

# How dormant origins promote complete genome replication

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**Many replication origins that are licensed by loading MCM2-7 complexes in G1 are not normally used. Activation of these dormant origins during S phase provides a first line of defence for the genome if replication is inhibited. When replication forks fail, dormant origins are activated within regions of the genome currently engaged in replication. At the same time, DNA damage-response kinases activated by the stalled forks preferentially suppress the assembly of new replication factories, thereby ensuring that chromosomal regions experiencing replicative stress complete synthesis before new regions of the genome are replicated. Mice expressing reduced levels of MCM2-7 have fewer dormant origins, are cancer-prone and are genetically unstable, demonstrating the importance of dormant origins for preserving genome integrity. We review the function of dormant origins, the molecular mechanism of their regulation and their physiological implications.**

## The problem of ensuring precise genome duplication

During S phase of the metazoan cell cycle, replication forks are initiated at replication origins that are organised into clusters, each comprising two to five adjacent origins. A timing programme sequentially activates different clusters, leading to complete duplication of the genome (Figure 1, normal replication). To preserve genome integrity, it is crucial that these origins are properly regulated. Unless a sufficient number of origins and origin clusters are activated, there is a danger that sections of the genome remain unreplicated when cells enter mitosis (Figure 1, under-replication). It is crucial also that replication origins fire no more than once, and never fire on sections of DNA that have already been replicated, otherwise DNA would be amplified in the vicinity of the over-firing origin (Figure 1, over-replication). Cells prevent re-replication of sections of DNA by dividing the process of replication into two non-overlapping phases (Figure 2) [1–3]. From late mitosis until the end of G1, before DNA synthesis begins, cells license replication origins for use in the upcoming S phase by loading them with double hexamers of the MCM2-7 (minichromosome maintenance) proteins. During S phase, MCM2-7 complexes are activated to form a central part of the helicase that unwinds DNA at the replication fork [4]. As active MCM2-7 complexes move with the replication fork, replicated origins are converted to the

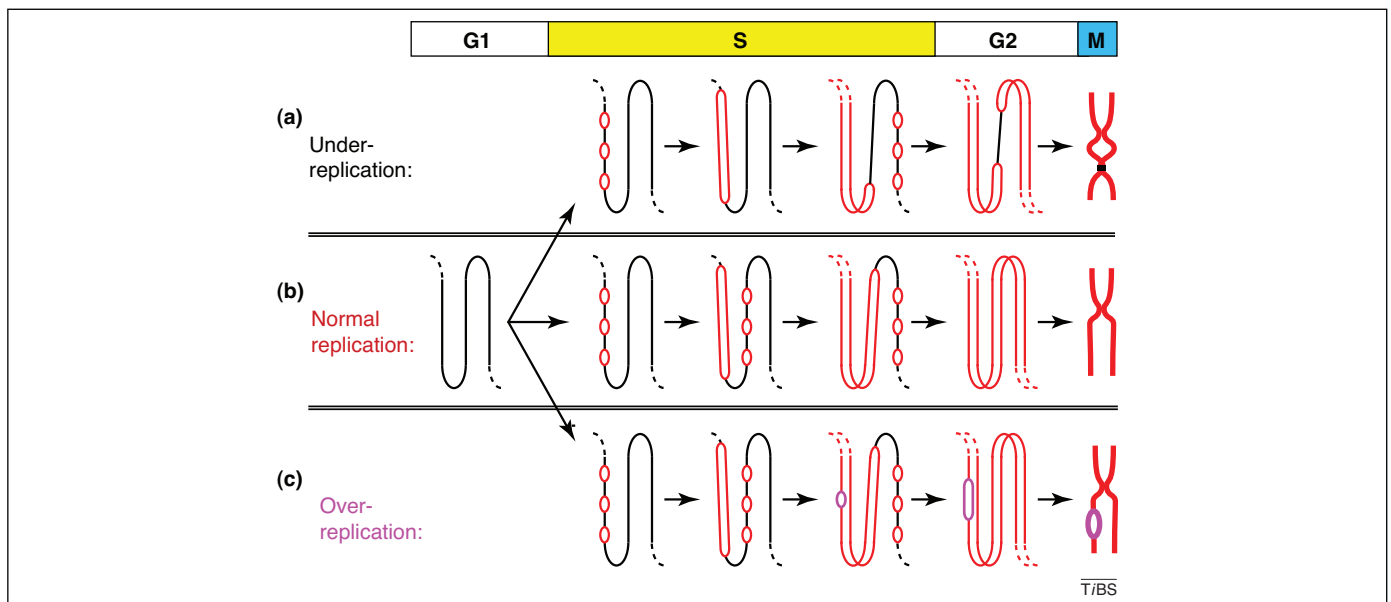
unlicensed state. Because no more MCM2-7 can be loaded onto DNA once S phase has started, no origin can fire more than once in a single S phase [1,2]. Cells rely on the presence of MCM2-7 to mark origin DNA that has not been replicated in the current cell cycle.

Thus, it is important for cells to ensure that sufficient origins are licensed before entering S phase. This is accomplished by a checkpoint (the licensing checkpoint) that monitors the number of licensed origins in G1, and delays entry into S phase if the number is insufficient [5,6]. In addition to being regulated during different phases of the cell cycle, the licensing system is inactivated when cells exit the cell cycle either reversibly into G<sub>0</sub> or irreversibly as a consequence of terminal differentiation or senescence. Notably, defects in the regulation of the licensing system are implicated in the development of genome instability and cancer [7–12].

As licensing occurs only before the onset of S phase, no new origin can be licensed if problems arise during S phase; for example, if replication forks stall on encountering DNA damage or tightly bound proteins. When fork stalling occurs, the DNA can sometimes be repaired or the blockage removed, but sometimes replication forks break down, leading to an irreversible fork arrest. Replication origins initiate a pair of bi-directional forks when they fire (most likely by using the pair of MCM2-7 heterohexamers loaded onto each origin [1,3,13–15]), and this provides some protection against the consequences of fork stalling: if one of a pair of converging forks stall, the other fork can compensate and replicate all of the intervening DNA (Figure 3a). However, if two converging forks both stall, replication of the intervening DNA is compromised (Figure 3b). A new origin cannot be licensed between the two stalled forks, because new origin licensing is prohibited once S phase has begun. All experimental evidence to date suggests that re-activation of the licensing system during S phase causes MCM2-7 complexes to be reloaded onto replicated DNA, leading to over-replication of DNA and consequent irreversible duplication of chromosomal segments [1,2,6,12].

