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DNA replication timing: temporal and spatial regulation of eukaryotic DNA replication

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Abstract

Eukaryotic genomes are replicated from tens of thousands of replication origins that are scattered along the chromosomes. In yeasts, timing of firing of these origins is regulated by *cis*-acting sequences and factors that may interact with them. Competition for limiting initiation factors also could regulate the order of origin firing in yeasts. In higher eukaryotes, replication timing may be regulated on a larger genome scale. This so called "replication domains" may be generated by formation of chromosome domains that are spatial and temporal units of simultaneously fired origins present in them. A conserved factor, Rif1, is a key regulator of replication domains, and determines the replication timing for the coming S phase at the end of M phase. Individual origins within the replication domain may be stochastically activated, although local transcription activity and histone modification may affect the firing efficiency. Replication domain is developmentally regulated and thus is a cell type-specific trait. Physiological significance of replication timing regulation will be discussed.

Introduction

Faithful and complete chromosomal DNA replication is essential to conserve genomic integrity and to prevent abnormal cell growth observed in cancer cells. DNA replication is initiated from replication origins and mediated by sequential recruitment of the replication factors such as ORC, Cdc6, Cdt1, and MCM to establish pre-replicative complex (pre-RC), followed by that of other factors including Cdc45, GINS and DNA polymerases (Masai et al. 2010). Two highly conserved S phase kinases, Cdc7 kinase and Cyclin-dependent kinase (CDK), phosphorylate pre-RC components and others to initiate replication (Remus et al. 2009; Labib 2010). In contrast to prokaryotes in which DNA replication is generally initiated from a single replication origin, many replication origins are present on the eukaryotic genomes and each of them is activated throughout the S phase at different timing or not activated at all. Although the pre-RC complexes are assembled at potential replication origins at early G1 phase, only a subset of them are activated at early S phase and others are fired late or not activated but replicated passively. In higher eukaryotes, genomes are more clearly divided into "replication domains" which are replicated at different timing within S phase. The delineation of genomes into these "domains" is intimately related to the three-dimensional chromatin structures within nuclei and assembly of these domains are under developmental regulation (Rhind and Gilbert 2013).

The choice of replication origins to be fired is flexible, adaptive and maybe stochastic (Bechhoefer and Rhind 2012; Gindin et al. 2014; Renard-Guillet et al. 2014). Cells may unexpectedly encounter replication fork blocks caused by DNA damage, DNA binding factors blocking the fork progression, unusual secondary structures of the template DNA, nucleotide depletion and others. In order to circumvent the problems caused by stalled forks, cells would use late/ dormant origins to complete the replication of unreplicated segments. Late/ dormant origins are prevented from firing, at least in the presence of replication stress signals, by checkpoint. Thus, inactivation of checkpoint generally causes activation of late/ dormant origins even when cells are arrested at early S phase. Sequential firing of early and late/ dormant origins is regulated also by transcription, chromatin structures, and chromatin architecture (Aparicio 2013).

In this short article, we will summarize the most recent information on mechanisms that regulate temporal and spatial program of chromosome replication. We will then discuss the potential physiological significance of replication timing.

1.Various aspects on regulation of replication timing

1-1. Decision of replication timing during cell cycle

pre-RCs are generated on chromosomes at late M/ early G1 to designate the potential replication origins that will be utilized in S phase. G1 phase appears to be crucial also for establishing replication timing program. Earlier works by Gilbert indicated that the temporal replication domains may be established at early G1 phase concomitant with nuclear repositioning after the nuclear envelope assembly in mammalian cell. This was termed replication timing decision point (TDP) (Dimitrova and Gilbert 1999). Replication timing may be determined in G1 phase in budding yeast as well. Separation of an origin from telomere *in vivo* by using site-specific recombination demonstrated that the signal for late activation is established between mitosis and START in the G1 phase, and once established, the signal can persist through the next S phase even when the origin is physically separated from the telomere (Raghuraman and Fangman 1997).

