Chapter 2
Choice of Origins and Replication Timing Control in Budding Yeast

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Abstract A complete and exact replication of every eukaryotic chromosome within each cell division cycle is essential to maintain stable genomes during cell proliferation. Abundant origins of DNA replication where the replication machinery assembles into replisomes to initiate DNA synthesis are widespread along chromosomes. DNA replication shows characteristic spatio-temporal patterns of origin usage and replication timing during S phase, which are conserved through evolution and are cell type specific, indicating an active process of regulation. Important advances have recently been made to elucidate the determinants and molecular mechanisms that regulate the patterns of origin activation. Among these, *cis*-acting elements, chromatin determinants, the timing of origin licensing and factors regulating the choice of origins and the firing timing during S phase have been described in *Saccharomyces cerevisiae*. Much less understood is the biological significance of this replication programme, but it could be significant in providing both robustness and plasticity to the DNA replication process in terms of replication completion and the maintenance of genome integrity.

Keywords Budding yeast • DNA replication origins • Cell cycle regulation • Origin specification • Origin activation • Firing timing • Replication completion • Genome stability

Introduction

Life perpetuates through the continued generation of daughter cells and requires the complete and exact replication of an accurate genome in every cell division cycle. The transmission of inexact genetic contents threatens the stability of progeny with potentially harmful consequences for viability or health. Reaching and maintaining cell populations in unicellular and multicellular organisms require vast numbers of cell divisions, providing ample opportunities for errors to occur. Successful DNA

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D.L. Kaplan (ed.), *The Initiation of DNA Replication in Eukaryotes*,
DOI 10.1007/978-3-319-24696-3_2

replication is thus a significant process that is further complicated in eukaryotic cells by the large size and fragmentation of eukaryotic genomes into chromosomes, the complex structure of chromatin and the pressure to complete replication in the relatively short duration of the S phase before segregation of sister chromatids starts in anaphase. To initiate DNA synthesis all eukaryotes display very abundant origins of DNA replication [1] that collectively expedite DNA synthesis, but that by being so numerous complicate their individual regulation to block re-replication while ensuring that no regions are left incompletely replicated.

Validating the 'replicon model' proposed by Jacob, Brenner and Cuzin in 1963, the initiation of eukaryotic DNA replication relies on the bipartite system of origins and initiation factors that are both necessary and together sufficient to initiate DNA synthesis [2]. The factors involved in the two-step mechanism of origin activation are now well known (for recent detailed reviews see [3, 4]). Briefly, in the first step, known as origin licensing, pre-replicative complexes (pre-RC) [5] form at origins by the sequential binding of the origin recognition complex (ORC), Cdc6, Cdt1 and two head-to-head Mcm2-7 hexamers. In the second step, known as origin firing, licensed origins are selected to initiate DNA synthesis, by the attraction of additional factors including Sld3, Sld7 and Cdc45 to the pre-RCs and by the phosphorylation by the Dbf4-dependent kinase (DDK) of at least some subunits of Mcm2-7, to form the pre-initiation complex (pre-IC) [6]. In parallel, a pre-loading complex (pre-LC) [7] containing GINS, Sld2, Polε and Dpb11 forms outside origins. Phosphorylation of Sld2 and Sld3 by the S-phase cyclin-dependent kinases (S-CDKs) [8, 9] is essential for the pre-LC to be recruited to pre-IC origins. The active Cdc45/Mcm2-7/GINS (CMG) helicase assembles [10, 11], and upon the attraction of additional replication factors two replisomes form which depart from each origin in opposite directions after DNA unwinding. This reaction leaves the origin in an inactive post-replication state in which it is bound only by ORC and with which it forms a post-replicative complex (post-RC) [5]. In synchrony with the cell cycle, licensing only occurs from late mitosis and during the G1 phase up to START, depending on the expression or recycling of the licensing factors and the inactivity of the S-, mitotic- and G1-CDKs. In late G1 phase the activity of G1-CDKs precludes licensing, while the lack of S-CDK impedes firing [12]. Firing initiates as soon as S-CDKs activate at the beginning of S phase. The persistence of active licensing-inhibitory CDKs up to the metaphase-to-anaphase transition prohibits new licensing. This dependency of licensing on the absence of CDK activity, and of firing on the presence of S-CDK, ensures that the activation of any origin is unique to each cell cycle.