In this Review, we describe how cells solve this problem by licensing additional origins that normally remain dormant but can be activated when forks stall. We discuss a simple stochastic model for how replication forks can initiate from dormant origins within replicon clusters that are currently engaged in replication. We then discuss how checkpoint kinases activated by replicative stresses suppress activation of new replicon clusters. We explain how

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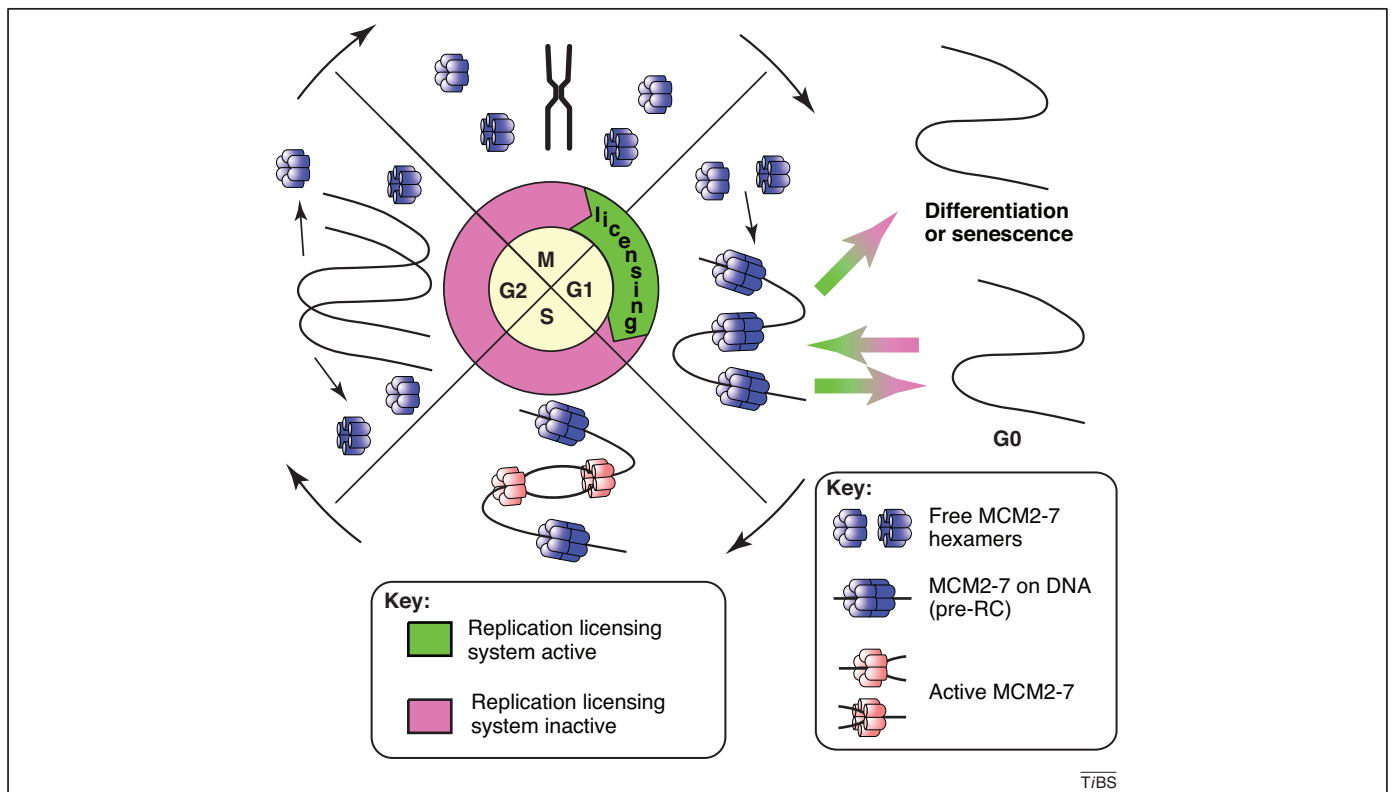


**Figure 1.** Ensuring precise chromosome replication. The small segment of chromosomal DNA shown consists of three domains, each replicated from three replication origins. The domain is shown at different stages of the cell cycle: G1, early-, mid- and late-S phase and G2; a whole chromosome containing the chromosomal segment is shown in mitosis. (a) The DNA is under-replicated as a consequence of origins in the middle cluster failing to fire. When sister chromatids are separated during anaphase, the chromosome is likely to be broken near the unreplicated section. (b) Origins are correctly used and chromosomal DNA is duplicated successfully. (c) One of the origins fires for a second time in S phase. The local duplication of DNA in the vicinity of the over-firing origin represents an irreversible genetic change and might be resolved to form a tandem duplication.