In *Xenopus*, mitotic remodeling is crucial for resetting the chromatin structure of differentiated adult donor cells for embryonic DNA replication. This involves topoisomerase II-dependent shortening of chromatin loop domains and an increased recruitment of replication initiation factors onto chromatin, leading to a short inter-origin spacing characteristic of early developmental stages (Lemaitre et al. 2005). The velocity of DNA replication fork in S phase also influences the chromatin loop size in the following S phase, which will be reset at mitosis (Courbet et al. 2008). Thus, resetting of chromatin structures at

M phase would play crucial roles for setting up a new program for the following S phase. Decision made at the end of M phase may also be affected by various factors including cellular metabolism, environmental stress, and previous S phase progression. A factor that may play a central role in this process will be discussed later.

1-2. Limited replication factors regulate replication timing

At the onset of the S phase, Cdc7 and S phase Cyclin-dependent kinase (S-CDK) sequentially function to assemble CMG (Cdc45-Mcm-GINS) helicase at the origins, and establish a replisome complex containing DNA polymerases and other fork factors (Kamimura et al. 2001; Gambus et al. 2006; Tanaka et al. 2007; Zegerman and Diffley 2007; Masai and Arai 2002). In yeasts, some of the key replication factors are limited in numbers and need to be recycled to activate all the origins. Indeed, overexpression of Sld3-Cdc45-Sld7 and Cdc7-Dbf4 results in activation of late replication origins in the presence of HU (Tanaka et al. 2011; Mantiero et al. 2011). Activation of late origins which are located within rDNA repeats by Sir2 deletion leads to repression of early-firing origins due to depletion of initiation factors (Yoshida et al. 2014). In fission yeast, overexpression of, Cdc45 or Hsk1-Dfp1 (the homologue of Cdc7-Dbf4 complex) increased the numbers of early-firing origins (Wu and Nurse 2009). It is not known whether initiation factors are limiting in higher eukaryotes.

1-3. Replication timing coordinates with gene expression

Chromatin structure and its subnuclear position affect accessibility of replication and transcription factors, and thus replication timing and gene expression may be correlated with each other. Although DNA replication mostly starts at mid S phase and there are no strong relationship between transcription and DNA replication in budding yeast, over 40% of gene expression is correlated with initiation of DNA replication at early S phase in *Drosophila melanogaster* (Raghuraman and Fangman 1997; Schubeler et al. 2002). Indeed, a subset of RNA polymerase II binding sites overlaps with ORC-binding sites and greater amount of RNA polymerase II is observed at early-firing replication origins compared to late-firing origins, suggesting that transcription influences replication timing (MacAlpine and Bell 2004). Mouse immunoglubin heavy chain locus (IgH) is rearranged by V(D)J recombination in differentiation from immature B cells. Replication timing is converted from late to early at the IgH locus during early stage of development in B cells, concomitant with activation of gene expression and altered chromatin modification and subnuclear localization (Norio et al. 2005; Oettinger 2004). In Chinese Hamster Ovary cells, over 20 potential initiation sites are detected between the dihydrofolate reductase (DHFR) and 2BE2121 genes. Suppression of

DHFR expression by depletion of its promoter led to decline of the origin activity at early S phase (Saha et al. 2004). Furthermore, allele-specific gene expression with genomic imprinting also supports positive relationship between transcription and initiation of DNA replication. Female cells have two X chromosomes and one of them is inactivated during differentiation through deacetylation of histone H4 followed by expression of X chromosome inactivation gene (Xist). Replication timing is switched from early to late on the X chromosome that is silenced (Koehane et al., 1996, Note that H4 deacetylation is not necessary for inactivation of the X chromosome). In addition to the X chromosome, some of disease-related genes are expressed in a paternal- or maternal-specific manner (mono-allelic expression). The transcription of an imprinted gene associates with allele-specific replication timing and its replication timing is established in the gametes (Simon et al., 1999). An imprinted chromosome is regulated through allele-specific DNA methylation and histone modification resulting in expression of only one allele (either paternal or maternal). The gene of Prader-Willi syndrome (PWS) or of Angelman syndrome (AS) responsible for neurodegenerative disorders is located at chromosome 15q11-13 and are exclusively expressed on the paternal or maternal allele, respectively. The early replication timing at the PWS region is correlated with its gene expression level in neuroblast and suppression of SNRPN gene, a candidate causative gene for PWS, results in loss of late replication timing in lymphocyte (Gunaratne et al. 1995). Furthermore, allele-specific replication timing was observed in the cells derived from these patients (Knoll and Lalande 1994).