However, in spite of this common machinery of origin activation, only a subset of origins is selected for firing during S phase, and origins display characteristic origin efficiencies (the percentage of firing in a cell population) and firing timing, leading to characteristic spatio-temporal patterns of replication initiation [13–18], both evidencing the active regulation of origin choice. In contrast to these conclusions obtained from cell populations, a stochastic choice of origins among single cells has been found when individual cells have been studied, revealing randomness in origin selection [19, 20]. The combination of global control in the order of firing and local stochastic competition among origins for firing has led to the suggestion of a 'controlled stochastic' model of origin choice [21, 22]. The factors and molecular mechanism that control the choice of origins and the firing timing, and the significance

of having defined replication patterns, are as yet incompletely understood and are the focus of intense research.

Here I focus on the current understanding of the determinants of the choice of origins, and their effect on the timing of replication in budding yeast, and also compile evidence supporting the biological significance of this replication programme. In brief, knowledge of the precise map of origin location and the temporal replication profile in budding yeast has facilitated the discovery of determinants of origin usage. Multiple factors are found to influence origin selection and firing timing in budding yeast, including *cis*-acting sequences at origins, local chromatin structure and epigenetic marks, origin positioning within chromosomes, timing of pre-RC formation and maintenance, recruitment of firing timing factors and competition for limiting origin firing factors (reviewed in [23–26]). All these regulators commonly display differential influences among origins; indeed, they provide diversity to the population of origins. The competition among origins for limiting firing factors is a source of plasticity in origin selection. The integration of these multiple controls at each origin could explain the differential activation probability and timing of choice among origins observed in cell populations, and the stochastic origin selection observed in single cells [22, 24–28]. Together with the non-random distribution of exceeding origin numbers, this organisation presumably adds redundancy and robustness to replication, for example against incomplete termination in agreement with the 'origin redundancy' model [27, 29]. Importantly, altering this programme is found to have negative consequences for chromosome integrity and genome stability.

Cis-Acting Elements and Chromatin Determinants at Origins for Origin Selection

Origins were first found in budding yeast and defined as autonomous replicating sequences (ARS) because they confer autonomous replication and maintenance to plasmids and are the sites where bidirectional replication starts [30–33]. The study of some ARSs by scanning mutagenesis showed that origins are a modular combination of distinct *cis*-acting elements including an essential A element which is constant to origins, and a variable composition of a few individually non-essential B elements that provide diversity among origins [34]. The sequence conservation of A elements allowed the definition of an extended ARS consensus sequence (EACS) of 17 AT-rich base pairs [35, 36] that further extends up to 33 base pairs if the ACS–ORC binding consensus [37] is considered. The ACS is insufficient to define an origin; thousands of sequences match the ACS on the yeast genome but data from genome-wide studies reveal that only around 800 are confirmed or likely ARSs [38]. ACS-B1 provides a bipartite sequence for ORC recognition [39, 40] and the B2 element of ARS1 facilitates pre-RC formation or maintenance [41]. In spite of the sequence specificity of pre-RC formation in *S. cerevisiae* in vivo, ORC can bind and load Mcm2-7 complexes to non-origin sequences [42] and support plasmid replication in vitro [43], which is similar to forced ORC binding to DNA in *Drosophila* [44].