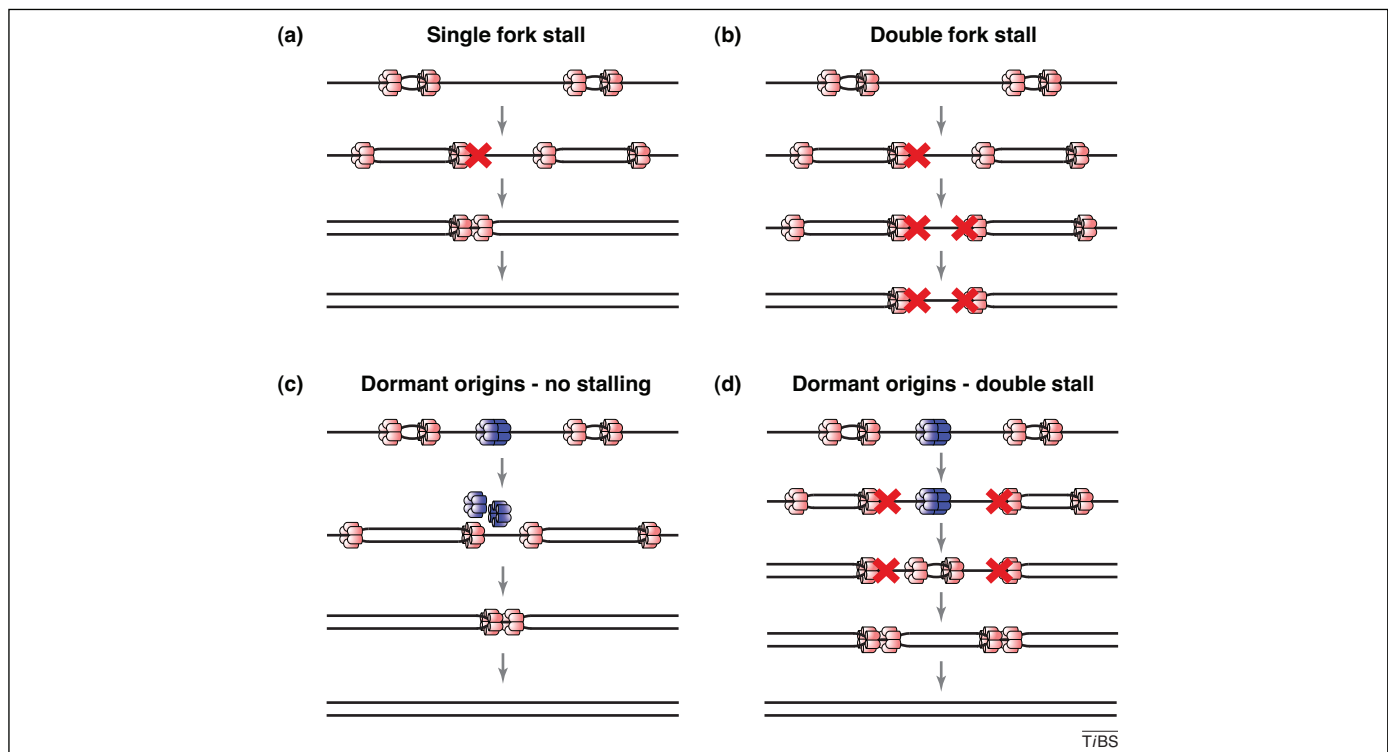
dormant origin activation and new cluster suppression act together to promote complete genome duplication. In the final section, we report how mice with hypomorphic MCM mutations suggest that dormant origins play an important role in maintaining genetic integrity.

#### Licensing excess (dormant) origins can prevent under-replication

MCM2-7 complexes are loaded onto DNA in a 3- to 10-fold excess over the number of replication origins that are normally used to complete S phase [16–20]. MCM2-7



**Figure 2.** The licensing cycle. The small segment of chromosomal DNA that is shown encompasses three replication origins. At the end of mitosis (M), the replication licensing system is activated (light green), which causes MCM2-7 complexes (blue hexamers) to be loaded onto potential replication origins (origin licensing). The licensing system is turned off at the end of G1. During S phase, some MCM2-7 complexes are activated as helicases as origins fire (pink hexamers). MCM2-7 complexes are removed from replicated DNA, either during passive replication of unfired origins or at fork termination. In this way, replicated DNA cannot undergo further initiation events until passage through mitosis.



**Figure 3.** The effect of fork stalling on completion of replication. A small segment of chromosomal DNA is shown with either two or three licensed origins. MCM2-7 complexes at unfired origins are shaded blue, MCM2-7 complexes activated as replicative helicases are shaded pink. Irreversibly stalled replication forks are marked with a red X. (a) One fork stalls, but all the intervening DNA is replicated by the fork originating at an adjacent origin. (b) Each of the two converging forks stalls. Replication cannot be completed because no new MCM2-7 complexes can be loaded onto DNA once S phase has begun. (c) A dormant origin is inactivated by a fork coming from the left. (d) Two converging forks stall, but a dormant origin between them allows replication to be completed.

loading is directed by the origin recognition complex (ORC) (Box 1). Again, the quantity of MCM2-7 loaded onto DNA is much greater than the amount of bound ORC [19,21] and MCM2-7 can be distributed at significant distances away from where ORC is bound [22]. These excess MCM2-7 complexes do not appear to be required for the bulk of DNA replication because cells continue to synthesise DNA at approximately normal rates when the level of MCM2-7 is reduced [19]. However, in *Xenopus laevis* egg extracts, at least, the vast majority of the MCM2-7 complexes loaded onto DNA are fully functional and capable of initiating replication forks [23]. Any excess MCM2-7 complexes that are not engaged in synthesis are displaced from DNA by replication forks originating from other origins (Figure 3c).

Decreased rates of fork elongation, which occur when DNA polymerase activity is inhibited or when DNA is damaged, cause 'replicative stress' and frequently result in fork stalling or collapse. Recent work has shown that the excess MCM2-7 licenses 'dormant' replication origins that normally remain inactive but which can be activated when replicative stress occurs [23–25] (Figure 3b). Activation of dormant origins can be demonstrated by analysing active replicons on stretched DNA fibres (Box 2), which shows a higher density of active origins when fork elongation is reduced [26–32]. Importantly, the potentially catastrophic events linked to fork collapse (Figure 3b) can be mitigated by activating dormant origins in the vicinity of inhibited forks. The high density of dormant origins ensures that if converging forks fail, there is likely to be an unfired (otherwise dormant) origin between them, which can be

activated to allow replication of the intervening DNA (Figure 3c and d).

Notably, dormant origins are important for cells to survive replicative stress. A reduction of chromatin-bound MCM2-7 by ~70% in human tissue culture cells caused no observable defect: replication rates, average origin spacing and cell cycle checkpoint activity were essentially normal [24]. However, when challenged with replication inhibitors, cells with this partial MCM2-7 function activated fewer dormant origins, progressed more slowly through S phase, and survived less well than control cells [24]. Similarly, *Caenorhabditis elegans* with partial knock-downs of MCM5, MCM6 or MCM7 exhibited proliferation defects specifically when challenged with the replication inhibitor hydroxyurea [23].