1-4. Replication timing and epigenetic regulation

Post-translational modifications of histone dictate chromatin structure and influence multiple fundamental cellular functions including transcription and DNA replication (Campos and Reinberg 2009). Histone H3 deacetylase, Rpd3L, suppresses about 100 replication origins in budding yeast. In *Drosophila*, the tethering of Rpd3 decreases replication origin activity, whereas an opposite effect was observed with Chameau, the putative fly orthologue of human HBO1, Histone H4 acetylase (Aggarwal and Calvi 2004; Knott et al. 2009). These results suggest that the alteration of histone modification can cause a switching of replication timing from late to early, or from early to late. The human β-globin domain replicates late in non-erythroid cells, whereas it replicates at early S phase in erythroid cells with concomitant increase of the histone H3 and H4 acetylation level. Tethering of histone deacetylase to β-globin locus decreases the histone acetylation level over this region and changes replication timing from early to late in a manner independent of β-globin gene transcription (Goren et al. 2008). Genome-wide analyses show that the histone modification

correlates with replication activity in different cell lines. Acetylation and methylation of Histone H3 and H4 appear to affect replication timing (Workman 2006; Eaton et al. 2011). H4K20me1 and H3K27me3 may be associated with early- and mid-S replication, respectively (Picard et al. 2014).

HBO1 is required for efficient MCM recruitment to origins. The H4 acetylation peaks at G1 phase, consistent with increasing HBO1 activity from M to G1 phase. HAT activity of HBO1 is required for MCM loading. Geminin inhibits DNA replication by preventing the interaction of HBO1 with Cdt1, which enhances HBO1 activity (Iizuka et al. 2006; Miotto and Struhl 2010). Thus, histone acetylation promotes DNA replication by changing condensed chromatin into open chromatin, which may facilitate pre-RC assembly as well as its activation step. Replication timing of an individual origin can be altered by manipulating the localization or activity of histone modification factors. On the other hand, replication timing may affect histone modification. This possibility is also consistent with the first appearance of changes in replication timing prior to the changes of transcription or histone modification in X chromosome inactivation (Keohane et al. 1996; Lande-Diner and Cedar 2009).

1-5 Checkpoint regulation and replication timing control

It was reported in budding yeast that a mutation in the checkpoint kinase *rad53* led to early-firing of late-firing origins in the presence of hydroxyl urea (HU) that prevents replication fork progression due to depletion of nucleotide precursors (Shirahige et al. 1998; Santocanale and Diffley 1998). HU induces fork stall and induces replication checkpoint signaling, which inhibits the firing of late-origins. RFC-Ctf18 was reported to be required for suppression of late origin firing in response to stalled forks (Crabbe et al. 2010). The question here is whether checkpoint regulators are involved in replication timing regulation in the absence of replication stress. In a mutant lacking checkpoint effector kinase Cds1, the replication foci assume a novel distribution that is not present in wild-type cells even in the absence of DNA damage (Meister et al. 2007). This suggested that the checkpoint kinase contributes to replication timing regulation during normal cell growth. In contrast, it was reported that low concentration of HU slows down S phase without changing the order of origin firing (Alvino et al. 2007). Mrc1 was reported be required for this “scaling” of the ordered origin firing in the cells with extended S phase (Koren and Barkai 2010). Mrc1 is known to have both checkpoint-dependent and -independent functions (Szyjka and Aparicio 2005), and it is not known which functions are involved in the origin “scaling”. It was suggested that Mrc1 may suppress firing of weak early-firing origins in a Chk1-independent manner in unperturbed S phase (Hayano et al. 2011; Matsumoto et al. 2011 and in

preparation; see below). Future investigation will be needed to clarify the issue of origin firing regulation by checkpoint in the absence of HU.

