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Order from clutter: selective interactions at mammalian replication origins

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Abstract

Mammalian chromosome duplication progresses in a precise order and is subject to constraints that are often relaxed in developmental disorders and malignancies. Molecular information about the regulation of DNA replication at the chromatin level is lacking because protein complexes that initiate replication seem to bind chromatin indiscriminately. High-throughput sequencing and mathematical modelling have yielded detailed genome-wide replication initiation maps. Combining these maps and models with functional genetic analyses suggests that distinct DNA–protein interactions at subgroups of replication initiation sites (replication origins) modulate the ubiquitous replication machinery and supports an emerging model that delineates how indiscriminate DNA-binding patterns translate into a consistent, organized replication programme.

Before each cell division, mitotic cells create exact duplicates of their entire genome to ensure transmission of complete sets of genetic and epigenetic information to daughter cells. Genomic DNA replicates at distinct nuclear locations during specific time intervals, and the decision about where and when DNA synthesis occurs has a crucial role in ensuring genetic stability. Cells that exhibit incomplete or excessive DNA replication often suffer deleterious consequences in the form of mutations and structural variations[1,2]. The preponderance of cancer-predisposing mutations within DNA replication genes[3–5] also suggests that the orderly progression of DNA replication is essential to cancer prevention.

Given the relatively large sizes of eukaryotic genomes, complete genome duplication requires DNA replication to start concomitantly at numerous locations (replication origins), which results in many discrete chromosomal loci replicating in parallel[6,7]. Indeed, some metazoan embryos initiate replication during early stages of development from abundant origin sites that are selected with limited or no specificity and can completely duplicate their genomes within minutes[8,9]. Differentiated somatic metazoan cells, however, exhibit slower DNA synthesis and initiate replication from fewer origins that are activated intermittently, each origin initiating replication in only a proportion of cell cycles[8–11]. In these cells, adjacent groups of replicating chromosomal sequences (replicons), each containing a replication origin, start DNA synthesis within a short time frame — probably within minutes

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Competing interests statement

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of each other — thereby forming megabase-scale ‘replication-timing domains’ (REFS 11,12) (FIG. 1). Although not all origins are activated in every cell during each cell cycle, at the cell population level, replication-timing domains are activated in a consistent order and share distinct chromatin interactions[6,7,11,12]. Metazoan replication domains often overlap with topologically associated domains (TADs)[7], which divide the genome into subnuclear compartments and are similar in size to replication domains[12]. The marked association between replication-timing domains and TADs suggests that the timing of replication initiation reflects a fundamental structural property of chromatin.

As rapid and accurate genome duplication can proceed in early embryos in an apparently indiscriminate order[8], distinct initiation sites and organized replication-timing domains are probably not required merely to complete DNA synthesis. Rather, consistent replication origins and organization of specific replication-timing domains might facilitate higher-order chromatin interactions[7,10] that help to coordinate replication with transcription and chromosome condensation. Recent studies using whole-genome analyses and mathematical models[11,13–19] have aimed to elucidate how chromatin-associated molecular interactions signal the initiation of DNA replication at replication origins to form the basis for timely, complete and orderly genome duplication.

Our understanding of the metazoan signalling cascades that lead to the activation of replication origins is limited because metazoan replication origins do not share strong consensus DNA sequences that could anchor origin-specific DNA–protein interactions. Ubiquitous chromatin-bound pre-replication complexes required for DNA replication initiation (BOX 1) bind to both replication origin and non-origin sequences with equal efficiency *in vitro*[20]. In agreement, chromatin association studies of replication machinery components in both *Drosophila melanogaster*[21] and mammalian cells[22,23] have not revealed strong sequence specificity despite preferential binding to chromatin features characteristic of replication origins. In addition, whole-genome and single-fibre analyses indicate that, in many metazoan loci, replication initiates alternately within clusters of adjacent origins such that each cell in a population uses slightly different combinations of replication origins[6,24]. It was suggested that this flexibility might help to coordinate DNA replication with transcription and other nuclear processes in a cell type-specific manner, as well as to facilitate recovery when replication is perturbed (see REF. 25 and references therein). Both the flexibility in establishing replication initiation sites and the lack of DNA consensus sequences raise the challenge of understanding how nonspecific chromatin interactions can lead to accurate initiation at consistent origins.

This Review describes the complex regulation of DNA replication in metazoans, focusing on recent insights into the genetic and epigenetic features of eukaryotic replication origins and the molecular interactions taking place at replication initiation sites. We discuss genome-wide mapping, functional analyses and mathematical simulations in a wide range of species, addressing the seemingly contradictory relationships — evident primarily in mammalian cells — between nonspecific interactions at replication origins and well-ordered, coordinated genome duplication. The mechanics of replication origin activation by the replication machinery and the distribution of replication-timing domains will not be discussed in detail, as these subjects have been recently summarized elsewhere[25–28].

Characteristics of replication origins

Beyond consensus: properties of replication origin sequences.

Given that replication origins function as nucleation points for pre-replication complexes, considerable effort has been directed at mapping origins in various organisms and cell types, as well as identifying distinct sequence and chromatin features that delineate replication origins. High-throughput sequencing analyses have yielded detailed genome-wide maps of replication initiation events and identified some characteristic features of metazoan replication origins[11,14–17,24,29]. However, these mapping studies have failed to identify sequence-specific DNA–protein interactions characteristic of all replication origins that could provide a mechanistic explanation for orderly mitotic genome duplication. Instead, genome-wide replication initiation maps and functional genetic analyses support a model in which overall indiscriminate DNA-binding patterns and combinatorial DNA–protein interactions at groups of origins translate into a consistent, organized replication programme.

A regulatory framework for the replication initiation process was postulated by the replicon model[30], which proposes that the ability to start replication at particular chromosomal locations requires specific DNA sequences known as replicators. In this model, replicators bind to initiators, which are protein complexes that recruit elements of the cellular replication machinery to chromatin. Indeed, replicators can be identified in prokaryotes and eukaryotes on the basis of their ability to facilitate replication when removed from their chromosomal context and cloned into plasmids (bacterial and yeast replicators)[31–33] or inserted at ectopic chromosomal sites (metazoan replicators)[34–37]. Initiators have also been identified in many prokaryotes and eukaryotes on the basis of their ability to bind to replicators and facilitate DNA replication initiation[6,7,10]. In eukaryotes, the initiator anchor — termed the origin recognition complex (ORC) — is a highly conserved complex that binds to chromatin and recruits pre-replication complex components during the G1 phase of the cell cycle[6,38] (BOX 1). An ORC is essential for the replication licensing process that renders chromosomes competent for replication, which is consistent with the role of the ORC as an initiator. In yeast, the ORC binds to replication origins in a sequence-specific manner essential for DNA replication initiation[38,39]. The metazoan ORC is also required for initiation of DNA replication, but metazoan ORC proteins do not exhibit sequence-specific DNA binding *in vitro*, and they also bind to non-origin regions on chromosomes[20,22,23,40]. The actual initiation of DNA replication is mediated by the replicative minichromosome maintenance (MCM) helicases, and the frequency and timing of initiation events is determined by the abundance of MCM complexes on chromatin[18,41], suggesting that MCM complexes might also partially fulfil the roles of initiators. The lack of sequence specificity of ORC and MCM binding seemingly contradicts the sequence-specific ability of metazoan replicators to initiate replication when inserted at ectopic chromosomal sites[34–37,42,43]. This suggests that nonspecific interactions with the ubiquitous pre-replication complexes provide necessary, but not sufficient, support for initiation of DNA replication in metazoans.

Metazoan initiation sites share some genetic features[10,24] but do not exhibit robust sequence similarities or a common ‘consensus’ sequence motif that specifies origin activity[6,10,17,44,45]. For example, replication origins are preferentially located near

transcriptional start sites (TSSs), CpG islands (CGIs), regions of DNase hypersensitivity, asymmetric G-rich sequences, TAD borders or origin G-rich repeated elements (OGREs)[11,14,24,46] (FIG. 2). A group of OGREs colocalize with CGIs and are associated with two flanking replication origins[24]. OGREs can potentially form G-quadruplex structures, which exhibit enriched association with replication origins[24], but G-quadruplexes are overall more abundant than replication origins[47], and many origins do not contain G-quadruplexes[46,48]. Replication origins are also enriched in asymmetric (A:T- and G:C-skewed) tracts, which might facilitate DNA unwinding and DNA– protein interactions, whereas they are depleted of purine-rich structures that can form DNA triplexes[11]. Consistent with a role for G:C-rich sequences in delineating replication initiation sites, detailed single-nucleotide polymorphism (SNP) analyses of phased genomes indicate a genetic link between strand asymmetry and replication origin activity[11].