Origin sequences and chromatin structure can modulate the probability of origin specification by influencing ORC recruitment and pre-RC assembly during licensing, and contribute to the timely selection and efficiency of origin activation during S phase. In support of origin sequence features influencing origin specification and activity in *S. cerevisiae*, it is found that mutations in the *cis*-acting sequences modify origin efficiency [45, 46], presumably by distinct abilities of differing sequences to attract or retain pre-RC factors (Fig. 2.1a). In further support, direct ORC–DNA chromatin-independent interactions also contribute to ORC recruitment to origins and to replication timing, because origins with this interaction are enriched for late firing [47] (Fig. 2.1a). Consistently, distinct sequence elements within origins influence that origins are differentially tolerant to the mutation of licensing factors or to CDK deregulation in the G1 phase, supporting a hierarchy of replication origins [48, 49]. Similarly, certain origin sequences predispose origins to re-replication, presumably by increasing the competency of origins to recruit or maintain pre-RC factors [50].

Chromatin determinants also regulate the dynamics of origin specification by licensing, and influence the activation timing. The positioning of origins close to those with earlier or more efficient activation can favour passive replication, so that in proximal origins the choice of one inactivates the others [51]. Pioneering experiments in budding yeast showed that the chromatin environment and origin position determine origin efficiency and firing timing independently of origin sequences, as evidenced by moving an early-firing origin to a subtelomeric late-replicating region or a late-firing origin to a plasmid [52]. This work also predicted the existence of *cis*-acting elements that determine the firing timing of proximal origins. Indeed, surrounding sequences and not the origin itself advance the firing timing of nearby origins [53]. Centromeres are normally early replicating, and they influence the replication timing of close regions as shown by the relocation of a functional centromere to a late-replicating region advancing the replication timing of surrounding origins even at long distances [54, 55] (Fig. 2.1b). Mechanistically, the effect of centromeres on the early firing timing of nearby origins can be contributed by kinetochores attracting DDK to recruit Sld3 and Sld7 to promote early replication [54, 55] (Fig. 2.1b), in a similar manner to the finding that the HP1 protein stimulates Sld3 loading and binding of Dfp1/Dbf4 for early replication of pericentromeric chromatin in the fission yeast [56]. Telomeres cause replication origins to fire late, as short telomeres replicate subtelomeric origins early [57, 58]. Telomeres and subtelomeric regions are frequently silenced in transcription, and origin firing is delayed to late in S phase by telomeric suppression of origin activation by the histone deacetylase (HDAC) Sir2 [52, 59], or by the Ku complex [58, 60] (Fig. 2.1b). Loss of function of Sir2 suppresses the *cdc6-4* mutation, and rescues DNA synthesis and plasmid stability of other pre-RC mutants, implying that Sir2 regulates initiation of DNA replication [61]. The Sir2-dependent inhibition of origin activity is differential among origins suggesting that it relies on origin sequences or structure, and mechanistically it could be explained by the presence of an inhibitory sequence (I^s) on ARSs that requires Sir2 [62] (Fig. 2.1b). Further supporting the idea that histone acetylation regulates the selection of origins for firing timing, the loss of the HDAC Rpd3 causes advanced firing timing and Cdc45 recruitment of the subset of late

