essential for prevention of rereplication. *Genes Dev.* 2007; 21:184–94. [PubMed: 17234884]
47. Sivaprasad U, Machida YJ, Dutta A. APC/C--the master controller of origin licensing? *Cell Div.* 2007; 2:8. [PubMed: 17319958]
48. Lehman NL, Verschuren EW, Hsu JY, Cherry AM, Jackson PK. Overexpression of the anaphase promoting complex/cyclosome inhibitor Emi1 leads to tetraploidy and genomic instability of p53-deficient cells. *Cell Cycle.* 2006; 5:1569–73. [PubMed: 16861914]
49. Verschuren EW, Ban KH, Masek MA, Lehman NL, Jackson PK. Loss of Emi1-dependent anaphase-promoting complex/cyclosome inhibition deregulates E2F target expression and elicits DNA damage-induced senescence. *Mol Cell Biol.* 2007; 27:7955–65. [PubMed: 17875940]
50. DePamphilis ML, Blow JJ, Ghosh S, Saha T, Noguchi K, et al. Regulating the licensing of DNA replication origins in metazoa. *Curr Opin Cell Biol.* 2006; 18:231–9. [PubMed: 16650748]
51. Petersen BO, Lukas J, Sorensen CS, Bartek J, Helin K. Phosphorylation of mammalian CDC6 by Cyclin A/CDK2 regulates its subcellular localization. *EMBO J.* 1999; 18:396–410. [PubMed: 9889196]
52. Saha P, Chen JJ, Thome KC, Lawlis SJ, Hou ZH, et al. Human CDC6/Cdc18 associates with Orc1 and cyclin-cdk and is selectively eliminated from the nucleus at the onset of S phase. *Mol Cell Biol.* 1998; 18:2758–2767. [PubMed: 9566895]
53. Alexandrow MG, Hamlin JL. Cdc6 chromatin affinity is unaffected by serine-54 phosphorylation, S-phase progression, and overexpression of cyclin A. *Mol Cell Biol.* 2004; 24:1614–1627. [PubMed: 14749377]
54. Kim J, Feng H, Kipreos ET. C. elegans CUL-4 prevents rereplication by promoting the nuclear export of CDC-6 via a CKI-1-dependent pathway. *Curr Biol.* 2007; 17:966–72. [PubMed: 17509881]
55. Lontos M, Koutsami M, Sideridou M, Evangelou K, Kletsas D, et al. Deregulated overexpression of hCdt1 and hCdc6 promotes malignant behavior. *Cancer Res.* 2007; 67:10899–909. [PubMed: 18006835]
56. Petersen BO, Wagener C, Marinoni F, Kramer ER, Melixetian M, et al. Cell cycle- and cell growth-regulated proteolysis of mammalian CDC6 is dependent on APC-CDH1. *Genes Dev.* 2000; 14:2330–43. [PubMed: 10995389]
57. Nishitani H, Lygerou Z, Nishimoto T, Nurse P. The Cdt1 protein is required to license DNA for replication in fission yeast. *Nature.* 2000; 404:625–8. [PubMed: 10766248]
58. Davidson IF, Li A, Blow JJ. Deregulated replication licensing causes DNA fragmentation consistent with head-to-tail fork collision. *Mol Cell.* 2006; 24:433–43. [PubMed: 17081992]
59. Zhu W, Dutta A. An ATR- and BRCA1-mediated Fanconi anemia pathway is required for activating the G2/M checkpoint and DNA damage repair upon rereplication. *Mol Cell Biol.* 2006; 26:4601–11. [PubMed: 16738325]
60. Green BM, Li JJ. Loss of rereplication control in *Saccharomyces cerevisiae* results in extensive DNA damage. *Mol Biol Cell.* 2005; 16:421–432. [PubMed: 15537702]
61. Arentson E, Faloony P, Seo J, Moon E, Studts JM, et al. Oncogenic potential of the DNA replication licensing protein CDT1. *Oncogene.* 2002; 21:1150–8. [PubMed: 11850834]
62. Gonzalez S, Klatt P, Delgado S, Conde E, Lopez-Rios F, et al. Oncogenic activity of Cdc6 through repression of the INK4/ARF locus. *Nature.* 2006; 440:702–6. [PubMed: 16572177]
63. Seo J, Chung YS, Sharma GG, Moon E, Burack WR, et al. Cdt1 transgenic mice develop lymphoblastic lymphoma in the absence of p53. *Oncogene.* 2005; 24:8176–86. [PubMed: 16261166]
64. Murakami H, Yanow SK, Griffiths D, Nakanishi M, Nurse P. Maintenance of replication forks and the S-phase checkpoint by Cdc18p and Orp1p. *Nature Cell Biol.* 2002; 4:384–388. [PubMed: 11988741]