Although OGREs and TSSs associate with replication origins, it is unclear whether these genomic elements are required for replication initiation, or whether they share a preference for similar chromatin features (such as enhanced chromatin accessibility) with origins. For example, although G-quadruplexes and origins colocalize, a causal relationship between G-quadruplex formation and origin activity was not observed in a SNP analysis of phased human genomes[11]. Given that G-quadruplexes can interfere with the replication process[49], the frequent presence of G-quadruplexes near replication origins might reflect a different selective factor, such as negative selection against both G-quadruplexes and origins in gene bodies[47] and/or a selective evolutionary pressure that destabilizes G-quadruplexes formed at origin-distal regions[50]. In another example, although replication origins strongly associate with TSSs, colocalization depends on transcriptional activity. Replication origins are enriched in TSSs of moderately transcribed genes but are depleted of TSSs of highly transcribed genes[14]. This finding suggests that interactions at TSSs are not strictly required for initiation, which can often occur at downstream sequences. The exclusion of initiation from highly transcribed TSSs might reflect steric hindrances that prevent colocalization of highly active transcription initiation complexes and replication initiation complexes.

Chromatin structural features associated with replication origins.

As mentioned above, the lack of a single, clear consensus sequence that facilitates replication origin activity contrasts with sequence-specific replication initiation activity exhibited by several replication origins at ectopic chromosomal sites[6,34–37,42]. These apparently contradictory observations might be reconciled if replicators modulate chromatin accessibility for the replication machinery rather than directly mediating initiation. For instance, replicators could facilitate initiation by affecting particular histone modifications[36,51–53], creating a chromosomal context permissive for both transcriptional activity and replication initiation[10,54,55] (FIG. 2). One example of sequences that create permissive environments are ubiquitous chromatin-opening elements (UCOEs)[56], which are used in gene therapy because they are resistant to heterochromatin-mediated silencing[55]. UCOEs are characterized by CGIs spanning dual, divergently transcribed promoters of housekeeping genes, and a highly permissive chromatin structure (characterized by histone H3 and H4 hyperacetylation, histone H3 lysine 4 trimethylation (H3K4me3) or lack of histone H1). Interestingly, UCOEs frequently colocalize with replication origins[53]. However,

well-defined replication origins can be mapped in heterochromatin (see REFS 6,24,48,57 and references therein), suggesting that the ability to create an open chromatin environment is not a universal determinant of origin activity.

Recent genome-wide analyses have identified distinct DNA and chromatin modifications that associate with origin activity[15,24,29,58–61]. Early-replicating origins associate with euchromatic histone modifications (such as H3K4 methylation (H3K4me), H3K4me2, H3K4me3, H3K9 acetylation (H3K9ac), H3K18ac, H3K36me3 and H3K29ac), whereas late replication is associated with repressive chromatin modifications (such as H3 and H4 hypoacetylation, H3K9me, H3K9me3 and H3K27me3)[17,62] (FIG. 2). Consistent with a role in genome organization, origin associations with chromatin modifications vary with cell differentiation status[8,10] and might reflect coordination between replication and transcription. Replication origins that initiate replication in many cell types are preferentially associated with unmethylated CGIs and with the euchromatin markers H3K4me3 and H3K9ac, whereas origins that initiate replication only in distinct cell types or lineages preferentially colocalize with the heterochromatin marker H3K9me3 (REFS 48,57,63). Interestingly, DNase 1-hypersensitive sites are enriched in regions of enhanced origin activity and preferentially interact with ORCs[22,23], but do not mark origins locally[48].

Specific histone modifiers affect global origin activity. In mammalian cells, methylation of histone H4 lysine 20 by KMT5A (also known as PRSET7 or SET8) promotes loading of pre-replication complexes onto chromatin to facilitate replication licensing[64], as the H4K20me2 interacts with the bromo-adjacent homology (BAH) domain of ORC1 (REF. 65). The histone acetyltransferase HBO1 facilitates initiation of DNA replication[66], and DOT1L catalyses H3K79 methylation, preventing re-replication[58]. In *D. melanogaster*, the histone acetyltransferases CREB-binding protein (Cbp) and Chameau (the homologue of human HBO1) regulate both DNA replication initiation and developmental oogenesis[67], and methylation of H4K20 is crucial for genomic stability, as it prevents DNA damage in late-replicating origins[68]. This observation suggests that chromatin modifiers may play a part in coordinating replication and transcription during developmental transitions[25]. In plants, H3K27 methylation is required to induce re-replication[69].

Beyond local chromatin modifications, replication origin activity is associated with distal interactions between origins and DNA elements that facilitate chromatin remodelling (FIG. 3). Such elements include a 40-kb region upstream of the *HBB* replication origin[70], the promoter of the Chinese hamster *Dhfr* locus[71] and an enhancer of the T_H2 locus[72]. In addition, replicator sequences themselves can affect chromatin structure. For example, replicators from the *HBB* and *LMNB2* loci can prevent transcriptional silencing by facilitating interaction between a locus control region and a chromatin remodelling complex[52,54,70]. The genome-wide correlation between TADs and replication-timing domains[7,12] also implies that long-range chromatin associations have an impact on the activation of replication origins. Proteins that regulate chromatin interactions, such as cohesins[73] and RIF1 (REFS 50,74,75), affect origin distribution, as reflected in the temporal distribution of replication initiation events.

Intermittent initiation, origin ‘dormancy’ and the determinants of origin choice.

The replication initiation programme exhibits remarkable flexibility, and many origins initiate DNA replication at disparate frequencies in different cell lineages[14,16,24,48]. In *Saccharomyces cerevisiae*, potential origins can initiate replication on plasmids, but origin activity can be suppressed by the chromosomal context[76,77]. The timing of ORC binding in yeast origins also correlates with the efficiency of initiation. Stable binding of ORCs in the G2 phase of the cell cycle, accompanied by nucleosome remodelling, is associated with efficient origins, whereas in the G1 phase ORCs transiently bind to origins that exhibit less frequent initiation[76].

In metazoan cells, most origins do not initiate DNA replication in all cell cycles (that is, they are not constitutive origins[6]) but exhibit flexible initiation and are active in only a proportion of cell cycles[6,10,28,57,78]. Initiation frequency can vary with chromosomal context, as exemplified by reduced initiation frequency in an *LMNB2* replicator transferred to an ectopic location[43]. Both constitutive and flexible origins are detectable in whole-genome analyses (FIG. 4), and many common origins identified in several cell lines are likely to be flexible origins. Origin choice may explain the observation that inter-origin distances measured by whole-genome sequencing are shorter than those measured by single-fibre analyses[6] (FIG. 4). Common and cell type-specific origins exhibit distinct epigenetic signatures[48], which is consistent with the differentiation-and developmental-stage-dependent reorganization of replication domains[7,79].

By contrast, dormant origins are not detectable in whole-genome analyses and might be activated only if replication from adjacent origins is stalled (FIGS 4,5). Activation of such novel dormant origins was demonstrated following DNA damage at the Chinese hamster *Gnai3* locus[80] and in association with repeat expansion in human fragile sites (reviewed in REFS 81, 82). In other cases, DNA damage and slow replication fork progression increase the frequency of initiation from flexible origins[83,84].

Origin flexibility is also observed within each cell population, as potential origins and pre-replication complexes are more numerous than observed replication initiation sites[6,7,41,85]. Components of the pre-replication machinery, such as MCM proteins, are in excess, and more MCMs are loaded onto chromatin during the G1 phase than are used during replication[6,7,41]. In yeast, an ORC might be able to load several MCM helicases, with more MCMs on early-replicating regions[18,41]. The excess (dormant) MCM-loaded origins will not initiate replication during an unperturbed S phase but will do so when adjacent replication forks stall[78,86,87] (FIG. 5). In metazoans, proteins involved in the cellular response to DNA damage — including Fanconi anaemia group I protein (FANCI)[88] and MMS and UV-sensitive protein 81 (MUS81)[83], which are both associated with the Fanconi anaemia pathway — can modulate dormant origin activation. FANCI, which was shown to bind some replication origins, affects dormant origin activation that is inhibited when FANCI is phosphorylated by ataxia telangiectasia and RAD3-related protein (ATR)[88]. MUS81 modulates rates of replication fork progression and the frequency of initiation from flexible replication origins during unperturbed growth[83], and promotes efficient replication after exposure to DNA damage[89]. The interactions between modulators of initiation frequency

and cellular pathways that respond to DNA damage highlight the potential role of replication origin choice in maintaining genomic stability.

Molecular interactions at origins

DNA–protein interactions that activate or repress initiation.