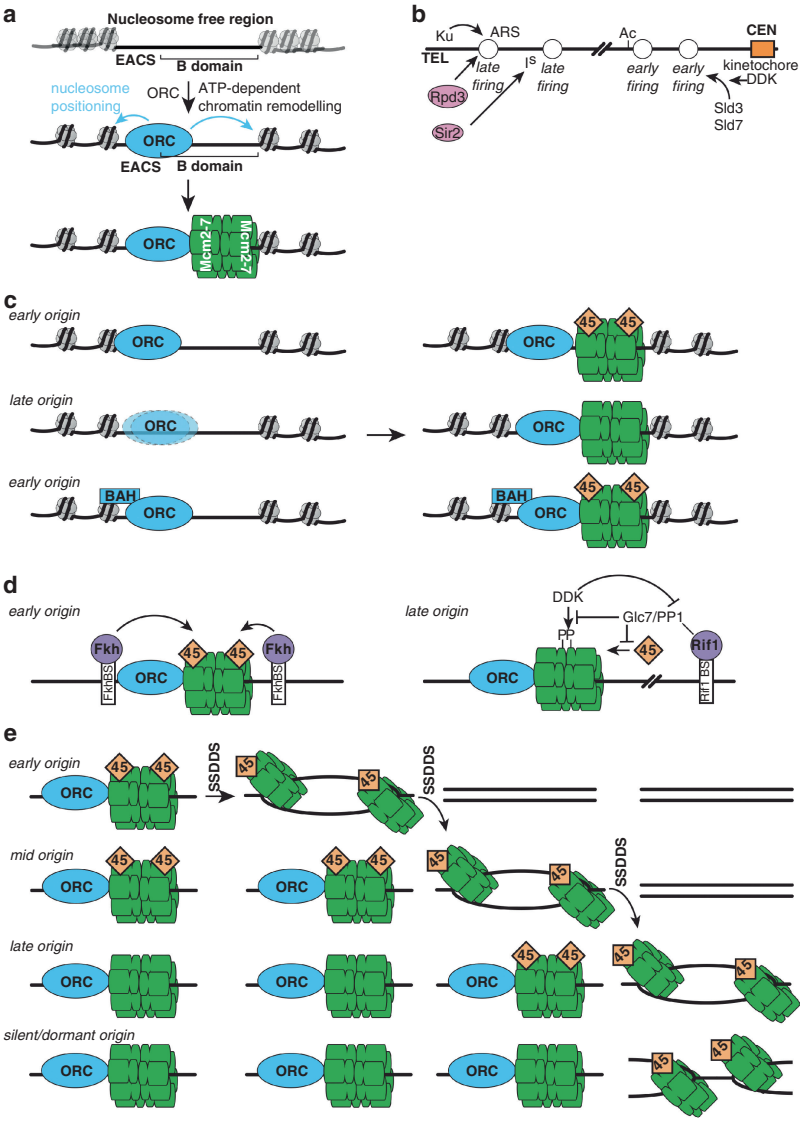

Fig. 2.1 Factors determining origin specification and choice in the control of replication timing. (**a**) *Cis*-acting elements at origins (EACS and B domain) influence origin efficiency and also the surrounding chromatin structure by maintaining a nucleosome-free region window that facilitates ORC recruitment; ORC–DNA binding and an ATP-dependent chromatin remodelling activity position flanking nucleosomes for pre-RC formation in the G1 phase. (**b**) Local and global chromatin determinants influence the choice and firing timing of origins, including centromeres and telomeres that influence the replication timing (early or late) of surrounding origins, and HDACs like Rpd3 and Sir2. Ac, histone acetylation. (**c**) The strength and/or the cell cycle timing of the ORC–chromatin interaction influence pre-RC formation and the timing of origin firing. Earlier or more stable ORC binding (*solid line*), or the presence of the BAH domain, associates with earlier pre-RC formation and with early origins; more labile or later ORC binding (*dashed line*) associates with late origins. The diamond represents Cdc45. (**d**) Apart from the general initiation machinery, specialised factors like Fkh1/2 and Rif1 influence the timing of origin activation by modulating the maturation of pre-RCs into active replisomes during origin firing. FkhBS, Fkh1/2-binding site; Rif1 BS, Rif1-binding site. (**e**) Rate-limiting availability of firing factors and sequential usage by origins govern the distributed timing of origin firing during S phase in the budding yeast. SSDDS, Sld2, Sld3, Dbf4, Dpb11, Sld7