Mice that are hypomorphic for *MCM2* (*MCM2*<sup>IRE5-CreERT2</sup>) or *MCM4* (*MCM4*<sup>Chaos3</sup>) have been described [7,8]. Both mutations appear to affect primarily the total amount of MCM2-7 loaded onto DNA rather than the biochemical activity of MCM2-7, and both show a reduction in dormant origin activation after challenge with replicative stress [9,10]. However, even in the absence of exogenously applied replicative stress, cells from the mutant mice displayed evidence of replication defects. *MCM2*<sup>IRE5-CreERT2</sup> mutant cells exhibited a small increase in basal levels of p21<sup>CIP1</sup> and a small increase in the number of foci of  $\gamma$ -H2AX and 53BP1, indicative of DNA damage [7,9]. *MCM4*<sup>Chaos3</sup> mutant cells had an increased number of stalled replication forks, a small increase in DNA damage foci containing RAD51, RPA32 and RAD17, a 50% increase of FANCD2

### Box 1. Origin licensing

Origin DNA must be licensed before undergoing replication. Licensing is the loading of MCM2-7 complexes onto DNA. This occurs from late mitosis to early G1 phase and marks all potential origins of replication for use in the upcoming S phase. MCM2-7 is a hetero-hexameric complex comprising each of the six highly related MCM2, MCM3, MCM4, MCM5, MCM6 and MCM7 proteins that are assembled into a ring-shaped structure. The process of origin licensing involves the clamping of two MCM2-7 hexamers in an antiparallel conformation around DNA [13–15]. This clamp-loading process is ATP-dependent and additionally involves proteins ORC, CDC6 and CDT1 [3,13–15]. ORC is composed of six polypeptides (ORC1–ORC6) that can bind DNA in the presence of ATP. Although ORC recognises origin-specific DNA sequences in *Saccharomyces cerevisiae*, it does not appear to do so in other eukaryotes, although it has a preference for asymmetric A:T-rich DNA. Other features of chromatin presumably enhance ORC binding in these organisms. Once bound to DNA, ORC recruits CDC6 to form a stable complex with ORC-DNA. In *S. cerevisiae* the ORC-CDC6 complex has higher DNA sequence specificity than ORC binding alone because the CDC6 ATPase activity promotes its dissociation from non-origin DNA [68]. CDT1 is then recruited to the CDC6-ORC-DNA complex [69]. The C-terminal domain of CDT1 can interact with MCM2-7 and plausibly functions to recruit MCM2-7 complexes to the origin. Following the clamping of MCM2-7 around DNA, ATP hydrolysis by ORC resets the CDC6-ORC-DNA complex for a new cycle of licensing. MCM2-7 complexes loaded onto origins are inactive as helicases until they associate with CDC45 and GINS proteins during S phase [4]. Once loaded, MCM2-7 complexes can slide along double-stranded DNA without unwinding it [13,14] thus potentially allowing multiple MCM2-7 double hexamers to be loaded onto DNA by a single molecule of ORC [70].

foci (a Fanconi anaemia protein involved in resolving stalled replication intermediates) and >2-fold increase of abnormal mitoses [10]. Similarly, yeast cells harbouring the *MCM4*<sup>Chaos3</sup> mutation or human T cells with reduced MCM2-7 levels are genetically unstable [33,34]. These results suggest that the use of dormant replication origins is required for cells to deal properly with spontaneous errors that occur during DNA replication, even when no exogenous replicative stress is applied. Most significantly, both *MCM2*<sup>IRIS-CreERT2</sup> and *MCM4*<sup>Chaos3</sup> mutant mice showed a dramatic increase in cancer (see below).

### Regulation of dormant origins in active clusters

In order for dormant origins to rescue stalled replication forks there must be a mechanism that allows them to be activated when required. Although it is not fully understood how metazoan origins are normally selected for activation, it is clear that this process involves significant stochasticity. Within cell populations, few, if any, origins are used in every cell cycle and many appear to be active in only a small proportion of S phase cells [31,35–38]. For the small number of loci that have been studied in detail, the available data suggest that during a typical S phase, most potential origins are not used and instead remain dormant. This implies that apart from differences in intrinsic firing efficiency, there is no qualitative difference between relatively efficient origins and origins that frequently remain dormant; an inefficient origin might be inactive (dormant) in one cell cycle but active in another, purely because of stochastic features of origin activation.

In contrast to the stochasticity with which individual origins are used, ~1 Mbp segments of the genome, which

probably represent individual origin clusters or groups of clusters, replicate predictably at specific times of S phase [37–39]. A simple explanation for this behaviour is that within an individual cluster, the activation of potential origins is essentially stochastic, with different origins having different intrinsic efficiencies, but that larger segments of DNA containing clusters of origins are activated with a more strictly defined temporal order during S phase. These larger segments of DNA probably correspond to foci of DNA that are replicated in discrete replication factories (Box 3) [37–40].

With these considerations in mind, we recently modelled the behaviour of origin activation within a single 250 kb origin cluster [41]. Origins were assigned a certain initiation probability per unit time and were then activated stochastically during S phase (Figure 4a). Model parameters (mean origin efficiency and density of licensed origins) were varied to fit experimental data obtained in living cells. In the model, when origins initiate, forks move bidirectionally away from them until they encounter another fork and terminate, which creates a series of troughs (initiation sites) and peaks (termination sites) on a replication timing map (Figure 4b). When a fork encounters an origin that has not yet fired, the origin is passively replicated and inactivated. When replication forks are slowed (broken blue lines in Figure 4b), it takes longer for origins to be passively replicated, meaning that there is an increased likelihood that otherwise dormant origins will fire. In the particular case shown in Figure 4b, slowing forks by 75% allowed the firing of three additional origins. This simple model, involving no special signal to activate dormant origins, provides a good match to *in vivo* data if there are three or four dormant origins for each origin that fires [41]. It shows how dormant origins protect against double fork stalls (Figure 3b) that leave unreplicable sections of DNA between them.

Interestingly, the model shows that the density of licensed origins on DNA determines the degree of protection against double fork stalling, with the efficiency of origin firing being largely irrelevant [41]. If this is the case, why do most origins remain dormant (unfired) in animal cells that are not experiencing replicative stress? One possible explanation is that it is too costly to have a very large number of replication forks simultaneously active, all of which require many proteins (probably >50) to function properly. Another possible explanation is that if there are too many stalled forks present in a cell at any given time, there is a dangerously high risk of recombination occurring inappropriately between DNA at different stalled forks, or of apoptosis being induced in preference to DNA repair.