Genome-wide analyses have shown that subsets of replication origins are differentially regulated and have distinct genetic and epigenetic features. As interactions with the ubiquitous pre-replication machinery are not sequence specific, the decision of whether or not an origin will be activated under specific circumstances may require distinct replicator-interacting proteins at particular groups of origins[6,7,10,90]. Metazoan replication origins can therefore be divided into categories, each associated with a specific modifier protein (or protein combination) that determines origin usage according to cell type and developmental stage. Accordingly, tissue-specific replication programmes would then reflect the activity of several origin-binding factors, each interacting with a subgroup of origins that exhibit common features, such as association with distinct transcriptional regulatory regions or nuclear locations. Sequence-specific origin-binding proteins and associations with distinct histones might also regulate *cis*-acting distal genomic elements[8,10,91–93] by forming chromatin loops that determine where and when replication initiates and probably coordinate replication with transcription.

Components of large chromatin complexes that regulate transcription and mitotic segregation affect the selection of eukaryotic replication origins (TABLE 1). In yeast, binding of the transcription factor Ars-binding factor 1 (Abf1) to autonomously replicating sequence 1 (Ars1) seems to alter the chromatin structure and the activity of origins[94]. The evolutionarily conserved transcription factors forkhead box protein 1 (Fkh1) and Fkh2 interact with distinct groups of yeast replication origin (for example, Fkh1 interacts with early origins) and regulate replication initiation timing by recruiting the initiation factor cell division control protein 45 (Cdc45)[95,96]. Notably, Fkh1 and Fkh2 cluster replication initiation events in the yeast nucleus to facilitate long-range chromatin interactions, suggesting a link between spatial organization of the genome and origin selection[95,96].

Sequence-specific interactions that recruit pre-replication complexes, possibly via an RNA molecule, are observed in other unicellular eukaryotes. For instance, the *Tetrahymena thermophila* ORC interacts with an integral noncoding RNA subunit that can bind to its corresponding DNA target in the ribosomal DNA (rDNA) replication origin[97]. This interaction regulates differential replication of the rDNA locus during sequential stages of the *Tetrahymena thermophila* life cycle.

In metazoans, site-specific interactions at replication origins are known to regulate the replication of DNA tumour viruses, which use components of the cellular replication machinery for genome propagation. Such viruses rely on viral origin-specific DNA–protein interactions (for example, papillomavirus proteins E1 and E2, Epstein–Barr virus (EBV) nuclear antigen 1 (EBNA1)) to load cellular pre-replication complexes and initiate DNA replication[98,99]. Proteins involved in these interactions also tether viral genomes to cellular chromatin via chromatin modifiers[100,101]. For example, papillomavirus E2 interacts with

Bromodomain-containing protein 4 (BRD4), a chromatin-binding protein and transcription activator[102,103], and with the DNA helicase CHLIR1 (also known as DDX11)[104].

In *D. melanogaster*, follicle cells undergo a specialized amplification process in which a few specific origins repeatedly initiate DNA replication, leading to amplification of the chorion gene clusters involved in eggshell synthesis. E2F1 and the *D. melanogaster* orthologue of retinoblastoma-associated protein (RB) form a complex that selectively binds to the replication origins in the chorion locus[105]. The large MYB–MUVB complex binds to amplified *D. melanogaster* origins to recruit histone acetylases[106], whereas tethering of the histone deacetylase Rpd3 (also known as HDAC1) at the chorion gene clusters inhibits amplification[107]. The transcription factor MYB binds to ORC2 and ORC6, and may modulate origin amplification by controlling ORC2 localization106.

Direct effects of transcription factors on origin localization are also evident in frog egg systems. Injection of a hybrid protein comprising the yeast transcriptional activator Gal4 and part of the herpes simplex virus protein VP16 (Gal–VP16) into *Xenopus laevis* eggs led to site-specific replication of a recombinant DNA containing the *myc* TATA box next to Gal4-binding sites[8]. Although binding of transcription factors located initiation events, active transcription was not required for the formation of site-specific origins, suggesting that transcription factors might facilitate site-specific initiation by recruiting other proteins, including histone-modifying enzymes.

In mammalian cells, examples of DNA–protein interactions at specific origins include the binding of DNA-unwinding element-binding protein B (DUEB; also known as D-tyrosyl-tRNA(Tyr) deacylase 1) to the *MYC* replicator, which results in CDC45 recruitment to form pre-initiation complexes[108,109]. The RECQ helicases RECQ1 and RECQ4 also associate with replication origins[110], and are required for unperturbed initiation and replication[110,111]. Origins also interact with the histone acetylase HBO1, which is a co-activator of the licensing factor CDT1 (REFS 112–114). The DDB1–CUL4-associated factor RepID (also known as PHIP or DCAF14) is essential for DNA replication initiation within a particular origin group[115]. RepID participates in a long-range interaction between the replication origin and the human *HBB* locus control region, which regulates both transcription and replication patterns[54,70,116] Glutamate-rich WD40-repeat-containing protein 1 (GRWD1), which also interacts with a subset of replication origins, colocalizes with the CDC6 component of the pre-replication complex and is required for MCM loading onto chromatin[117]. These observations support the notion that distinct DNA–protein interactions at specific replicator groups dictate replication initiation. Interestingly, both RepID and GRWD1 interact with DDB1–CUL4 (REFS 118,119), suggesting that this ubiquitin E3 ligase complex may influence specific origins.

Site-specific interactions at subsets of replication origins might also have other functions that are unrelated to transcriptional activity. For instance, H3K79me2 associates with a distinct proportion of replication initiation sites and can mark replicated chromatin during S phase[58]. H3K79me2 is not required for DNA replication initiation, but this modification prevents re-replication from occurring within a single cell cycle.

The selection of replication initiation events might also be regulated by differentiation-specific transcription factors such as the homeobox protein HOXC13, which interacts with the *LMNB2* origin120. HOXC13 binding facilitates subsequent ORC4 recruitment to the origins of human *LMNB2, TOP1* and *MCM4*. Other HOXB orthologues (such as HOXD13 and HOXA11) bind to the pre-replication complex component CDC6, along with human *LMNB2* and origins near *MYC, FMR1, MCM4* and *TOP1* (REF. 121).

DNA–protein interactions that selectively affect replication timing.

Organization of the genome into replication-timing domains requires activation of replication origins at adjacent replicons concomitantly or in rapid succession. Chromatin modifications, distal chromatin interactions and several nuclear-scaffold structural components affect the distribution of replication initiation events and of replication factories throughout the nucleus[6,7,57]. Transcription factors[95] and chromatin modifiers[76], which associate with chromatin as DNA synthesis progresses and have a crucial role in chromatin re-assembly following replication[122], differentially affect activation timing in subsets of yeast origins. In *D. melanogaster*, H4K20 methylation is required for complete replication and prevents the accumulation of DNA damage lesions in late-replicating regions[68]. In mammals, the interactions of histone-modifying enzymes and transcription factors with chromatin correlate with changes in replication timing[79,123]. Dynamic replication order, however, is subject to some constraints and is partially regulated by *cis*-acting elements that dictate the time of replication in genomic regions ranging in size from single loci to entire chromosomes[52,84,91,124,125]. For example, *cis*-acting elements that determine replication timing on a whole-chromosome level encode monoallelically expressed long noncoding RNAs that regulate both replication timing and mitotic chromosome stability in mammals[91].

Sequence-specific interactions can also modulate distal interactions that affect origin activity and might coordinate replication with transcription. Overexpression of the yeast transcription factors Fkh1 and Fkh2 advances initiation of many origins, resulting in increased initiation in S phase[96]. In *Schizosaccharomyces pombe*, the heterochromatin protein 1 (HP1) homologue Swi6 associates with ORCs, Cdc18 (the homologue of human CDC6 homologue) and the DBF4-dependent kinase (DDK) homologue Hsk1, and is required for centromere replication[126,127]. Swi6 is also involved in pericentromeric heterochromatin formation and maintenance, and may have both positive and negative roles in centromere replication[126]. By recruiting DDK to pericentromeric heterochromatin, Swi6 promotes DNA replication initiation. However, as disruption of the Swi6–Cdc18 complex accelerates centromere replication[126], Swi6 may negatively regulate replication timing, although the mechanistic details of this interaction are currently unclear.

S. pombe Taz1, an orthologue of mammalian proteins telomeric repeat-binding factor 1 (TRF1) and TRF2, is required for telomere maintenance and plays a major part in replication timing[128]. Taz1 binds to internal chromosomal repeats in a sequence-specific manner and forms facultative heterochromatin islands, promoting late replication of nearby origins by preventing early DDK-dependent loading of Sld3 (the yeast homologue of the human treslin) on origins, while concomitantly repressing gene expression[129]. The *S. pombe*

centromeric protein Shugoshin 2 also regulates replication timing at telomeres by limiting Sld3 loading[130].