origins of non-telomeric regions [63, 64] (Fig. 2.1b). Consistently, targeted histone acetylation by recruitment of the histone acetyltransferase (HAT) Gcn5 to the late-firing origin ARS1412 advances the firing timing. In line with this, the deletion of Gcn5 compromises minichromosome maintenance, alters the chromatin structure, decreases the level of Mcms at origins and finally a high dosage of Gcn5 suppresses the thermosensibility of *ORC* and *MCM* mutants defective in initiation of DNA replication [64, 65]. Acetylation of H3 and H4 has been shown to be present around a replication origin in a minichromosome, and acetylation of multiple lysine residues is important for efficient chromosomal origin activation and DNA replication during S phase [66]. Indeed, other HDAC, such as the Sum1-Rfm1-Hst1 complex that binds to a subset of origins, is required for normal initiation activity, and removal of a binding site decreases origin activity [67]; furthermore, deletion of this HDAC increases H4K5 acetylation and decreases origin activity [68]. Other histone marks such as methylation also influence origin activity [69, 70]. H3K36me by Set2 aids the binding timing of Cdc45, and H3K36me3 is inhibitory to this process [69].

Regarding the implication of chromatin structure and remodelling in origin activation and replication initiation, almost all origin sequences in *S. cerevisiae* maintain a nucleosome-free region (NFR) which starts from the ACS [71, 72]. Leading-strand synthesis preferentially initiates within the NFR [73], and the NFR is directed by the origin sequences since the absence of ORC does not abolish the NFR [37]. Nucleosome positioning affects the function of ARS1 [74]. The NFR presumably provides access for ORC binding, which in turn positions nucleosomes flanking the origin together with an ATP-dependent chromatin remodelling activity at almost every origin and facilitates the initiation of DNA replication [37, 75, 76] (Fig. 2.1a).

Dynamics of Origin Licensing and Timing of Origin Firing

The choice of which origins are to be fired during S phase can only be made from those origins that have previously been specified by licensing. The timing programme is established to be coincident with the licensing period (between late mitosis and the end of the G1 phase), at least for subtelomeric late-replication regions where a subtelomeric late origin excised in G2/M, and not in the G1 phase, switches the activation time to early firing [77]. Hence, the schedule of origin licensing can contribute to the timing of origin activation.

The chromatin structure may influence the activity of origins by regulating the accessibility of initiation factors to origins, both globally at entire chromatin regions because origin activity correlates with nuclear positioning [78] and origins organise into foci of multiple origins that fire at similar times [79], and locally at specific origins under the influence of the chromatin environment. The NFR at origins can accommodate Mcm2-7 hexamers, and disruption of nucleosome positioning by ORC interferes with pre-RC formation [76]. Significantly, the nucleosome positioning which is established during the G1 phase differs between early and late origins, and is modulated during origin activation in the cell cycle [80]. Supporting the view

that the association of ORC with chromatin during the cell cycle is a determinant of origin efficiency, a detailed analysis of DNA proteins by MNase footprint mapping at origins [81] has shown a cell cycle regulation of ORC binding to origins and consistent nucleosome remodelling. This approach discriminates between 30 % of origins showing a detectable ORC-dependent footprint in G2, and another 15 % of origins having a footprint detected only in G1 (consistent with transient ORC binding in G2 or with no binding until G1) (Fig. 2.1c). Significantly, ORC binding in G2 is a determinant for efficient or early origin activation, although the effect is not global [81]. In *S. pombe* the timing of ORC binding and pre-RC formation during mitosis and G1 influences origin efficiency and firing timing during S phase [82]. Origins with earlier Mcm2-7 loading could have further time for additional Mcm2-7 recruitment, thus increasing the probability of attracting firing timing factors and therefore of firing earlier during S phase [21, 26] (Fig. 2.1c).