1-6. Replication timing and spatial organization of chromatin in nuclei

Chromosomes are organized and packaged in nuclei with characteristic subnuclear positions which will have significant impact on transcription, replication, recombination and repair. This sort of nuclear chromatin architecture is related to chromatin loop networks that are generated by tethering of distant chromosome loci as well as by interaction of chromatin with particular nuclear structures (Cremer et al. 2006). In general, inactive and closed chromosomes are located at nuclear periphery and active and open chromatin is at the interior of nuclei.

DNA replication is spatially regulated. It has been known that locations of DNA replication within nuclei change during S phase; many fine foci spreading all over nuclei in the early-S, discrete foci at the nuclear periphery and around nucleoli in mid-S, and bigger foci that overlap with heterochromatic regions in late-S (Dimitrova and Berezney 2002). In budding yeast, the telomere region is deacetylated and highly condensed. The late-replicating subtelomeric region preferentially occupies the nuclear periphery segments during G1 phase. Thus, mid/ late replication is generally associated with nuclear periphery or other nuclear structures. However, tethering of an early-firing origin to nuclear periphery does not alter replication timing, indicating that the nuclear positioning alone is not enough to dictate replication timing (Heun et al. 2001; Ebrahimi et al. 2010).

Recent analyses revealed genome-wide distribution of replication timing domains in mammalian cells, reinforcing the idea that replication domains are closely correlated with chromatin proximity maps generated by high-C analyses. Thus, spatial organization of chromatin in nuclei is a major determinant for replication timing in mammalian cells. The next question would be what determines the domains and how are they generated?

2. Mechanistic insight into regulation of replication timing

2-1. *cis*-acting sequences that regulate replication timing

In budding yeast, replication origins were first isolated as ARS (autonomously replicating sequence) which includes a 11 to 17 bp consensus sequence (ACS; ARS consensus) that has been shown to be essential for replication initiation (Palzkill and Newlon 1988; Marahrens and Stillman 1992; Theis and Newlon 1997; Chuang and Kelly 1999). The origin recognition complex (ORC) has been identified as a protein complex which binds to ARS through ACS in budding yeast and was later shown to be conserved in other eukaryotes including human (Bell and Stillman 1992). ACS itself cannot regulate replication timing,

since it is present in both early-firing and late-firing origins. Early pioneering studies indicated the presence of another *cis*-acting sequence distal from a replication origin that may determine the timing of origin firing. For instance, ARS1, a well characterized early-firing origin, fired late in S phase, when it was relocated to the position of ARS501 which is located in the subtelomere region and replicates late in the S phase. (Ferguson and Fangman 1992). An element near the late-replicating origin on the chromosome XIV contains a sequence contributing to its late replication. This element can convert an early-firing origin from other locus to late-firing (Friedman et al. 1996). In fission yeast, late-consensus sequence (LCS) and tandem telomeric repeat are found to be present close to late replication origins and are sufficient for suppression of firing of origins placed nearby (Yompakdee and Huberman JA 2004).

In Drosophila, an element named ACE3 is selectively bound by ORC and is required in *cis* for activation of DNA replication at Ori-β and at nearby origins (Austin and Bell 1999; Beall et al. 2002; Zhang and Tower 2004). Deletion of a 13.5kb segment from the intergenic segment 3' to the Chinese Hamster DHFR (dihydrofolate reductase) gene results in complete loss of early replication at a distal origin (Kalejta et al. 1998). Mammalian α-globin locus is replicated in late S phase in non-erythroid cells, but is replicated early in erythroid cells. Replication timing is developmentally regulated through the locus control region (LCR), a 16 kb segment that is located 6 to 22 kb upstream of β-globin gene and is important for DNase-I resistance at the β-globin locus. LCR is sufficient to delay replication timing and change gene expression but not to trigger chromatin modification (Forrester et al. 1990; Simon et al. 2001; Feng et al. 2005). The *cis*-acting sequence does not act as an origin but is necessary to recruit DNA replication initiation factors. Cells might mark late replication origins on the chromosome through histone modification and nucleosome condensation that regulate gene expression in advance.