Hp1, a structural component of heterochromatin, is required for both very late centromeric DNA replication and early pericentromeric DNA replication in *D. melanogaster*[131]. Another heterochromatin-binding protein, ORC-associated protein (ORCA; also known as LRWD1), interacts with the pre-replication complex ORC1 in mammalian cells. ORCA stabilizes ORC binding to chromatin, and binds to and regulates both H3K9me3 and HP1 (REF. 132); these observations are consistent with the hypothesis that ORCA binds to a group of late replication origins in heterochromatin.

The telomere-binding protein Rif1 is involved in regulating replication timing in *S. pombe*[28,50,75,133]. Rif1 is a structural component of the nucleus that also associates with chromatin loops[50] and coats late-replicating domains[50,74,75,133]. Rif1 also mediates cell-cycle signalling by promoting dephosphorylation of the replicative MCM helicase to dynamically regulate DNA replication initiation. Specifically, Rif1 targets protein phosphatase 1 to Mcm4, a component of the MCM helicase, preventing its phosphorylation by Hsk1 to delay the activation of the helicase and ensure appropriate replication timing[75].

Similarly to its yeast counterpart, mammalian RIF1 acts as a nuclear organizer, coats late replication domains and interacts with a group of late-replicating regions to delay replication[74]. RIF1 modulates the frequency of chromatin interactions in late-replicating domains, restricting interactions during the G1 phase of the cell cycle. In both human and mouse cells, RIF1 depletion alters replication-timing patterns and, in particular, advances the replication timing of chromosomal regions that normally replicate mid-S phase[74,133]. RIF1 also affects the size of chromatin loops[133], supporting the notion that distal chromatin interactions have a role in establishing replication-timing patterns.

Replication origin locations are sufficient to determine replication timing without a separate ‘timing factor’.

Distinct chromatin modifications, which are often altered during development, can associate with regions that exhibit particular replication timing. Although replication proceeds randomly in the inactive X chromosome[134], replication-timing domains are clearly delineated in most chromosomes, and these domains often correspond to particular TADs[7]. Both TADs and replication timing are cell type-specific in mammalian cells. TADs are described as stable regulatory units of replication timing; early DNA synthesis begins within transcription-rich TADs and subsequently progresses into neighbouring later-replicating and transcription-poor TADs[10]. Thus, replication domains that exhibit early replication tend to be gene-rich, associated with active chromatin markers and located near the nuclear interior[7]. Late-replicating regions are enriched in heterochromatin, in areas corresponding to lamin-associated domains, and tend to be located near the nuclear periphery[7]. The temporal order of chromosome replication correlates with biases in evolutionary mutation rates[135,136]. Replication-timing domains are conserved in syntenic regions[137–139] and affect the frequency of somatic copy number alterations in cancer cells[136]. The frequently observed variation in replication-timing domains in cancer and developmental disorders[2,27,140,141]

might indicate that the order of replication helps to ensure stable genetic and epigenetic inheritance.

Consistent with the role of chromatin in determining replication timing, a large proportion of the human genome exhibits changes in the order of replication during nuclear reorganization associated with differentiation and development[7,12,27]. The precise timing of origin activation within replication-timing domains is determined anew after each mitotic cell division[142], facilitating dynamic and flexible changes in replication order[27]. One possible mechanism underlying rapid changes in replication order is the dynamic distribution of one or more limiting replication factors that can preferentially associate with potential origins in accessible chromatin during the G1 phase of the cell cycle[143,144]. These limiting factors can be sequentially released from origins after replication, subsequently binding to and facilitating initiation from later-replicating origins. In concordance with this model, recent simulation studies based on mathematical models[13,18,19] suggest that the locations of replication origins and the relative efficiency of initiation at these locations are sufficient to determine the dynamic high-level organization of replication domains.

In one simulation study[13], global replication order was accurately predicted in human cells using a model that had two variables: an ‘initiation probability landscape’, corresponding to a known distribution of replication origins determined by DNase 1-hypersensitive sites, and the assumption that replication initiation is restricted by the availability of a single rate-limiting activator. The model was able to predict replication-timing domains without assuming any particular spatial genomic organization, which suggests that the distribution of DNase 1-hypersensitive sites contains sufficient information to contribute to genome conformation, possibly through histone displacement by transcription factors. This model implies that replication timing does not require a separate regulatory mechanism distinct from the location of initiation events and is ultimately determined by chromatin structure, which itself is determined by DNA sequences through the distribution of genes and other regulatory elements.

A second simulation study[19], based on a model assuming spontaneous stochastic initiation within euchromatin and facultative heterochromatin, was also able to predict the general progression of DNA replication on the basis of the distribution of chromatin zone sizes and higher chromatin organization. Importantly, the second model predicted a three-dimensional spatial organization of replication events by assuming concomitant inhibition of replication initiation at distances below the size of chromatin loops and by taking into account a domino-like effect in which replication at a particular origin would induce initiation from adjacent origins. The model also predicted a transition between early and late S phase with both spontaneous and induced initiation in heterochromatin. In concordance, a third simulation study[18] based on a model relying on the density of MCM replicative helicases, assuming a high level of MCMs at early origins, predicted replication timing in yeast with high accuracy.

Together, these models predict that the timing of origin activation could be determined on the basis of the locations of origins competing for a limiting factor. As these models did not assume the existence of an independent chromatin feature that determines replication time,

they support the notion that a higher density of origin activation is sufficient to increase the chance of early replication in regions containing those origins. Hence, the replication-timing programme seems to be determined by local interactions that signal to licensed replication origins, which are in turn activated by a combination of sequence-specific DNA–protein interactions and chromatin modifications. Thus, the results of the simulation studies imply that tissue-and differentiation-specific replication-timing programmes are dictated by chromatin features that determine the efficiency of initiation, combined with the constraints that limit replication initiation such that it only occurs once per cell cycle.

Cells might require a consistent replication-timing programme to establish and maintain specific nuclear compartments, such as heterochromatin. For example, late replication of heterochromatin might prevent rapid, massive chromatin decondensation and re-condensation, which could affect gene expression, alter the structural integrity of the nucleus and lead to DNA damage[145]. Within heterochromatin, however, replication can proceed randomly with no apparent replication-timing domains, as exemplified by the inactive human X chromosome[134]. This observation again suggests that a consistent replication programme is required neither for completion of the replication process nor to establish heterochromatin, but rather to coordinate replication with other chromatin transactions, primarily transcription. A consistent replication-timing pattern that coordinates with chromatin transactions requires origin activation to be co-regulated with programmed changes in chromosome packaging. The simulation studies discussed above imply that replication origin distribution is sufficient to dictate replication timing. Thus, molecular interactions that determine the likelihood of origin activation can affect both the spatial and temporal properties of the replication landscape.

The central role of origin choice

The remarkable flexibility of DNA replication is facilitated by an excess of potential replication origins. Excess origins permit flexible replication times and also enable alterations in initiation frequency. For example, replication initiation patterns change during transcriptional activation in lymphocyte immunoglobulin genes[123,146]. Overall, only half of active origins exhibit consistent genome-wide initiation frequency across many cell lines, whereas a large proportion of potential origins exhibit cell type-specific initiation[16,48].

Genomic stability in the face of replication stress seems to hinge on the excess of potential origins and pre-replication complexes. Potential replication origins that do not initiate replication during unperturbed growth can do so to complete the duplication of DNA sequences adjacent to collapsed or stalled replication forks or when nucleotide pools are depleted[78,80,86,147] (FIG. 5). A role for excess origins is evident from the slow replication and loss of genomic stability that occur when the number of potential pre-replication complexes is reduced, for example in a mouse model harbouring a mutation in the gene encoding the replicative helicase MCM4 subunit[147,148]. Similarly, RepID-depleted cells, which do not initiate replication from a proportion of origins, exhibit diminished initiation frequency, slower replication fork elongation and frequent replication fork stalling events[115] Initiation from origins adjacent to stalled replication forks is activated by checkpoint signalling cascades that concomitantly inhibit DNA replication initiation in distal origins

that are programmed to replicate later[82,83,89,149–151] Signalling cascades activated upon replication fork stalling (such as those involving checkpoint kinase 1 (CHK1), CDC25, cyclin-dependent kinases (CDKs) and DDK) are also involved in modulating the activation of origins during unperturbed growth[6,83].