However, the determinants of ORC binding to specific origins and during the cell cycle are not known. In metazoans, the conserved chromatin-binding module bromo-adjacent homologous (BAH) domain of Orc1 [83] recognises and binds to H4K20me2 methylated histones, but this function is not conserved in Orc1BAH in yeasts [84]. Instead, in *S. cerevisiae* the BAH domain of Orc1 is important for origin selection within chromatin [85] (Fig. 2.1c). *orc1bahΔ* cells show reduced ORC and Mcm2-7 association with chromatin. Consistent with the BAH domain not being a general regulator of origin activation, the effect is differential among origins so that some origins are *orc1bah*Δ sensitive and others *orc1bah*Δ resistant. Furthermore, there are differential responses among sensitive origins, and the loss of the BAH domain does not completely remove ORC/Mcm2-7 binding to *orc1bahΔ*-sensitive origins, but replication initiation, efficiency of origin firing and plasmid maintenance are compromised in sensitive and not in resistant origins [85]. Importantly, the BAH domain is not the determinant of origin efficiency, as efficient and inefficient origins have been found among *orc1bahΔ*-sensitive and -resistant origins [85]. Significantly, ORC binds more stably to origin-containing chromatin than to naked DNA suggesting that ORC at origins is stabilised through the interaction with nucleosomes, and is independent of the BAH domain [86]. Origins relying more on local chromatin determinants, defined as chromatin dependent, are enriched in early-firing origins [47].

Hence, it seems that origin selection can be viewed as the intrinsic origin sequence capability modified by several local chromatin determinants that differentially merge at each origin and modulate the characteristic origin probabilities of efficiency or firing timing during S phase.

Factors Regulating the Timing of Origin Activation

Although firing at all origins occurs by the maturation of licensed origins from pre-RCs to pre-ICs and replisomes, there are conserved distinctive activation times and efficiencies between different origins [13–15], and more origins are licensed than

are actually selected to initiate replication [50], evidencing a timely choice of origins for firing. Identification of the factors that regulate timing of origin firing is of relevance considering that the replication profile results predominantly from the kinetics of origin firing [73]. The determinant factors of firing timing had remained elusive until recently, and key discoveries have now shed light on the process.

The transcription factors Fkh1 and Fkh2 are determinants of the origin firing timing programme because they promote early firing to a subset of early origins through ORC binding, clustering of early origins and association with Cdc45 during pre-RC maturation in the G1 phase [87] (Fig. 2.1d). Fkh1/2-binding sites on origins are limited to early origins, although the presence of these sites at origins is insufficient to confer early firing (which is also dependent on the close proximity to the ACS). Furthermore, not all early origins contain Fkh1/2-binding sites, and the introduction of Fkh1/2-binding sites at late origins is insufficient to confer early replication [87, 88]. The position and number of Fkh1/2 sites relative to the ACS seem to be important for origin activity and only a subset of origins contain two sites in a position which flanks the NFR in a precise localisation relative to ACSs [80, 88] (Fig. 2.1d). This regulation by Fkh1/2 in tethering early origins together is consistent with these clusters being poles of attraction for firing factors, including Cdc45, which concentrate spatially and temporally leading to early replication [87] (Fig. 2.1d), and also with evidence that early origins frequently interact [89].

The telomere-binding protein Rif1 is also a conserved regulator of the replication timing programme in normal cell cycles from yeast to human cells [90–93]. In its absence there is a premature activation of origins at telomeres and earlier replication [58]. Rif1 regulates the firing timing of late/dormant origins in internal and subtelomeric chromosome regions in *S. cerevisiae* [92] (Fig. 2.1d). Both in fission and budding yeast Rif1 binds to telomeres and along chromosomes, and although binding is close to some Rif1-regulated origins there is no specific enrichment at origins [91, 92, 94]. The details of the mechanism by which Rif1 controls the firing timing of origins have been elucidated recently. In *S. pombe* the binding of Cdc45, but not of Mcm4, to origins is affected in *rif1*Δ cells, where it was shown that Cdc45 was bound to late origins in contrast to wild-type cells, suggesting that Rif1 influences the steps after pre-RC assembly [91]. In *S. cerevisiae* Rif1 contains two Glc7/protein phosphatase 1 (PP1) interaction motifs at the N-terminus, which enable Rif1 to target PP1 activity to pre-RCs to counteract the DDK phosphorylation of Mcm4 that is critical for the Rif1-repressive effect on the firing timing of late origins [95–97] (Fig. 2.1d). Importantly, Rif1 is also regulated in its binding to Glc7 by interaction with DDK and by DDK-dependent phosphorylation [95–97], and by Tel1 phosphorylation at short telomeres [98] (Fig. 2.1d).