65. Clay-Farrace L, Pelizon C, Santamaria D, Pines J, Laskey RA. Human replication protein Cdc6 prevents mitosis through a checkpoint mechanism that implicates Chk1. *EMBO J.* 2003; 22:704–712. [PubMed: 12554670]
66. Oehlmann M, Score AJ, Blow JJ. The role of Cdc6 in ensuring complete genome licensing and S phase checkpoint activation. *J Cell Biol.* 2004; 165:181–90. [PubMed: 15096526]
67. Hermand D, Nurse P. Cdc18 enforces long-term maintenance of the S phase checkpoint by anchoring the Rad3-Rad26 complex to chromatin. *Mol Cell.* 2007; 26:553–63. [PubMed: 17531813]
68. Kan Q, Jinno S, Kobayashi K, Yamamoto H, Okayama H. CDC6 determines utilization of P21waf1/cip1-dependent damage checkpoint in S phase cells. *J Biol Chem.* 2008
69. Borlado LR, Mendez J. CDC6: from DNA replication to cell cycle checkpoints and oncogenesis. *Carcinogenesis.* 2008; 29:237–43. [PubMed: 18048387]
70. Blow JJ, Tanaka TU. The chromosome cycle: coordinating replication and segregation. Second in the cycles review series. *EMBO Rep.* 2005; 6:1028–34. [PubMed: 16264427]
71. Aggarwal P, Lessie MD, Lin DI, Pontano L, Gladden AB, et al. Nuclear accumulation of cyclin D1 during S phase inhibits Cul4-dependent Cdt1 proteolysis and triggers p53-dependent DNA rereplication. *Genes Dev.* 2007; 21:2908–22. [PubMed: 18006686]
72. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature.* 2006; 444:638–42. [PubMed: 17136094]
73. Bartkova J, Rezaei N, Lontos M, Karakaidos P, Kletsas D, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature.* 2006; 444:633–637. [PubMed: 17136093]
74. Di Micco R, Fumagalli M, d'Adda di Fagagna F. Breaking news: high-speed race ends in arrest—how oncogenes induce senescence. *Trends Cell Biol.* 2007; 17:529–36. [PubMed: 17980599]
75. Chong JP, Mahbubani HM, Khoo CY, Blow JJ. Purification of an MCM-containing complex as a component of the DNA replication licensing system. *Nature.* 1995; 375:418–421. [PubMed: 7760937]
76. Kubota Y, Mimura S, Nishimoto S, Takisawa H, Nojima H. Identification of the yeast MCM3-related protein as a component of Xenopus DNA Replication Licensing Factor. *Cell.* 1995; 81:601–609. [PubMed: 7758114]
77. Madine MA, Khoo C-Y, Mills AD, Laskey RA. MCM3 complex required for cell cycle regulation of DNA replication in vertebrate cells. *Nature.* 1995; 375:421–424. [PubMed: 7760938]
78. Ekholm-Reed S, Mendez J, Tedesco D, Zetterberg A, Stillman B, et al. Deregulation of cyclin E in human cells interferes with prereplication complex assembly. *J Cell Biol.* 2004; 165:789–800. [PubMed: 15197178]
79. Tsuji T, Ficarro SB, Jiang W. Essential role of phosphorylation of MCM2 by Cdc7/Dbf4 in the initiation of DNA replication in mammalian cells. *Mol Biol Cell.* 2006; 17:4459–72. [PubMed: 16899510]
80. Stuermer A, Hoehn K, Faul T, Auth T, Brand N, et al. Mouse pre-replicative complex proteins colocalise and interact with the centrosome. *Eur J Cell Biol.* 2007; 86:37–50. [PubMed: 17157410]
81. Lambert S, Carr AM. Checkpoint responses to replication fork barriers. *Biochimie.* 2005; 87:591–602. [PubMed: 15989976]
82. Coxon A, Maundrell K, Kearsley SE. Fission yeast cdc21+ belongs to a family of proteins involved in an early step of chromosome replication. *Nucleic Acids Res.* 1992; 20:5571–7. [PubMed: 1454522]
83. Lei M, Kawasaki Y, Tye BK. Physical interactions among Mcm proteins and effects of Mcm dosage on DNA replication in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1996; 16:5081–90. [PubMed: 8756666]
84. Liang DT, Hodson JA, Forsburg SL. Reduced dosage of a single fission yeast MCM protein causes genetic instability and S phase delay. *J Cell Sci.* 1999; 112:559–67. [PubMed: 9914167]
85. Lengronne A, Schwob E. The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G(1). *Mol Cell.* 2002; 9:1067–78. [PubMed: 12049742]