2-2. Factors affecting replication timing

Recent studies identified potential regulators that may regulate replication timing by recognizing the *cis*-regulatory sequences. Telomeres in yeasts are replicated very late in spite of the presence of a large amount of bound Mcm proteins. A number of proteins bind to telomere and subtelomere regions to regulate telomere lengths and its functions. Ku is one of these telomere binding proteins and its inactivation in yeast leads to shortening of telomere as well as activation of pre-RC located within the 80kb segment from the telomere end early in S phase (Cosgrove and Donaldson 2002). *pif1* mutation could restore both telomere length and its late replication in the *yku70*Δ background, suggesting that Ku regulates replication timing through size of the telomere. Origins on the arm segments were

not affected in *yku70*Δ cells.

A conserved telomere binding protein Rif1 was shown to regulate DNA replication timing not only at telomeres but also along the entire chromosomes (Kanoh and Ishikawa 2001; Hayano et al., 2012). Rif1 was rediscovered as a bypass mutant that could restore the growth of *hsk1*Δ cells. Not only the dormant origins in the subtelomere regions but also late/ dormant origins on the chromosome arms are extensively deregulated in *rif1*Δ cells in the presence of HU. Rif1 does not affect the pre-RC formation but inhibits loading of Cdc45 onto pre-RC at late/ dormant origins. LCS-like sequences were found near the Rif1 binding sites. It would be interesting to examine whether this sequence is involved in chromatin binding of Rif1 (Yompakdee and Huberman 2004).

Rif1 is conserved in evolution, but its roles in telomere regulation are not obvious in higher eukaryotes (Silverman et al. 2004). Further studies showed that Rif1 protein has a major impact on replication timing domain structures in mammalian cells as well. Chromatin binding pattern of Rif1 closely resembles foci pattern of mid-S replication, localizing at nuclear periphery and around the nucleoli. It tightly binds to nuclease-insoluble nuclear structures at the end of M phase and stays bound all through the interphase. Thus, decision for replication timing appears to be made at the end of M phase/ early G1. Rif1 was shown to affect the chromatin loop sizes. Thus, it might regulate replication timing through dictating subnuclear chromatin positioning (Yamazaki et al. 2012). Rif1, in conjunction with topo II-mediated chromatin resetting at M phase (Lemaitre et al. 2005), may generate mid-S replication chromatin domains by tethering chromosomes at nuclear periphery or at nucleoli periphery, which would be refractory to actions of initiation factors until the mid-S phase.

Another telomere binding protein, Taz1, binds to selected arm segments by recognizing a telomere-like sequence, GGTTAC and its tandem repeats, and suppresses firing of selected late-firing/ dormant origins in fission yeast. The effect of Taz1 on replication timing is dependent on Rif1 protein (Tazumi et al. 2012). It is interesting to note that telomere binding factors function on the chromosome arms to regulate the timing of origin firing.

2-3. Novel mechanisms of regulation of replication timing

Mrc1, an adaptor protein for replication checkpoint, is essential for replication checkpoint, and loss of *mrc1* results in activation of late/ dormant origins. This is exemplified also by the fact that *mrc1*Δ can bypass the requirement of *hsk1* (cdc7 homologue of fission yeast) (Matsumoto et al. 2011). Checkpoint-deficient mutant of *mrc1* (*mrc1-3A*) as well as *cds1*Δ (downstream effector kinase) can activate late origins in the presence of HU, and also can weakly suppress *hsk1*Δ. The suppression of *hsk1*Δ by *mrc1-3A* is not as efficient as by

*mrc1*Δ, suggesting the presence of the checkpoint-independent pathway for suppression of *hsk1* mutation (Matsumoto et al. 2011). Indeed, a mutant *mrc1* was identified that is checkpoint-proficient but can rescue *hsk1* mutation. In this mutant, enhanced firing at weak early-firing origins are observed. Mrc1 binds specifically to early-firing origins before the firing event by Hsk1 kinase, and thus, it was proposed that Mrc1 may mark the early-firing origins and exerts inhibitory functions on origin firing (Hayano et al., 2011; Matsumoto et al, in preparation).