A more global determinant of origin firing timing seems to be the limiting step of pre-RC maturation towards active replisomes. Indeed, while the earliest origins recruit Cdc45 (although loosely during the G1 phase), late origins remain unbound until late S phase [99] (Fig. 2.1e). Work in *S. pombe* has indicated that the recruitment of rate-limiting initiation factors to origins controls origin efficiency by ordered ORC and Mcm2-7 binding, and of firing timing by limiting DDK [19, 82]. Furthermore, Fkh1/2 and Rif1 influence the schedule of Cdc45 recruitment and of

DDK phosphorylation to origins to regulate the firing timing of origins [87, 95–97] (Fig. 2.1d). Work in budding yeast has shown that the essential initiation factors for pre-RC maturation Sld2, Sld3, Sld7, Cdc45, Dpb11 and Dbf4 are rate limiting for origin association and influence the timing of origin firing as their combined overexpression advances the firing timing of late origins to earlier in S phase [100, 101]. Hence, sequential origin firing timing is ordered by the binding of rate-limiting factors to early origins, and further release and subsequent recycling by mid and late origins until complete replication (Fig. 2.1e). In this context, the prevention of late origin activation under replication stress or DNA damage by the S-phase checkpoint also operates. New firing events at licensed origins are prevented upon activation of the S-phase checkpoint [102, 103] mediated by phosphorylation of Dbf4 and Sld3, and inhibition of Cdc45 recruitment to late origins [99, 104–106].

Significantly, those determinants of firing timing actually operate in parallel. Fkh1/2-binding sites are excluded from the subset of origins regulated by Rpd3L [87], while the combined lack of Rpd3L-dependent late firing of dormant origins and overexpression of the rate-limiting firing factors is required for early firing of dormant origins [100].

Significance of Spatio-Temporal Programmes of Origin Activation

Replication timing patterns are more conserved across eukaryotic evolution than strict origin positioning (mainly of dormant origins), even in closely related species [107]. The function of performing regulated temporal programmes of origin choice and replication therefore seems important but is, as yet, incompletely understood, and a number of suggestions have been posed and modelled mathematically [21, 22, 24, 26]. The identification of factors that determine this control has allowed the consequences of its mutation for genome integrity to be addressed experimentally.

The replication timing programme can influence the mutagenic landscape of chromosomes (see [108]). Chromosomes contain an irregular distribution of distinct elements whose replication pattern provides evidence of preferred replication dynamics. The function or homeostasis of these elements may require specific replication control, which could explain the existence of replication timing programming. This is the case with centromeres that replicate early in budding and fission yeasts [109], presumably to ensure optimal chromosome segregation and prevention of aneuploidy [110]. It is also the case with fragile sites (where chromosomes break more frequently), which are present from yeasts to human cells, that frequently have specific chromatin structure or composition, display difficult replication, break under defective or slow replication dynamics, and correlate with a paucity of dormant origins along large chromosome regions and retarded replication in human cells [111–113]. Also of relevance is that mutagenesis is non-random across the genome. Replicative polymerases have distinct error rates and contribute differently to mutation rates by inducing compositional biases along DNA associated with the asymmetry of DNA replication, and accordingly active

origins establish a strand bias for mutagenesis [114, 115]. Late-replicating regions have been shown to have a higher incidence of mutagenesis than early regions [116]. Inserting a sequence at distinct replication-time positions along a chromosome reveals a strong correlation with timing and rates of mutagenesis, and consistently the deletion of an early origin leads to a mutagenic increase presumably by retarding replication of nearby regions [117].