86. Shreeram S, Sparks A, Lane DP, Blow JJ. Cell type-specific responses of human cells to inhibition of replication licensing. *Oncogene*. 2002; 21:6624–6632. [PubMed: 12242660]
87. Tanaka S, Diffley JF. Deregulated G1-cyclin expression induces genomic instability by preventing efficient pre-RC formation. *Genes Dev*. 2002; 16:2639–49. [PubMed: 12381663]
88. Feng D, Tu Z, Wu W, Liang C. Inhibiting the expression of DNA replication-initiation proteins induces apoptosis in human cancer cells. *Cancer Res*. 2003; 63:7356–64. [PubMed: 14612534]
89. Teer JK, Machida YJ, Labit H, Novac O, Hyrien O, et al. Proliferating human cells hypomorphic for origin recognition complex 2 and pre-replicative complex formation have a defect in p53 activation and Cdk2 kinase activation. *J Biol Chem*. 2006; 281:6253–60. [PubMed: 16407251]
90. Bailis JM, Luche DD, Hunter T, Forsburg SL. Minichromosome maintenance proteins interact with checkpoint and recombination proteins to promote s-phase genome stability. *Mol Cell Biol*. 2008; 28:1724–38. [PubMed: 18180284]
91. Burkhardt R, Schulte D, Hu D, Musahl C, Gohring F, et al. Interactions of human nuclear proteins P1Mcm3 and P1Cdc46. *Eur J Biochem*. 1995; 228:431–438. [PubMed: 7705359]
92. Mahbubani HM, Chong JP, Chevalier S, Thömmes P, Blow JJ. Cell cycle regulation of the replication licensing system: involvement of a Cdk-dependent inhibitor. *J Cell Biol*. 1997; 136:125–135. [PubMed: 9008708]
93. Edwards MC, Tutter AV, Cvetic C, Gilbert CH, Prokhorova TA, et al. MCM2-7 complexes bind chromatin in a distributed pattern surrounding the origin recognition complex in *Xenopus* egg extracts. *J Biol Chem*. 2002; 277:33049–33057. [PubMed: 12087101]
94. Cortez D, Glick G, Elledge SJ. Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. *Proc Natl Acad Sci USA*. 2004; 101:10078–10083. [PubMed: 15210935]
95. Tsao CC, Geisen C, Abraham RT. Interaction between human MCM7 and Rad17 proteins is required for replication checkpoint signaling. *EMBO J*. 2004; 23:4660–9. [PubMed: 15538388]
96. Ge XQ, Jackson DA, Blow JJ. Dormant origins licensed by excess Mcm2-7 are required for human cells to survive replicative stress. *Genes Dev*. 2007; 21:3331–41. [PubMed: 18079179]
97. Woodward AM, Gohler T, Luciani MG, Oehlmann M, Ge X, et al. Excess Mcm2-7 license dormant origins of replication that can be used under conditions of replicative stress. *J Cell Biol*. 2006; 173:673–683. [PubMed: 16754955]
98. Ibarra A, Schwob E, Mendez J. Excess MCM proteins protect human cells from replicative stress by licensing backup origins of replication. *Proc Natl Acad Sci U S A*. 2008; 105:8956–61. [PubMed: 18579778]
99. Ockey CH, Saffhill R. The comparative effects of short-term DNA Inhibition on replicon synthesis in mammalian cells. *Exp Cell Res*. 1976; 103:361–373. [PubMed: 137123]
100. Taylor JH. Increase in DNA replication sites in cells held at the beginning of S phase. *Chromosoma*. 1977; 62:291–300. [PubMed: 142621]
101. Anglana M, Apiou F, Bensimon A, Debatisse M. Dynamics of DNA replication in mammalian somatic cells: nucleotide pool modulates origin choice and interorigin spacing. *Cell*. 2003; 114:385–394. [PubMed: 12914702]
102. Gilbert DM. Replication origin plasticity, Taylor-made: inhibition vs recruitment of origins under conditions of replication stress. *Chromosoma*. 2007; 116:341–7. [PubMed: 17404750]
103. Shima N, Alcaraz A, Liachko I, Buske TR, Andrews CA, et al. A viable allele of Mcm4 causes chromosome instability and mammary adenocarcinomas in mice. *Nat Genet*. 2007; 39:93–8. [PubMed: 17143284]
104. Pruitt SC, Bailey KJ, Freeland A. Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer. *Stem Cells*. 2007; 25:3121–32. [PubMed: 17717065]
105. Spruck CH, Won KA, Reed SI. Deregulated cyclin E induces chromosome instability. *Nature*. 1999; 401:297–300. [PubMed: 10499591]
106. Loeb KR, Kostner H, Firpo E, Norwood T, K DT, et al. A mouse model for cyclin E-dependent genetic instability and tumorigenesis. *Cancer Cell*. 2005; 8:35–47. [PubMed: 16023597]
107. Lei M. The MCM complex: its role in DNA replication and implications for cancer therapy. *Curr Cancer Drug Targets*. 2005; 5:365–80. [PubMed: 16101384]