Conversely, replication rate could dictate the frequency of initiation events[81,86,150]. For instance, cells that exhibit low levels of DNA topoisomerase 1 (which is encoded by *TOP1*)[152] or are deficient in the repair endo-nuclease MUS81 (REF. 83) exhibit slower replication fork progression that is compensated for by increased replication initiation frequency. Mapping of replication origins in MUS81-deficient cells identified the same pool of replication origins as in MUS81-proficient cells[83], suggesting that the increased initiation frequency in cells with slow replication reflects the accrued activation of origins that exhibit a low initiation frequency when replication speeds up[153,154]. In other examples, however, perturbed replication induces novel replication initiation events in regions that do not initiate replication in the absence of perturbations[80,153]. In these cases, the pool of flexible origins was not sufficient to compensate for replication fork stalling, and so replication initiated at novel locations distinct from flexible origins, probably dormant (or cryptic) origins that are never used during non-perturbed growth. In other examples, exposure to drugs that inhibit histone deacetylases[87] and depletion of RepID[115] reduce initiation frequency and induce replication fork asymmetry, which often indicates chromosome breakage[123,152]. Locally, mutations in an adjacent replication origin can affect the expansion dynamics of triplet repeats in the rare fragile site *FMR1* (REFS 82,155). Hence, replication origins that are activated only in particular cell subtypes might confer a clear evolutionary advantage in facilitating specialized transcription and differentiation programmes.

Supporting the notion that excess replication origins might be used to complete DNA synthesis when replication is perturbed, regions that contain fewer replication origins are highly susceptible to genomic instability, including DNA breakage. For example, replication origin scarcity is a hallmark of common fragile sites, which correspond to slow replication zones in yeast. In these regions, reduced replication origin abundance increases the frequency of chromosome loss[156] and can trigger the DNA damage response[82,157–161]. Chromosome fragility can also be associated with early-replicating regions (early-replicating fragile sites[162]) that might reflect interference between transcription and replication. Dynamic changes in replication origin use that are associated with gene expression might allow cells to minimize such interference.

An excess of replication origins might be beneficial for two reasons. First, excess origins provide a mechanistic basis for flexible initiation that synchronizes with cell type-specific gene expression. Second, excess origins can be used as ‘backup’ origins in cases for which there is a need to rescue stalled replication forks during perturbed growth. Identifying the determinants of replication origin activation is crucial because unregulated changes in replication initiation frequency can lead to genomic instability and DNA damage, such as that caused by oncogene-facilitated DNA over-replication[163]. The pace of replication fork progression directly determines the cellular response to stalled replication forks. Consistent with a role in maintaining genomic stability, mutations in components of the pre-replication complex can cause developmental syndromes[164]. In addition, deregulated expression of pre-

replication complex components, driven by transcription factors such as E2F1 and MYC, is associated with a high risk of re-replication and cancer upon co-occurrence with inactivation of cell-cycle check-points that maintain genomic stability[165–167]. Oncogene activation also induces fragile sites, and activation patterns are oncogene specific (for example, RAS induces a set of fragile sites that is different from that induced by cyclin E)[168], suggesting that over-replication overwhelms the machinery that maintains replication equilibrium in sites with fewer origins.

Perspectives and future directions

The original replicon model[30], which proposes that interactions between replicators and initiators activate DNA replication, still provides the fundamental framework for understanding the regulation of chromosome replication. However, the diverse interactions that dictate replication initiation sites and timing for distinct sets of origins provide an additional regulatory layer to ensure orderly and accurate eukaryotic genome duplication. The recent observations summarized in this Review imply that diverse DNA–protein interactions contribute, possibly in a combinatorial manner, to the activation of pre-replication complexes and the initiation of DNA replication at distinct *cis*-acting sequences. The assumption that replication initiation patterns emerge from combinations of diverse sequence-specific DNA–protein interactions could resolve the apparent paradoxical finding of consistent initiation patterns at specific DNA elements that lack a single consensus protein-binding sequence. As combinatorial DNA–protein interactions can be modified by cellular circumstances (such as gene expression, developmental stage, and external and internal stress), the capacity to initiate replication at additional origins when necessary provides cells with the ability to adapt to a changing environment by modifying the replication programme.

Nevertheless, the following questions remain. First, it is still unclear whether replicators establish the location of initiation events by interacting directly with the replication machinery or by creating a chromatin environment that is conducive to initiation. The recently observed colocalization of TADs with replication-timing domains supports the latter[12], but direct DNA–protein interactions that facilitate initiation could still determine the locations of replication initiation events locally. Second, the mechanics of establishing the locations and timing of initiation remain to be clarified. In particular, emerging observations suggest that some components of the replication machinery associate with late-replicating origins only after early origins complete replication[28,169], implying that cells contain several subsets of potential licensed replication origins at different stages of assembly. To understand replication-timing patterns, it would be crucial to study how origins are assigned to each origin group: a group of early-replicating origins, associated with pre-replication complexes that are ready to start DNA replication at the onset of S phase, and groups of later-replicating origins, associated with some components of pre-replication complexes but awaiting one or more critical factors. Future studies should also ask if the assignment of potential origins to the earliest or subsequent replicating groups could be accomplished solely on the basis of the site and the initiation frequency from each origin, without an independent ‘replication-timing factor’, as deduced from simulation studies[13,19]. If replication timing is ingrained in the location of initiation events, DNA–protein interactions

at the origin level can provide a critical determinant underlying the abrupt changes in the replication-timing programme that are observed upon differentiation, activation of a gene expression programme or environmental stress.

Notably, the need for consistent origins and a clear replication-timing programme has not yet been clarified, as data from embryonic systems[8] and the inactive X chromosome[134] show that specific localization of replication initiation events and a structured replication-timing programme are not required for genome duplication. The past decade has revealed striking correlations between replication timing and chromatin structure at the megabase-scale domain level[7]. The strict temporal regulation of DNA replication and the consistency of initiation events also suggest that temporal and spatial regulation of the initiation of DNA replication help to coordinate DNA replication with gene expression. A consistent replication-timing programme might also be required to establish and maintain specific nuclear compartments, such as heterochromatin. The next challenge will be to better understand the detailed mechanisms linking replication initiation and the whole-genome replication-timing programme. For example, it would be pertinent to characterize the actual effect of RIF1 (and other proteins with similar roles) on modulating replication timing for a proportion of replication domains. Another challenge will involve investigating how the properties of replication domain structures reconcile with reports on genetic determinants of replication timing that are embedded in DNA sequences that delay or advance replication timing directly. Also, although mathematical models suggest that replication timing is stochastically determined, future studies should ask whether the replication initiation landscape that affects timing patterns is associated with specific chromatin marks that have yet to be identified. Finally, the recent realization that proteins involved in the progression of unperturbed replication also play a part in the cellular response to DNA damage[6] lends new urgency to studies aimed at understanding the molecular basis of genome duplication. Such dual roles in modulating both perturbed and unperturbed replication are perhaps not surprising, but a complete understanding of the role of cell-cycle checkpoint signalling in regulating replication patterns might help us to exploit the regulatory interactions that modulate replication for therapeutic purposes.

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Replication origins

Locations on chromatin from which replication forks emanate.

Replicons

Units of chromatin that are replicated from a single origin.

Replication-timing domain

A region on chromatin that exhibits a uniform replication time from multiple adjacent replicons.

Topologically associated domains

Discrete units of chromatin that tend to exhibit internal, rather than external, chromatin interactions that probably reflect distinct folding patterns.

Single-fibre analyses

Studies of chromosomal dynamics that are based on visualization of labelled, stretched single DNA molecules (for example, on microscope slides). Single-fibre replication analyses visualize the incorporation of nucleotide analogues into chromatin fibres.

Replicon model

A model proposing that replication initiates through an interaction between *cis*-acting elements (replicators) and *trans*-acting factors (initiators).

Replicators

Genetic elements that are required for the initiation of DNA replication from a particular chromosomal site.

Initiators

Proteins, or protein complexes, that interact with a replicator and are required for initiation of DNA replication.

CpG islands

Stretches of nucleotides (longer than 200 bp) that exhibit a high abundance of CpG dinucleotides.

G-quadruplex structures

Stacked, planar structures formed by intramolecular interactions among DNA sequences that contain multiple stretches of four guanines.

DNA triplexes

Non-B helix-type structures that contain a third strand.

Phased genomes

Assembled genome sequences that identify alleles of maternal and paternal chromosomes.

Negative selection

An evolutionary process by which genetic elements with potentially deleterious effects are removed from a population.

Constitutive origins

DNA replication origins that initiate replication in all cells.

Flexible origins

DNA replication origins that initiate replication in some cells but not in all cells.