Fkh1 (Forkhead homolog 1) and Fkh2 regulate expression of the genes related to cell cycle at G2-M phase (e.g.CLB2) by binding their promoters. Fkh1/2 were shown to be required for establishing early replication of clusters of early-firing origins (Murakami et al. 2010; Knott et al. 2012). They bind to these replication origins through interacting with ORC. Fkh1/2 may alter chromatin configuration of the early-firing origins by tethering these clusters of origins, which will increase the local concentration of limiting initiation factors and facilitate the early-firing at these origins.

It was recently reported that budding yeast and fission yeast Rif1 proteins interact with PP1 phosphatase through its PP1 interacting motifs. The recruitment of PP1 counteracts the Cdc7-mediated phosphorylation of Mcm on chromatin and inhibits the firing of nearby origins. It can also explain how loss of Rif1 partially restores the defect caused by Cdc7 mutation (Hayano et al. 2012; Hiraga et al. 2014; Dave et al. 2014; Mattarocci et al. 2014; Peace and Aparicio 2014). It would be conceivable that Rif1-mediated generation of specific chromatin architecture may assist the positional effect of recruited phosphatase.

3. Physiological functions of replication timing

Replication timing may directly or indirectly affect gene expression through its effect on localization of transcription factors on the chromosome or histone modification. This suggests a possibility that DNA replication timing may play a role in regulating differentiation/development as well as the pathogenesis of some diseases. Indeed, it has been reported that replication timing is distinct between different cell lines and cancer samples from different leukemia patients (Amiel et al. 1998). The human β-globin domain spans over a 200 kb segment and its replication timing changes during development (Aladjem et al. 2002). Alteration of DNA replicating timing is observed prior to drastic transcriptional shift at early-epiblast stage in mouse ES cells (Kitsberg et al. 1993; Simon et al. 2001; Hiratani et al. 2010). It was suggested that manipulation of replication timing could be exploited to inhibit abnormal growth of cancer cells by altering chromatin architecture or expression profiles (Amiel et al. 2001; Dotan et al. 2008; Korenstein-Ilan et al. 2002; D'Antoni et al. 2004). Also, DNA replication timing could be a more sensitive bio-marker for

cancer and other diseases (Allera-Moreau et al. 2012; Ryba et al. 2012).

However, fission yeast *rif1*Δ cells grow almost as normally as the wild-type cells, and they are resistant to various DNA damaging agents or replication stress, including MMS, UV and HU, in spite of the fact that replication timing regulation is grossly altered in this mutant (Hayano et al. 2012). Human cells depleted of Rif1 exhibit DNA stress, as exemplified by the phosphorylation of Chk1. Thus, temporal regulation of early and late origin firing may contribute to the maintenance of genomic stability. Nevertheless, they can complete S phase with almost normal duration in the absence of Rif1. These facts indicate that replication timing, although under regulation through conserved mechanisms, can be perturbed to a significant extent without losing viability. In contrast to the pre-RC formation that is regulated very strictly to permit once and only once replication and is absolutely essential for DNA replication, replication timing regulation is far more relaxed and adaptive to the intra- and extra-cellular conditions. It would be lethal for the cells if DNA synthesis is aborted before completion. Thus, cells are programmed to complete S phase, once they have made a commitment to initiate DNA replication. The presence of the pre-RCs on the genome far excess in number over what is actually utilized would be manifestation of how eukaryotic cells cope with the “emergency” that may threaten the completion of S phase (Aguilera and Garcia-Muse 2013; Santocanale and Diffley 1998; Shechter and Gautier 2004).