Although this model seems to account for many of the features of dormant origin activation [41], it is unlikely that things are quite this simple. In particular, DNA fibre analysis consistently demonstrates that adjacent active origins within origin clusters initiate with a high degree of synchrony, even though forks from neighbouring replicons might elongate with significantly differing rates [40,42,43]. When labelling is done for 15–30 min, enough time to complete ~50% of the synthesis of a typical replicon, it is notable that new initiation events are almost never seen after the initial set of synchronised initiation

## Box 2. DNA fibre technologies

The analysis of sites of DNA synthesis after spreading DNA fibres on a glass surface was first demonstrated more than 30 years ago using radio-labelled (tritium) replication precursor analogues and fibre autoradiography [32]. This approach allows the visualization of DNA tracks replicated by individual replication forks, and can be used to determine various features of replication fork movement and distribution. A significant limitation of the use of tritium is the long exposure time, typically months, required to give robust signals. More recently, alternative replication precursor analogues (e.g. BrdU, CldU or IdU and biotin-dUTP) and fluorescence-based detection methods have been used to dramatically increase the efficiency with which replication can be analysed using DNA fibre technology. Consecutive pulses of different nucleotide analogues can be used to distinguish different replication events, such as initiation, elongation and termination of forks (Figure 1 from [71]). In the first study to use this approach [40], cells synchronised at the onset of S phase were

labelled with BrdU or IdU during consecutive cycles. After labelling, cells were lysed on glass slides, the DNA spread and fixed on the glass surface for indirect immunofluorescence of the labelled replication forks. This experiment showed the efficiency with which origin initiation zones were activated at the beginning of S phase in the two cell cycles. One limitation of this approach is the difficulty in following DNA molecules over long distances when using standard spreading techniques. The use of molecular 'combing', where DNA molecules are tethered at one end before being drawn along the slide, provides more obvious DNA continuity because the tracks are spread unidirectionally and lie parallel on the slide. Another limitation of standard DNA fibre analysis is that the DNA sequences being visualized are anonymous. Locus-specific data for replicon structure on combed DNA fibres can be obtained by combining labelled deoxynucleotides with fluorescence *in situ* hybridization (FISH)-based identification of the target locus.

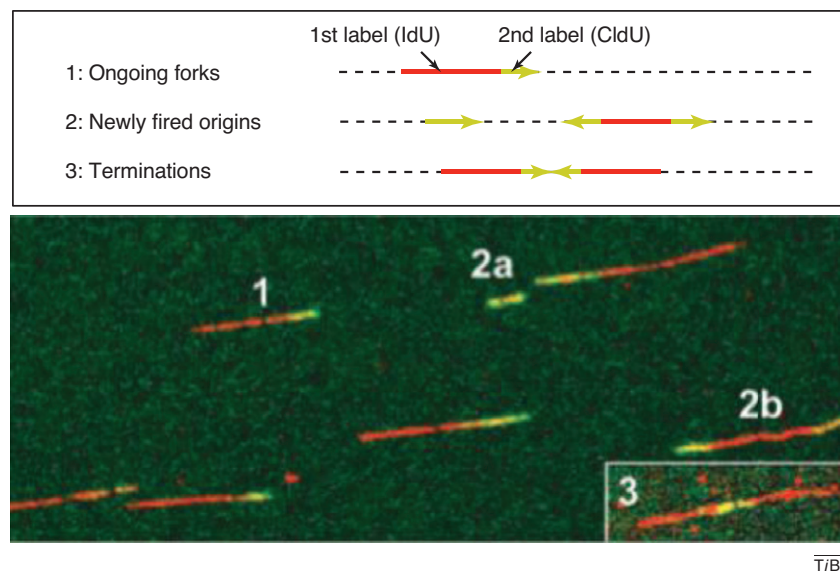


Figure 1. Use of consecutive pulses to distinguish replication events.

events. These observations are consistent with the idea that once sufficient origins have been activated to sustain a certain level of synthesis within a cluster, the activity of other nearby origins is suppressed.

ATR (ataxia telangiectasia and Rad3 related) and its downstream effector CHK1 play a major role in regulating the initiation of DNA replication in response to replication stresses [44–46]. Both of these kinases are activated when replication forks slow or stall, in part as a consequence of the increased amount of single-stranded DNA exposed when DNA synthesis is inhibited. CHK1 helps to limit the number of initiation events that occur within active origin clusters, and inhibition or knockdown of CHK1 leads to an increased origin density, as seen by DNA fibre analysis, both in the presence or in the absence of exogenous replication stress [23,24,43,47,48]. Because CHK1 helps to stabilise replication forks [49], this effect could be mediated, at least in part, by a 'passive' activation of dormant origins in response to fork stalling (Figure 4). In addition to mechanisms that suppress origin firing, it is possible that fork stalling actively promotes the firing of nearby dormant origins, which frequently occurs within ~10 kb of an arrested fork [43].

Mechanistically, one possible mediator of dormant origin activation might be the ATR kinase, which is activated at stalled or inhibited replication forks. ATR can phosphorylate MCM2-7 [50,51] and, although the function of this phosphorylation is unknown, it could promote initiation of dormant origins. The activation of dormant origins in the vicinity of stalled forks would be particularly efficient if chromatin-bound MCM2-7 complexes are able to migrate ahead of active replication forks without being displaced from DNA [13,14]. Notably, when chromatin is assembled in *Xenopus* egg extract, the distribution of chromatin-associated ORC and MCM2-7 implies that the position of MCM2-7 is not fixed after loading [22], consistent with the idea that they might be capable of moving ahead of elongating replication forks. Even so, it is important to stress that these mechanisms for actively promoting initiation in the vicinity of stalled forks are currently only speculation.

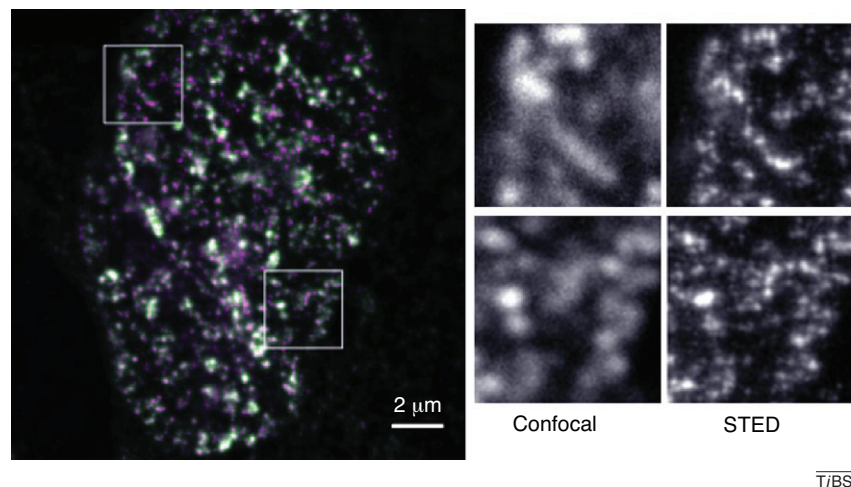
### Regulation of cluster activation

When replication forks are arrested, it only makes sense for dormant origins to be activated in the vicinity of the stalled forks and not elsewhere in the genome. So how are