108. Machida YJ, Teer JK, Dutta A. Acute reduction of an origin recognition complex (ORC) subunit in human cells reveals a requirement of ORC for Cdk2 activation. *J Biol Chem.* 2005; 280:27624–30. Epub 2005 Jun 7. [PubMed: 15944161]
109. Montagnoli A, Tenca P, Sola F, Carpani D, Brotherton D, et al. Cdc7 inhibition reveals a p53-dependent replication checkpoint that is defective in cancer cells. *Cancer Res.* 2004; 64:7110–6. [PubMed: 15466207]
110. Gillespie PJ, Khoudoli GA, Stewart G, Swedlow JR, Blow JJ. ELYS/MEL-28 chromatin association coordinates nuclear pore complex assembly and replication licensing. *Curr Biol.* 2007; 17:1657–62. [PubMed: 17825564]
111. Tercero JA, Diffley JF. Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint. *Nature.* 2001; 412:553–7. [PubMed: 11484057]

Figure 1.

Overview of origin licensing during the cell division cycle.

A small segment of chromosomal DNA, encompassing 3 replication origins is shown during the cell cycle. Mcm2-7 complexes (here shown as a double hexamer) are loaded onto origins during G1 when the licensing system is active (green). During S phase, Mcm2-7 complexes at licensed origins are activated to form part of the replication fork. When replication forks terminate, Mcm2-7 is displaced from the DNA. Prior to entry into S phase, the licensing system is shut down (pink shading) so Mcm2-7 cannot be loaded onto unreplicated DNA. This occurs as a consequence of geminin activation and Cdt1 degradation during S and G2, plus high CDK activity during mitosis. On exit from the cell cycle into G0, terminal differentiation or senescence, the licensing system is inactivated and Mcm2-7 are degraded.

Figure 2.
Mcm5 in normal and dysplastic cervical epithelium.
Frozen sections of normal cervix (a), low-grade (b) and high-grade (c) squamous intraepithelial lesions stained with antibodies against Mcm5. Magnification x108. Reproduced from 27.

Figure 3.
The DNA damage response.
Cartoon summarising major components of the DNA damage response (DDR) system that are active during S phase. Defects occurring at the DNA are sensed, and through the activation of the signal transducers leads to cellular responses.

Figure 4.**Consequences of re-replication**

A small segment of chromosomal DNA containing a replication origin is shown. (a) A single re-initiation event generates a bubble of re-replicated DNA. If the rate of re-initiation is high, forks be initiated sufficiently close together that they undergo head-to-tail collision (b-c)⁵⁸. The resultant bubble structures (d) may undergo recombination to form either extrachromosomal circles (e) or tandem chromosomal duplications (f). (Lines in (a-c) indicate single DNA strands, lines in (d-f) indicate double DNA strands).

Figure 5.

Dormant origins and replication fork stalling.

A small section of chromosomal DNA replicated by 2-3 origins is shown. Mcm2-7 double hexamers are shown as a pink oval. (a) If two converging forks stall with no dormant origin between them, the result is likely to be DNA breakage or rescue by recombination. (b) In the absence of fork stalling, the dormant origin between the two active origins does not initiate, and its Mcm2-7 proteins are passively displaced as they are replicated. (c) If the converging forks stall, the dormant origin between them can initiate and allow complete replication of the chromosomal segment.