Then, why DNA replication timing program? We think that the replication timing is installed as a consequence of chromatin regulation that facilitates the chromosome transactions needed to go through S phase. Transcription usually facilitates initiation of DNA replication, thus replication and transcription regulation may be locally coupled, which may be achieved by specific chromatin architecture generated by factors such as Rif1. The chromatin architecture would also affect potential coregulation of DNA replication and repair/ recombination. In fact, Rif1 facilitates NHEJ (non-homologous end-joining)-mediated DSB repair through 53BP1 (Silverman et al. 2004; Callen et al. 2013; Zimmermann et al. 2013). These systems are not essential for survival at a cellular level, but obviously play a crucial role in development of embryos into organs and tissues, as indicated by the phenotypes of Rif1 knockout mice. They are deficient in gene rearrangement in B cells, and display abnormal gene expression in ES cells, and also in developing fertilized eggs (Yamazaki et al.; Yoshizawa et al., unpublished data). Abnormal DNA replication program could contribute to tumorigenesis in a long term, since the presence of replication stress is the first step for cancer cell development (Bartkova et al. 2005; Bartkova et al. 2006; Ryba et al. 2012; Allera-Moreau et al. 2012). The replication timing regulation is known to be related to frequency of mutagenesis. Late replicating segments generally have higher mutation

frequency (Postnikoff et al. 2012; Chen et al. 2010; Pink and Hurst 2010). Thus, abrogation of replication program could potentially lead to increased mutagenesis. These issues need to be experimentally evaluated in the future.

Concluding remarks

Two aspects on regulation of eukaryotic DNA replication should be noted. A very strict one applies to achieve "once and only once replication", since re-replication can immediately cause genomic instability and needs to be strictly avoided. On the other hand, a relaxed regulation operates during S phase. The temporal and spatial program of genome replication is under regulation of chromatin context, chromatin architecture, transcription, availability of factors and materials (nucleotides), cellular environment and so forth. Once cells commit to DNA replication, there is no return. They need to complete DNA synthesis, otherwise they cannot survive. Thus, abrogation of replication program has minimal effect on progression of S phase. This is because cells are equipped with excess numbers of replication origins and only a subset is utilized and others are backups for emergency. Nevertheless, genomes are replicated under a program predetermined for each cell (Figure 1). Replication timing regulation may be a result of chromatin organization which cells adopt to maximize the efficiency and accuracy of nuclear transactions occurring on the chromosomes under each physiological and developmental condition.

Legend to figure

Figure 1. Regulation of DNA replication timing

DNA replication timing is coordinated by various different cellular functions such as transcription, histone modification, chromatin architecture and subnuclear positions as well as through competition for limited factors. (a) The numbers of some essential replication factors (e.g. Cdc7-Dbf4, Sld3, Cdc45 and Sld7) are less than that of replication origins and thus the competition for limited replication factors can be a determining element for replication timing. (b) and (c) Active transcription and histone modification affect replication timing. (d) Subnuclear positions of origins in nuclei, which may be specified at TDP (late M/ early G1) in each cell cycle, can be a determinant for the temporal and spatial regulation of replication timing in the next S phase. Rif1 could be a major mediator of this regulation. (e) *cis*-acting elements such as locus control region (LCR), late consensus sequence (LCS) or telomere-like sequence may be located near late-firing or dormant replication origins. In fission yeast, telomere binding factors (Rif1, Taz1) may recognize these sequences to regulate firing of some late/ dormant origins. In mammals, Rif1 may generate specific chromatin architecture at particular subnuclear locations through its ability to generate chromatin loops that may be closely related to replication timing domains. Rif1 may also recruit other factors (e.g. phosphatase) to counteract the actions of kinases essential for initiation. (f) A transcription factor (e.g. Fkh1/2) associates with subsets of early-firing origins and promotes early-firing by inducing their clustering. Black arrows indicate suppressive effect on origin firing, whereas the red arrow indicates stimulatory effect.