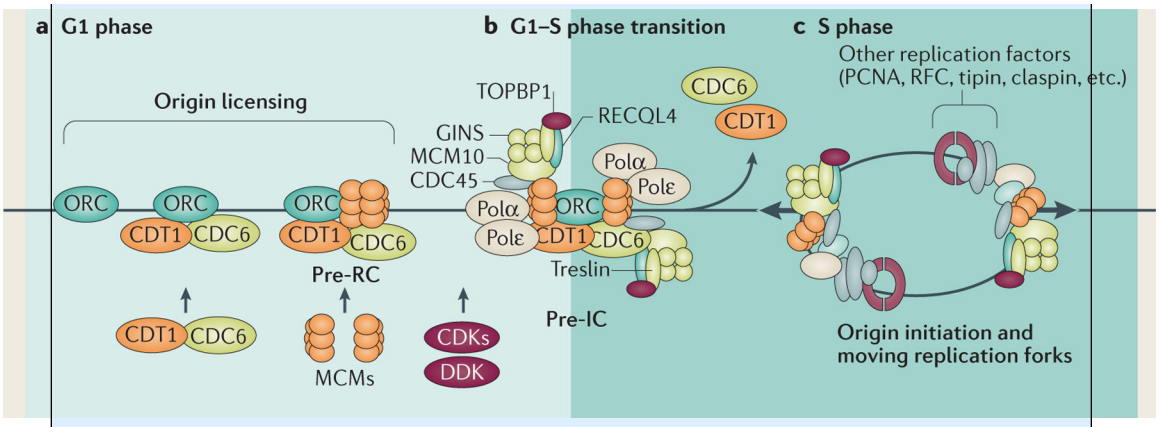
Dormant origins

DNA replication origins that undergo replication licensing but do not initiate replication during a typical, unperturbed cell cycle.

Box 1 |

Replication initiation mechanics

The initiation of eukaryotic DNA replication comprises two distinct steps[9,28,38]. The first step occurs shortly after the completion of cell division and involves the assembly of pre-replication complexes (pre-RCs) that include replicative DNA helicase components (a process known as replication origin 'licensing'; see the figure, part **a**). The replication origin licensing step requires binding of an origin recognition complex (ORC) to potential initiation sites and subsequent recruitment of a helicase (minichromosome maintenance 2–7 (MCM2–7)), which is mediated by the interaction of the licensing factors cell division control protein 6 (CDC6) and CDT1 with the chromatin-bound ORC. The resulting pre-RC includes an inactive form of the replicative helicase. In the second step (see the figure, part **b**), some pre-RCs recruit additional components to form larger pre-initiation complexes (pre-ICs) that activate the helicases and associate with DNA polymerases to start replication (a process known as origin activation). Pre-ICs are initiated by the recruitment and/or phosphorylation of additional proteins by DBF4-dependent kinase (DDK) and cyclin-dependent kinases (CDKs). Substrates of DDK and CDKs — including MCM10, RECQ-like DNA helicase type 4 (RECQL4), treslin and DNA topoisomerase 2-binding protein 1 (TOPBP1) — mediate the association of chromatin-bound MCM2–7 with additional components, such as CDC45 and GINS (*go-ichi-ni-san*), to form an active helicase[6,38,170]. Concomitantly, DNA polymerases associate with the active helicase complex and catalyse DNA synthesis (see the figure, part **c**). As replication progresses, pre-RCs dissociate from passively replicated origins, and high CDK activity prevents the assembly of new complexes[6,38,171–174]. The first step, origin licensing, is inhibited by CDKs and therefore only occurs when CDK levels are low during the G1 stage of the cell cycle. The second step, origin activation, requires CDK-mediated phosphorylation and occurs when CDK levels are high. As high CDK activity levels prevent further association of pre-RC components with DNA, no origins are licensed after replication initiates during the S phase of the cell cycle. The activation of replication origin complexes by CDKs couples genome duplication with cell-cycle signalling. Inhibition of the licensing step by the same CDKs ensures that the entire genome is duplicated only once during each cell division cycle.



PCNA, proliferating cell nuclear antigen; RFC, replication factor C; tipin, TIMELESS-interacting protein.

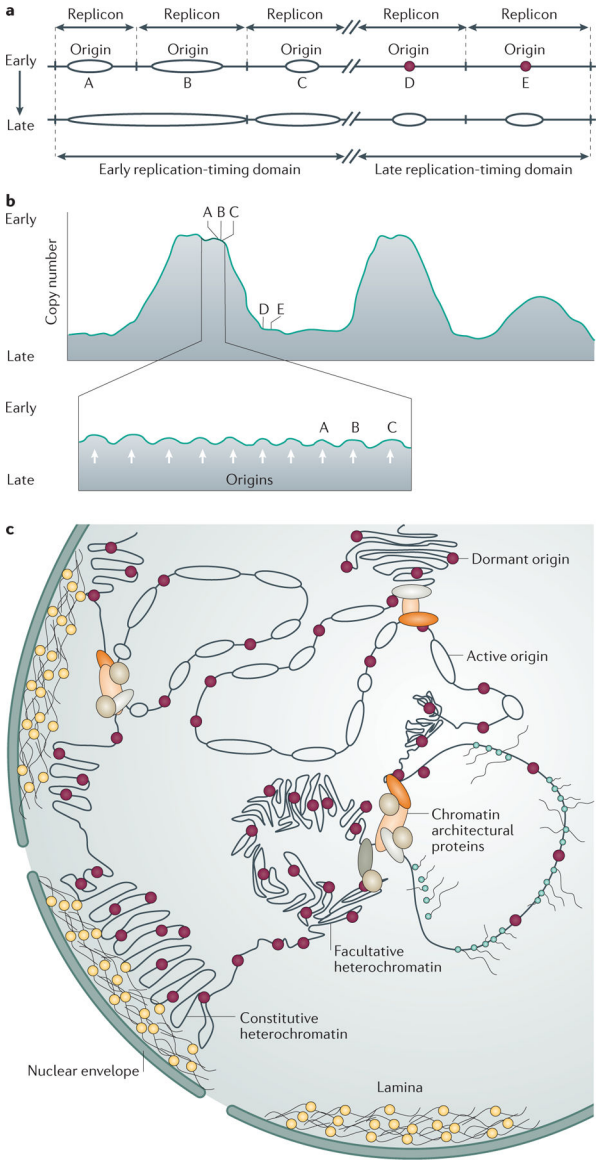


Figure 1. Organization of replication origins, replicons and replication-timing domains.

a | An illustration of a portion of a eukaryotic chromosome depicting five replicons, each defined by an origin of replication (A, B, C, D and E). Replication starts at different times during the S phase of the cell cycle. Origins A, B and C start replication first, concomitantly or in immediate succession, followed later by the initiation of DNA replication from origins D and E. The replicons containing origins A, B and C form an early replication-timing domain, and the replicons containing origins D and E form a late replication-timing domain. **b** | An example of possible replication-timing measurements revealing large megabase-sized replication-timing domains. Chromatin domains with similar replication timing (top panel) exhibit a similar copy number in an asynchronous cell population, with early-replicating domains (such as the domain including replicons A, B and C) containing more copies than late-replicating domains (such as the domain including replicons D and E). High-resolution mapping of replication timing (bottom panel) reveals subpeaks (indicated by white arrows)

in large replication domains that correspond to individual replicons driven by distinct replication origins (for example, the subpeaks for origins A, B and C are visible within the large early-replicating domain). **c** | Nuclear distribution of replicons and replication domains. Replicating DNA (represented by ellipses) tends to cluster, with each replication cluster containing adjacent, concomitantly replicating regions with several active origins. Early-replicating clusters usually associate with open chromatin (euchromatin; typically in the centre of the nucleus). Late-replicating clusters (here shown containing origins that have not yet started replication, depicted as dark red circles) associate with silent, transcriptionally inactive chromatin (facultative and constitutive heterochromatin; typically near the nuclear envelope). Highly transcribed chromatin often replicates early during S phase, but replication rarely initiates within actively transcribed regions.