### Box 3. Replication factories

DNA synthesis requires the intimate interaction between the DNA template and multiple proteins that form the replication machinery. The template is folded as chromatin into higher order DNA structures (DNA foci) that contain small clusters of replication units (replicons) within ~1 Mbp of DNA [57]. From their range of sizes, a diploid human cell will have ~10,000 of these chromatin superstructures [60]. Different classes of chromatin are replicated at discrete times of S phase as part of a temporally structured S phase programme [57], which possibly functions to preserve different epigenetic states that are encoded in post-translational histone modifications. When DNA foci are engaged in synthesis they become associated with replication machinery. This machinery is present within discrete structures – the replication factories. Individual factories appear to replicate the DNA within replicon clusters that are gathered together in individual foci. Replication factories have been characterised in detail using immuno-electron microscopy [72,73] and fluorescence-based light microscopy [61,74]. These techniques show that in early S phase factories have an average diameter of ~150 nm. Indirect immuno-staining and light microscopy studies showed that mammalian cells have 500~1000 replicating DNA

foci [40,58] which are labelled efficiently with nucleotide analogues such as BrdU and that these cells have a similar number of engaged replication factories containing replication fork proteins such as PCNA [74]. Using stimulated emission depletion microscopy to provide high-resolution light microscopy images [61], diploid human fibroblasts (MRC5) were recently shown to have, on average, 1230 PCNA-containing active sites. Interestingly, direct comparison of these high-resolution light microscopy structures reveals that most discrete foci seen by standard confocal microscopy are seen as small clusters of replication structures at higher resolution (Figure 1). The same organisation was revealed for the chromatin foci themselves using a variant high-resolution light microscopy technique [75]. During S phase, diploid human cells replicate ~50,000 replicons within ~10,000 chromatin foci. S phase in typical tissue culture cells is ~9 h long and the average time of synthesis for each foci is ~75 min [60]. Hence, about 14% of the genome is engaged in synthesis at any time, which is equivalent to 1400 foci and 7000 replicons. This is consistent with the number of active sites seen by high-resolution light microscopy and the model that each active site contains ~5 engaged replication units.



**Figure 1.** An S phase MRC5 cell labelled with anti-PCNA primary antibodies. Images were acquired sequentially, in normal confocal mode (green) and then by using the stimulated emission depletion microscopy (STED) setup (magenta). The lower panels are magnified regions of the cells, as indicated. Reproduced with permission from [61].

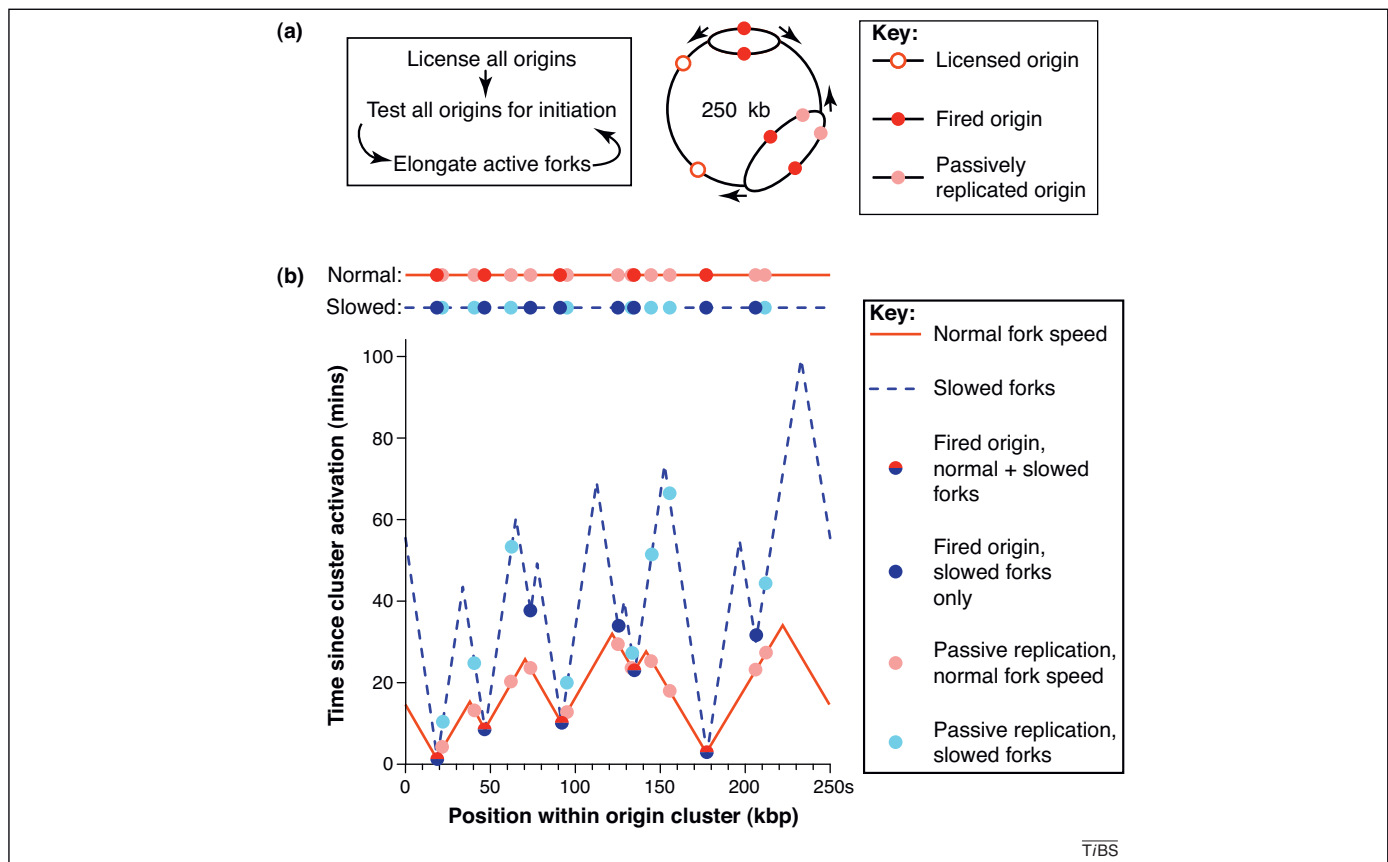
dormant origins regulated within the overall S phase DNA replication programme? When replication fork progression is inhibited, activation of the checkpoint kinases ATR and CHK1 promotes a number of different cellular responses. ATR and CHK1 stabilise stalled replication forks, delay mitotic entry and promote lesion repair [44–46]. They also inhibit further replication initiation and delay progression through the replication timing programme [47,49,52–54].