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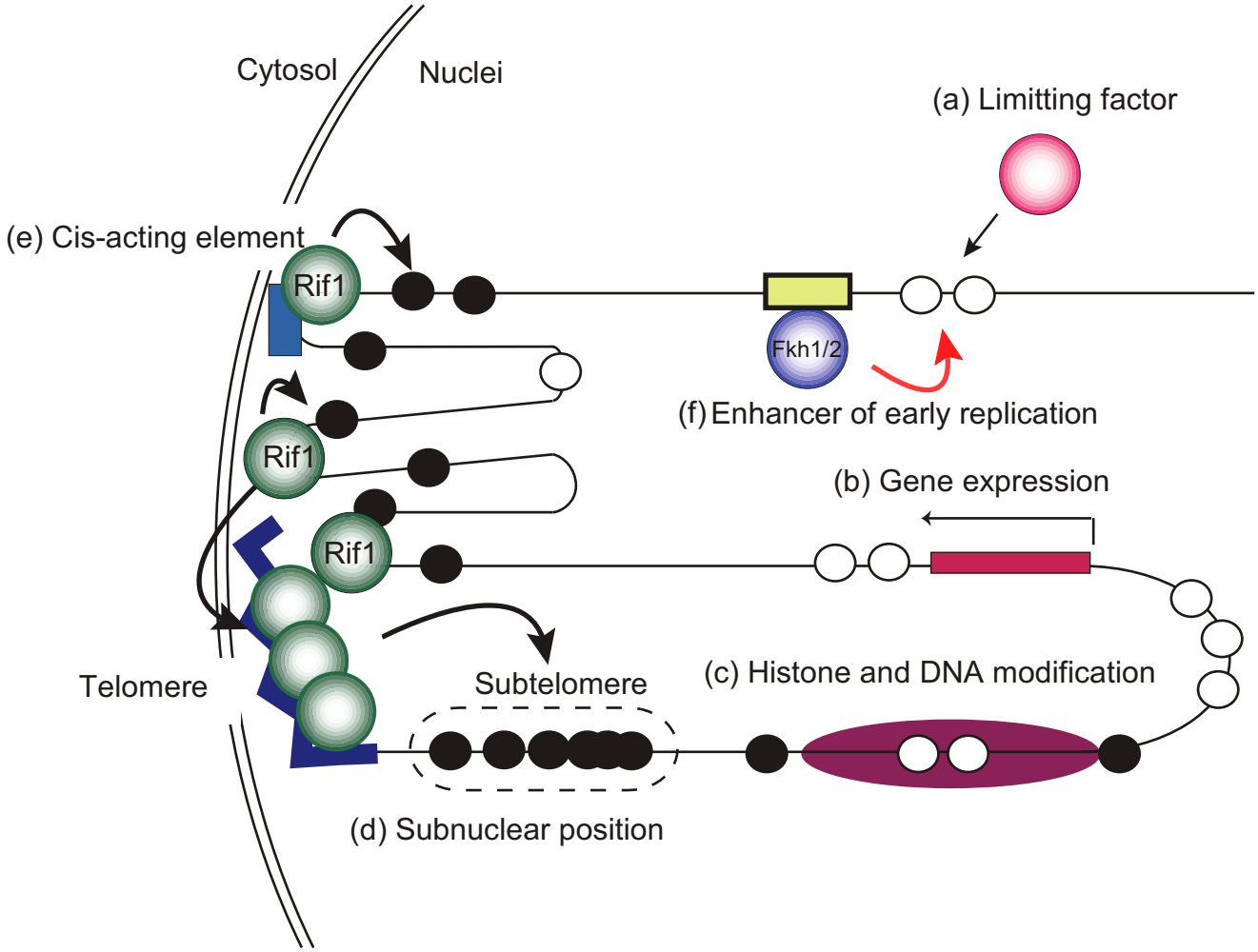

Figure 1 Hayano et al.