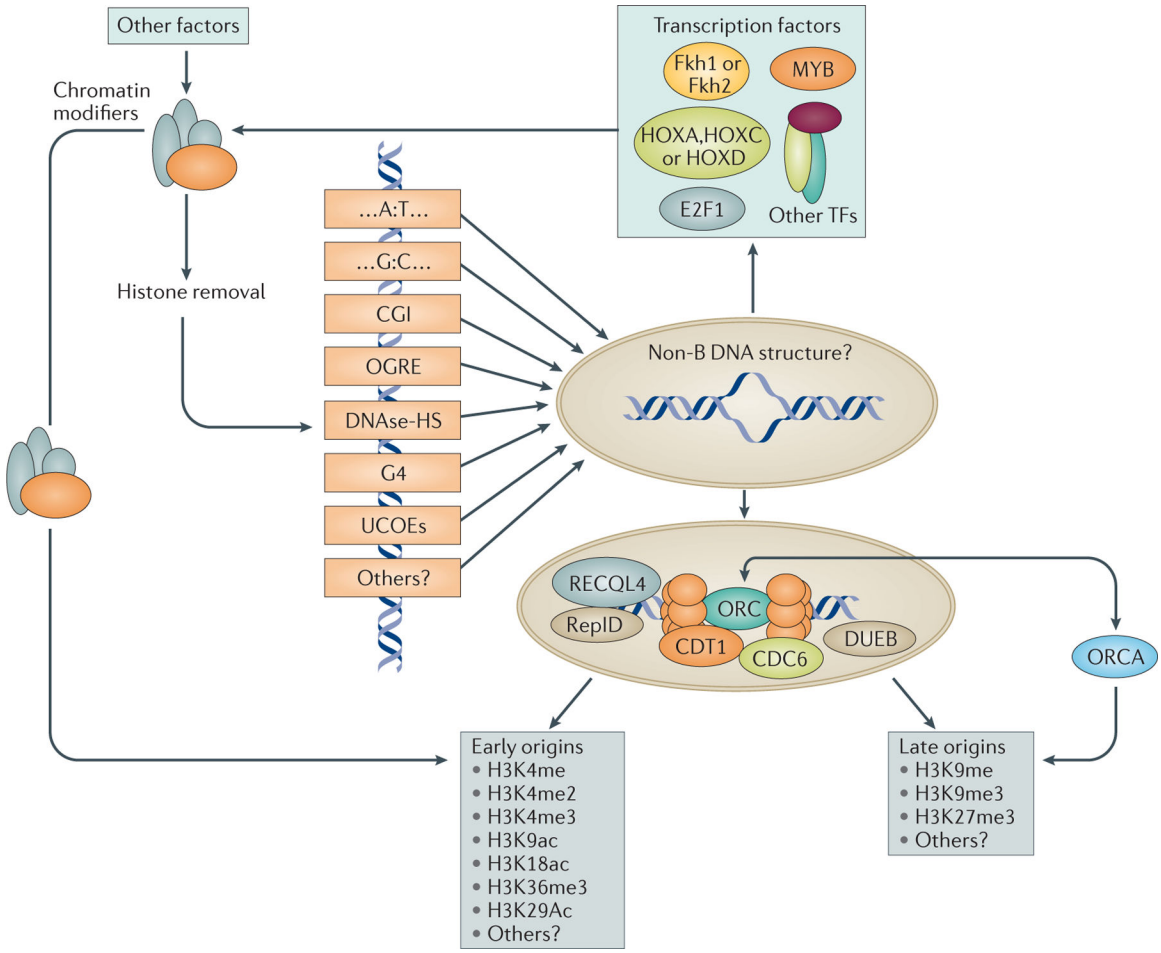


Figure 2. Genetic and epigenetic features of replication origins.

Although there are no strong consensus DNA sequences to anchor origins in metazoans, whole-genome sequence analyses have revealed several motifs that are present near or at origin sites. These include A:T- and G:C-rich motifs, CpG islands (CGIs), origin G-rich repeated elements (OGREs), DNase 1-hypersensitive sites (DNase-HS), quadruplex-like structures (G4) and ubiquitous chromatin-opening elements (UCOEs), which might increase the propensity of origins to unwind and adopt a non-B DNA structure. Groups of replication origins interact with proteins from the pre-replication complex and with proteins located at a subset of replication origins (such as replication-initiation determinant (RepID), DNA-unwinding element-binding protein B (DUEB) and RECQ-like DNA helicase type 4 (RECQL4)). Such interactions may also facilitate binding of transcription factors (TFs) such as forkhead box protein 1 (Fkh1), Fkh2, MYB, homeobox protein A (HOXA), HOXC, HOXD and E2F1) or other proteins that may regulate chromatin structure locally. Origins in early-replicating, open chromatin are preferentially enriched with euchromatin markers (such as H3K4 monomethylation (H3K4me), H3K4 dimethyation (H3K4me2), H3K4 trimethylation (H3K4me3), H3K9 acetylation (H3K9ac), H3K18ac, H3K36me3 and H3K29ac). Cell type-specific, late-replicating origins often associate with heterochromatin markers (such as H3K9me3, H3K27me3 and ORC-associated protein (ORCA), an ORC-binding protein that interacts with H3K9me3).

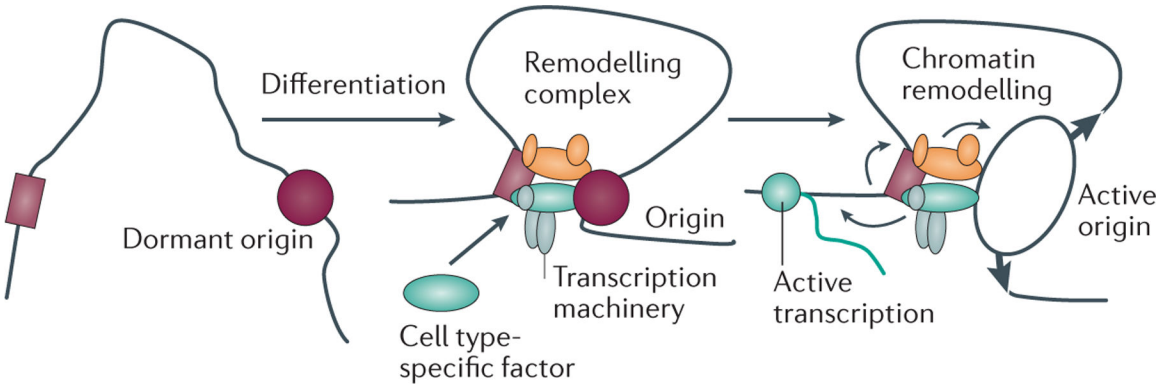


Figure 3. Long-range chromatin interactions can regulate origin activation and replication timing.

Cell type-specific transcription and/or chromatin-modifier complexes can bind both an origin of replication and a locus control region of a distant gene (dark red rectangle), modulating both the expression of a tissue-specific gene and replication initiation. Origin activation by distal interactions may enable local chromatin remodelling and the establishment of a more open chromatin configuration.

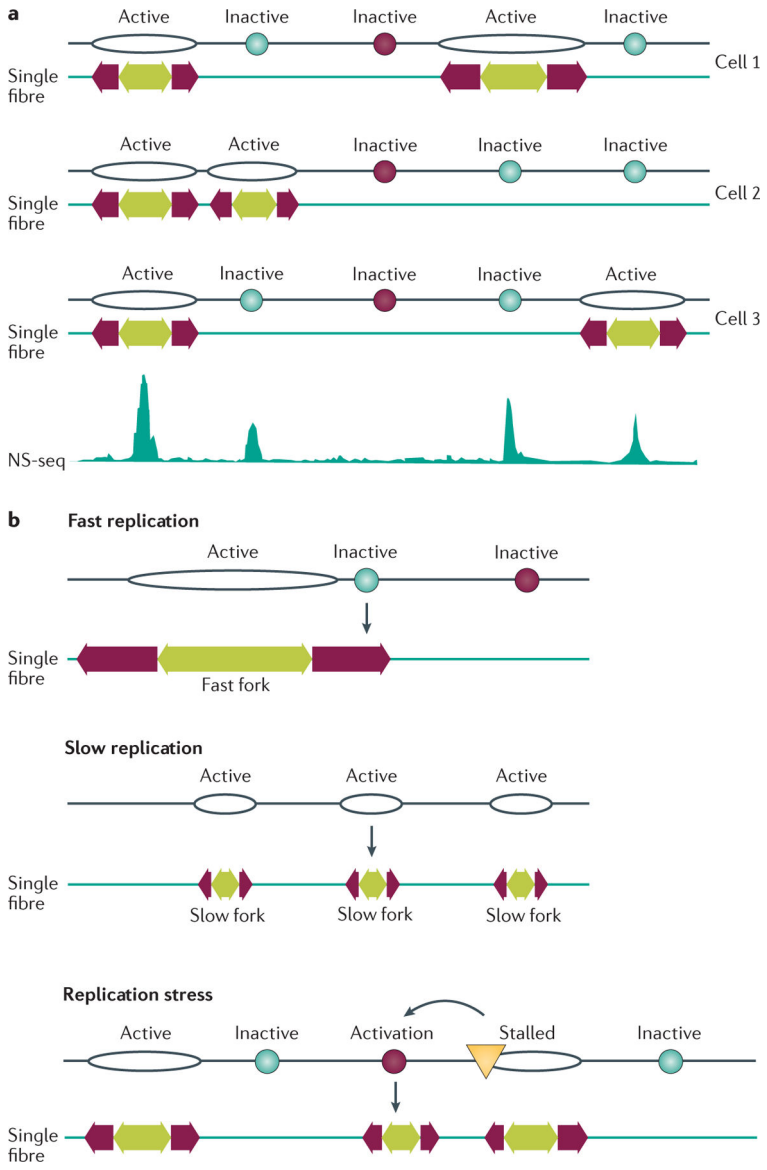


Figure 4. Replication origin choice and origin dormancy.