Excessive firing could be restricted to proceeding sequentially during S phase if replication proteins or other factors are rate limiting so that the progression during S phase must accommodate the rates of synthesis or recycling of those factors. This is the case of the rate-limiting firing factors (Sld2, Sld3, Dbf4, Dpb11, Cdc45 and Sld7) that impede inappropriate origin activation during S phase and control S-phase length in budding yeast [100, 101]. Otherwise, simultaneous firing can be deleterious. Indeed, dNTP pools are rate limiting and balanced for precise genome duplication by the ribonucleotide reductase [118]. The simultaneous firing of early and late origins in S phase by the overexpression of the rate-limiting firing factors in budding yeast imbalances replication by the elevated numbers of replication forks: dNTPs are deprived, replication stress arises, and the checkpoint kinase Rad53 is activated depending on dNTP levels [100].

Deregulated origin usage could alter the optimal distribution of initiation events along chromosomes needed to ensure replication completion according to the proposed 'random completion' or 'replication gap' problem [27, 29, 119]. For timely completion of replication, the 'origin redundancy' model [27] proposes two solutions: first, that a large excess of licensed origins are selected to fire during S phase in a regular distribution, and second that unreplicated regions retain initiation potential at licensed unfired origins whose activation would facilitate replication completion. A compatible proposed solution is that the efficiency of origin firing increases as S phase progresses at unreplicated regions [27, 29]. Indeed, the replication programme displays exceeding numbers of origins used below saturation during S phase, and non-random origin distribution. The features of regulated activation timing of origins (providing origin diversity) and some allowed stochastic origin selection (providing flexibility) together lead to strong origin redundancy in replication. This replication programme could thus provide the optimal organisation for completion of replication [120]. This is particularly important for two reasons: firstly, replisome progression is normally highly irregular due to eventual fork stalling or collapse [121], regulated pausing at programmed fork barriers [122–124] or delayed replication progression across chromatin regions that display difficult replication like fragile sites [111], and secondly, considering that every chromosomal sequence has a maximum of two opportunities of replication by incoming replisomes from each flank, new origin firing within the region can easily rescue irreversible fork arrest [125]. Consistently, reducing origin numbers compromises chromosome maintenance and integrity, and is further aggravated upon reducing origin diversity by the simultaneous deletion of dormant origins [126, 127]. Similarly, a paucity of origins delays replication completion leading to the expression of fragile sites in human cells [112]. Mutants of licensing factors also reduce the efficiency of origin activation and cause the loss of minichromosomes and

elevated rates of chromosomal rearrangements [45, 49, 128]; consistent upregulation of licensing-inhibitory CDK kinases in the G1 phase reduces origin licensing and the efficiency of origin firing, compromising the dynamics of S phase and genome stability possibly by incomplete genome duplication before the initiation of anaphase [129, 130]. Strongly linking genome instability to defective origin usage, the elevated rate of gross chromosomal rearrangements (GCR) caused by the deregulation of CDK activity in the G1 phase at a chromosome region is suppressed by increasing the concentration or distribution of origins in that region presumably by increasing the density of initiation events [130]. And consistently, the rates of GCR reflect the paucity of initiation events from active origins in that region [131]. Hence, compromising the number or choice of origins available during replication could reduce the flexibility of initiation and the robustness of S phase towards replication completion and genome maintenance.

Acknowledgments I apologize to those colleagues whose work could not be discussed or cited in the text owing to space constraints. Work in the laboratory was supported by the Spanish Ministry of Economy and Competitiveness (grant no. BFU2010-18225).

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http://www.springer.com/978-3-319-24694-9

The Initiation of DNA Replication in Eukaryotes
Kaplan, D.L. (Ed.)
2016, XII, 563 p. 87 illus., 84 illus. in color., Hardcover
ISBN: 978-3-319-24694-9