At first sight it appears paradoxical that replication inhibition simultaneously activates dormant origins and suppresses overall origin initiation via ATR and CHK1. We recently provided a resolution to this dilemma by showing that when cells experience low levels of replication fork inhibition, which leads to maximal activation of dormant origins, ATR and CHK1 predominantly suppress initiation by reducing the activation of new replication factories [55]. This means that the superactivation of origins is restricted to already active replication clusters [43,55]. Clusters of origins undergoing replication can be visualized in cells as discrete subnuclear foci, which contain ~1 Mbp of DNA, and these foci remain stable through multiple cell divisions (Box 3) [39,40,56–58]. During S phase, the temporal association of DNA foci with the replication factories occurs by

a ‘next-in-line’ mechanism where cluster activation propagates sequentially along chromosomal DNA [59,60].

Measurements of the rate of DNA synthesis occurring in individual factories showed that ~75% inhibition of replication fork speed caused an approximate doubling of replication forks per factory [55], in line with the doubling of fork density observed by DNA fibre analysis [24]. However, this inhibition of DNA synthesis also caused a reduction in the total number of active replication factories [55,61]. The decrease in factory number was due to the inhibition of *de novo* factory assembly and was dependent on CHK1 activity [55]. A role for CHK1 in inhibiting factory activation is supported also by the observation that CHK1 inhibition leads to an increase in factory number in the absence of replication inhibition [43,55].

It is unclear how factory activation is regulated and how it is suppressed by CHK1. Recent work has shown that modest changes in CDK activity preferentially alter the activation of new replication factories, leaving initiation within clusters relatively unaffected [62]. This might reflect the requirement for additional CDK substrates, distinct from those required for individual origins, that facilitate the initiation of all origins within a cluster or



**Figure 4.** Stochastic origin firing within a single cluster. An example of the computer model showing how stochastic origin firing leads to dormant origin activation if fork speed is slowed. (a) A cartoon of the modelling process, with initial origin licensing, followed by repeated steps of initiation and elongation. During each step, a licensed origin undergoes a random test, to determine whether it fires. Once an origin has fired, replication forks proceed away from it, as shown by the arrows. If a fork passes over an unfired origin (passive replication of a licensed origin), the origin is inactivated. In the cartoon, two of the five origins have fired and one has been passively replicated. Arrows show the direction of fork movement. (b) An example output of the computer model where 16 licensed origins were randomly spaced on a 250 kb origin cluster (x axis). Each origin was assigned an initiation probability randomly distributed around a mean of 0.00508/step. S phase was then enacted in steps of 25 s (y axis). Initiation events are marked by dark circles, passive replication is marked by faint circles and fork progression is represented by the lines. Line peaks represent termination events. Two simulations using identical origin parameters are shown: in red where forks proceeded at a normal speed (20 nt/s) and in blue where forks have been slowed to 5 nt/s. The pattern of origin usage is also shown on the linear DNA molecules at the top. Sample data were taken from [41].

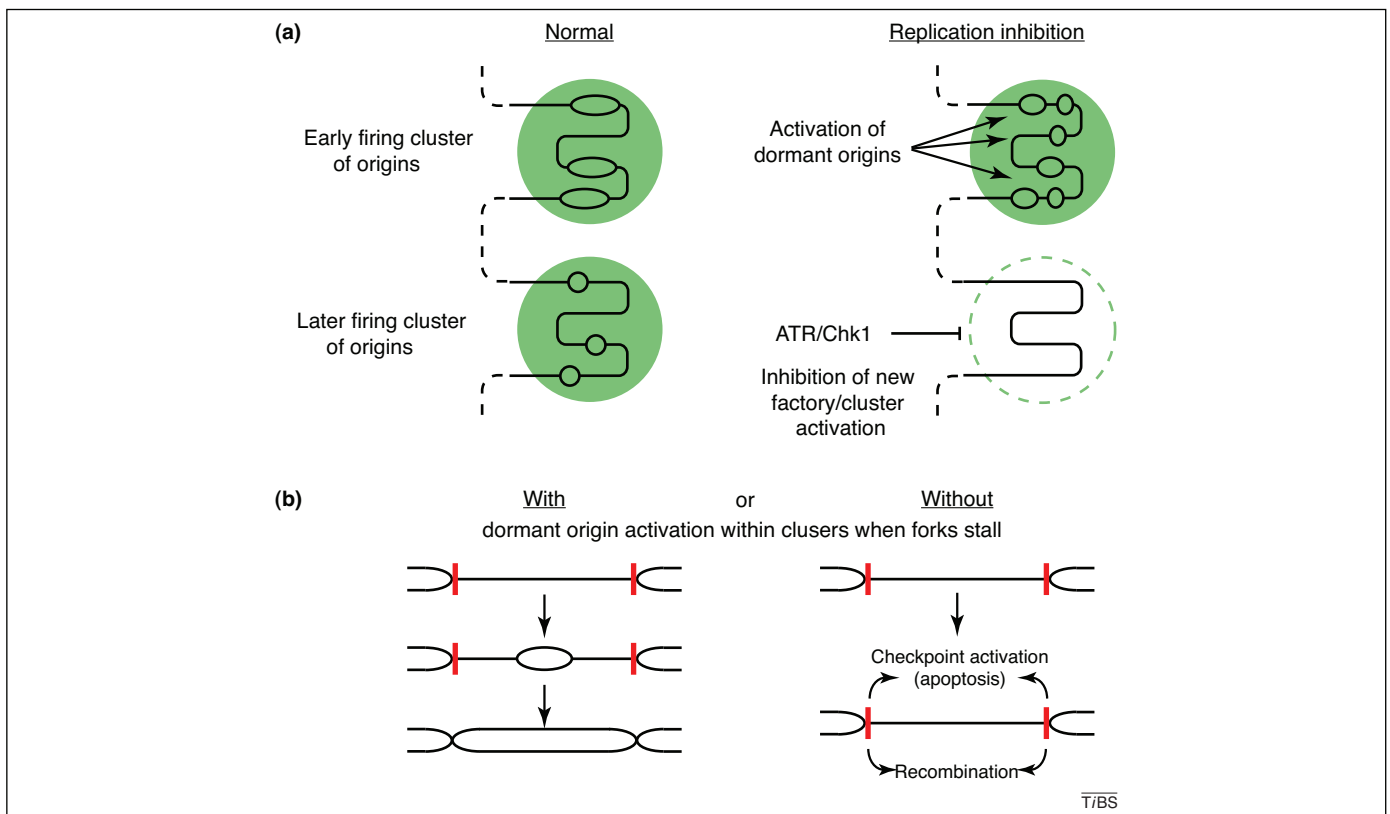
domain; alternatively, the firing of the first origin within a cluster (which is dependent on CDK activity) might propagate a change throughout the cluster to facilitate initiation at other origins [39,62]. Since CHK1 is known to reduce CDK activity at the G2/M transition [44–46,63], it is possible that CHK1-mediated inhibition of CDK activity during S phase causes the reduction in factory activation. However, we have found no evidence that total CDK activity is reduced when dormant origins are activated [55]. An alternative possibility is that CHK1 directly inhibits the CDK substrates that are required for factory activation [39,62].