Constitutive origins (depicted as black oval-shaped outlines) initiate replication at all times in all cells; flexible origins (turquoise circles) initiate replication intermittently with initiation frequencies that vary from cell to cell; and dormant origins (dark red circles) are licensed to initiate replication but never, or very rarely, initiate during an unperturbed cell cycle. Dormant origins can be activated, however, if they reside next to a stalled or damaged replication fork (yellow triangle). **a** | Top: initiation from both constitutive and flexible origins shown on three hypothetical fibres, with each cell exhibiting a unique pattern of initiation. Experimentally, single-fibre analyses indicate the selection of initiation sites from the pool of flexible origins. Bottom: a population-based assay based on the abundance of nascent strands (nascent strand sequencing (NS-seq)) captures short, newly replicated DNA from all origins. Overall, the mean distances between population-based origin peaks are shorter than those measured on single fibres, indicating origin choice. **b** | Initiation rates can

increase when DNA synthesis slows or when replication forks stall owing to DNA damage. Fast replication is associated with few origins on average because some potential initiation sites are passively replicated by adjacent replication forks before their own replication is actively initiated. Slow replication can increase the frequency of initiation from flexible origins, resulting in shorter inter-origin distances detected on single fibres. In addition, perturbed replication can activate dormant origins that do not normally initiate replication during unperturbed DNA synthesis.

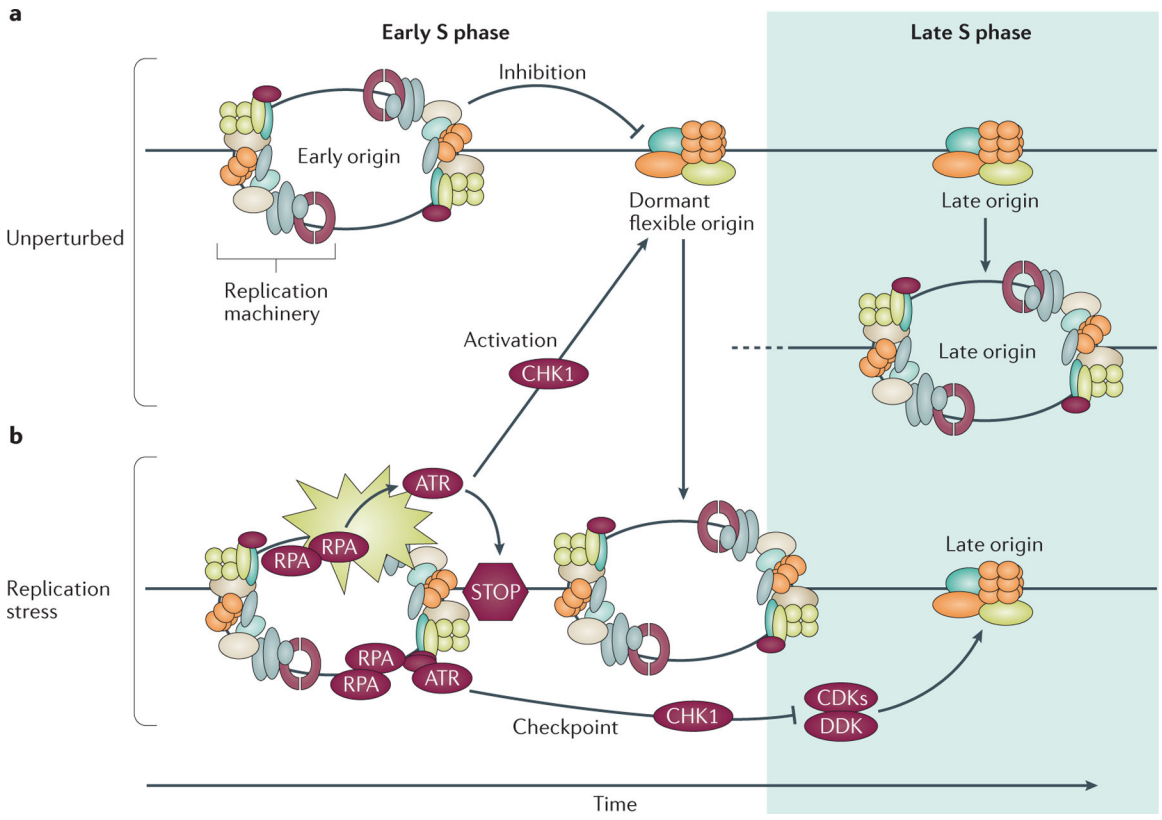


Figure 5. Origin inhibition and activation in unperturbed cells and after replication stress.

a | Only approximately 10% of origins are typically used in an unperturbed cell cycle; most origins remain dormant and are replicated passively by replication forks emanating from adjacent origins. **b** | Stalled replication forks contain replication protein A (RPA)-coated single-stranded DNA that is sensed by the checkpoint kinase ataxia telangiectasia and RAD3-related protein (ATR), activating a signalling cascade (indicated by the beige star shape) that prevents initiation at late replication origins through checkpoint kinase 1 (CHK1)-mediated inhibition of phosphorylation events catalysed by cyclin-dependent kinases (CDKs) and DBF4-dependent kinase (DDK). Concomitantly, CHK1 activation permits replication initiation from origins (dormant or non-activated flexible origins) located near the stalled forks to complete DNA replication locally.

Table 1 |

DNA–protein interactions at groups of replication origins

Name	Definition	Species	Interaction	Refs
Abf1	ARS-binding factor 1	*Saccharomyces cerevisiae*	Interacts with yeast replication origins in a sequence-specific manner	94
Fkh1 and Fkh2	Forkhead box protein 1 and forkhead box protein 2	*Saccharomyces cerevisiae*	Bind a subset of yeast replication origins (Fkh1 only binds origins that initiate replication early), regulate initiation timing by clustering and participate in long-range chromatin interactions	96
RB–E2F complex	A multiprotein complex containing a heterodimeric E2F transcription factor and an RB family member	*Drosophila melanogaster*	Interacts with ORCs and selectively binds replication origins at the chorion gene locus	105
MYB–MUVB	Part of the DREAM–MYB–MUVB complex containing RB	*Drosophila melanogaster*	Interacts with ORCs, binds amplified replication origins and recruits histone acetylases	106
XRCC5	X-ray repair cross-complementing protein 5 (also known as KU80)	Human	Interacts with primate replication origins in the G1 phase and with replication proteins	175
HOXC13	Homeobox protein HOXC13	Human	Binds the human *LMNB2* replication origin of the gene encoding human lamin and interacts with the pre-replication complex in a cell-cycle-dependent manner	120
DUEB	DNA-unwinding element-binding protein B	Human	Binds DNA-unwinding elements at replication origins in the human *MYC*, *LMNB2* and *SCA10* loci, and interacts with pre-replication complexes at those sites	176
ORCA	Origin recognition complex-associated protein	Human	Associates with human ORCs and stabilizes them on chromatin, and binds HP1 and H3K9me3	132
RepID	Replication-initiation determinant	Human	Binds to a group of human replication origins and is essential for their activation	115
LMO2	LIM domain only protein 2 (also known as rhombotin 2)	Human	Interacts with replication proteins and associates with some origins at promoters in human erythroleukaemic cells	177
H3K79me2	Dimethylated histone H3 lysine 79	Human	Binds a group of human replication origins and is required for limiting replication such that it occurs only once per cell cycle	58
GRWD1	Glutamate-rich WD40-repeat-containing protein 1	Human	Binds histones, CDT1 and CDC6 in human cells, colocalizes with CDC6 on chromatin and is required for MCM loading	117
HBO1	Histone acetyltransferase binding to ORC1 (also known as KAT7)	Human	Interacts with human CDT1 to activate replication origins	60, 112
RECQL4	RECQ-like DNA helicase type 4	Human	Recruited to the origins at the human *LMNB2*, *HBB* and *CSF2* loci, and is required for origin binding of MCM10 and CTF4	110, 111
BRPF3	Bromodomain and PHD-finger-containing protein 3	Human	Interacts with HBO1 to activate replication origins in open chromatin regions (for example, near TSSs) via histone acetylation	60

ARS, autonomously replicating sequence; CDC6, cell division control protein 6; *CSF2*, colony stimulating factor 2; CTF4, chromosome transmission fidelity protein 4 (also known as DNA polymerase α-binding protein); DREAM, DRE-antagonist modulator (also known as calsenilin); *HBB*, haemoglobin subunit-β; HP1, heterochromatin protein 1; *LMNB2*, lamin B2; MCM, minichromosome maintenance; MUVB, *Drosophila* orthologue of human RB; ORC, origin recognition complex; PHD, PH domain; RB, retinoblastoma-associated protein; *SCA10*, spinocerebellar ataxia type 10 protein (also known as ataxin 10); TSS, transcription start site.