Figure 5a summarises these conclusions about how dormant origins are regulated, showing a segment of genomic DNA that is normally replicated by two sequentially activating origin clusters. When replication forks are inhibited, dormant origins are activated within the active earlier-firing cluster, possibly as a simple consequence of the stochastic nature of origin firing. The inhibition of fork progression also activates ATR and CHK1, which suppresses the activation of later-firing/inactive clusters. The combination of these two features effectively diverts further initiation events away from unreplicated regions of the genome and toward active factories where

replication forks are inhibited. This ensures rapid rescue of stalled forks and minimises the risk of undergoing inappropriate recombination or apoptosis (Figure 5b). This model also provides a potential explanation of why adjacent origins are organised into clusters, which allows dormant origins to be activated where they are needed and allows pausing of replication by delaying activation of unreplicated clusters.

#### Dormant origins act as tumour suppressors

Because dormant origins can be activated within the normal programme of DNA replication, they can be considered as the cell's first line of defence against replication inhibition. Consistent with this idea, recent studies with mice hypomorphic for *MCM2* or *MCM4* suggest that dormant origins play an important role in maintaining genetic stability [7–10,64,65]. As described above, both of these mutations (*MCM2*<sup>IRES-CreERT2</sup> and *MCM4*<sup>Chaos3</sup>) cause defects in the activation of dormant origins and hypersensitivity to replicative stress. Significantly, mutant cells show evidence of genomic instability even in the absence of exogenously applied replicative stress. This suggests that spontaneous problems during DNA replication, such as fork stalling, are normally resolved by the use



**Figure 5.** Model for how cells respond to low levels of replicative stress. (a) Two adjacent clusters of origins (factories bounded by green circles) are shown on a single piece of DNA (black lines). Under normal circumstances (left), the upper factory is activated slightly earlier than the factory below, and each initiates three origins. Under low levels of replicative stress (right), replication forks are inhibited in the earlier replicating cluster, which promotes the firing of dormant origins as a direct consequence of stochastic origin firing. Replicative stress activates DNA damage checkpoint kinases, which preferentially inhibit the activation of the unfired later clusters/new factories. (b) A single piece of DNA (black line) is shown with two converging forks that have stalled (red bars). If a dormant origin is activated between them, replication can be rapidly rescued (left). If there is no dormant origin firing between the stalled forks (right), the DNA damage response can lead to recombination or induction of apoptosis. Reproduced with permission from [55].

of dormant origins. Importantly, mice homozygous for the *MCM2*<sup>IRE5-CreERT2</sup> or *MCM4*<sup>Chaos3</sup> mutations are cancer-prone. Combining the *MCM4*<sup>Chaos3</sup> mutation with hemizygoty of *MCM2*, *MCM6* or *MCM7* further reduced DNA-bound MCM2-7 and increased both genetic instability and the rate of tumour formation [64]. The original *MCM2*<sup>IRE5-CreERT</sup> mutant mice suffered mainly thymomas [7], whereas the original *MCM4*<sup>Chaos3</sup> mutant mice suffered mainly mammary adenocarcinomas [8], but it is now clear that the genetic background of the mutant mice is the major influence on the type of cancer arising rather than the specific MCM mutation [9,10]. Another interesting feature of the *MCM2*<sup>IRE5-CreERT</sup> mutant mice is a reduction in stem cell number and a spectrum of additional phenotypes characteristic of age-related dysfunction, indicating a defect in the proliferation or viability of stem cells or their precursors in mutant mice [7]. Together, these results suggest that even relatively minor defects in dormant replication origin usage can cause genetic instability thereby leading to cancer.

Despite DNA replication being a target of many anti-cancer drugs, it is unclear how S phase progression is affected by replicative stress and why some cancer cells are susceptible to chemotherapeutic drugs that target DNA replication [6]. Clearly, any predictive capacity to determine how specific cancers will react to chemotherapeutic drugs would be highly beneficial. The ability of cells

to survive replicative stress depends on the appropriate use of dormant origins and inappropriate regulation of this process provides an obvious target for anti-cancer drugs. The replication licensing checkpoint, which ensures that enough origins are licensed before progression into S phase, involves pathways that activate p53 and suppress Rb function during G1 [5,6,66,67]. These pathways are often defective in cancer, so that this checkpoint control is perturbed. The molecular mechanisms regulating factory activation following replicative stress are unclear, but some cancer cell lines appear to be defective in this response [55]. The inability of certain cancer cells to correctly regulate dormant origins and replication factory usage might determine their sensitivity to chemotherapy drugs. Understanding the molecular mechanisms that control the function of dormant origins might allow the development of assays that can predict the likely effectiveness of anti-cancer drugs that target DNA replication.

### Conclusions

The use of dormant origins is a newly discovered response to replication fork inhibition that plays an important role in maintaining genetic stability. Correct operation of this system requires the appropriate distribution of 'excess' MCM2-7 complexes along chromosomal DNA and requires the regulation of replication factories by checkpoint kinases. Neither of these processes is well understood at

present. There is much to be learnt about what determines where MCM2-7 complexes end up on chromosomal DNA and how this relates to the sites where the ORC and the rest of the licensing machinery are located. The molecular details of how replication factories and replicon clusters are activated remain obscure, but knowing that factory activation is regulated by both CDKs and CHK1 might help to tackle this problem. Perhaps most exciting is the prospect that the regulation of dormant origins could be defective in cancer cells. MCM hypomorphic mice show the potential importance of dormant origins, but it remains to be determined whether spontaneous cancers show similar defects and whether this information can be used to direct anti-cancer treatment more precisely.

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