Rif1 choreographs DNA replication timing

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Eukaryotic cells duplicate their genome in a pre-determined order that appears to reflect a fundamental property of chromatin. Each chromosomal region replicates at a consistent, developmental- and tissue-specific time during the S phase of the cell cycle, and regions that replicate at the same time form distinct patterns in three-dimensional nuclear space. Although orderly progression of DNA replication is important for insuring stable genetic and epigenetic inheritance, the mechanisms underlying replication patterns have yet to be elucidated. Two reports in *The EMBO Journal* now identify a protein, Rif1, as a novel global determinant of the mammalian DNA replication program, and provide a link between higher order chromatin assembly and proper cell-cycle progression.

Shortly after cell division, cells organize their genomes into large (> 1 Mb) ‘replication domains’ containing multiple replication initiation sites that replicate at the same time, share distinct chromatin conformations and localize to discrete chromatin-interaction compartments (Hiratani *et al*, 2009;

Ryba *et al*, 2010; Yaffe *et al*, 2010). Replication domain organization occurs during the G1 phase of the cell cycle at the so-called timing decision point, coincident with chromosome positioning. Early replicating chromatin primarily resides internally in the nucleus, commonly exhibits a decondensed (‘open’) conformation and includes active genes. Late-replicating chromatin resides in the nuclear periphery, is often densely packaged and primarily contains transcriptionally inactive genomic regions. During differentiation, transcriptional silencing frequently correlates with replication delay and transcriptional activation correlates with advanced replication timing (Hiratani *et al*, 2009; Eaton *et al*, 2011). The division into replication timing domains and the tissue specificity of replication timing exhibits high evolutionary conservation in syntenic regions (Ryba *et al*, 2010; Yaffe *et al*, 2010). Evolutionary conservation of replication patterns might reflect a role for distinct temporal and spatial order of replication in facilitating epigenetic inheritance by insuring correct packaging of DNA after replication. For example,

Figure 1 Rif1 binds chromatin during the G1 phase of the cell cycle. Rif1 binding occurs concomitant with the binding of the pre-replication complex (purple) and recruitment of replicative helicase components (blue), but the two binding events are independent of each other. In early replicating chromatin regions, Rif1 binding does not interfere with the activation of DNA replication by S phase-specific kinases acting on the replicative helicase component Cdc45. Replication therefore can occur early during S phase with the two DNA strands becoming separated by the activated helicase. By contrast, Rif1 bound to late-replicating chromatin domains prevents activation of DNA replication by S-phase kinases, possibly by interfering with the interaction of Cdc45 with chromatin. When Rif1 is absent, the replication timing program is disrupted. As proposed by Yamazaki *et al* (2012) (illustrated in Figure 6 of that publication), Rif1 might delay replication by facilitating the formation of tight nuclear matrix-attached loops in heterochromatin.

DNA methyltransferases and histone methylases, which are often more abundant in late S phase, might play a role in packaging of late-replicating heterochromatin. In accordance, histone modifying enzymes and transcription factors can indeed alter replication timing of some mammalian chromosomal regions (Hiratani *et al*, 2009; Demczuk *et al*, 2012) and inhibition of histone acetylation can alter the replication program and induce replication stress (Priour *et al*, 2011 and references therein). However, histone modifiers affect only a subset of replication domains and understanding how DNA replication is choreographed in space and time requires identifying the global determinants linking chromatin structure and replication timing.

Rif1, originally identified in yeast as a telomere binding protein, was recently shown to affect the replication timing program in fission yeast. Rif1 deficiency advanced the replication time of some genes that normally replicated late, suggesting that Rif1 confines early replication to a subset of genomic regions and delays the replication of other regions (Hayano *et al*, 2012). Continuing that study, Yamazaki *et al* (2012) now show that human Rif1 also affects spatial and temporal replication patterns. Human Rif1 associates with nuclear scaffold structures during interphase and binds chromatin tightly after mitosis, concomitant with the replication timing decision point. In Rif1-depleted cells, overall replication patterns in the nucleus (visualized as 'replication foci') were rearranged, eliminating the distinct spatial pattern typically associated with replication in mid-S phase. Mapping replication timing in a 42-Mb region of chromosome 5 revealed that replication order was disturbed, with delays in some domains and advanced timing in others. Interestingly, Rif1 depletion in S-phase cells did not prevent the initiation of DNA replication—on the contrary, more replication initiation events were observed, and some proteins involved in the initiation of DNA replication, including the replicative helicase component Cdc45, exhibited increased association with chromatin.

Cornacchia *et al* (2012) used murine cells to investigate the function of Rif1. In mice, Rif1 is an essential protein involved in DNA repair (Buonomo *et al*, 2009). Depletion of Rif1 in murine fibroblasts prevented many cells from starting replication, and fibroblasts that did enter S phase exhibited overall rearrangements of replication domains similar to those observed in human cells by Yamazaki *et al* (2012). Importantly, Cornacchia *et al* (2012) analysed replication timing for the entire genome to reveal an overall fragmentation of replication domains in Rif1-depleted fibroblasts. Such cells exhibited numerous small replication domains, resembling the domains typically observed in stem cells during early embryogenesis. In contrast, fibroblasts with intact Rif1 normally exhibit fewer, consolidated replication domains. The altered structure of replication domains therefore suggests that Rif1 depletion disrupts a pattern established during differentiation. Cornacchia *et al* (2012) also determined the subnuclear location of tagged Rif1 in relation to the spatial patterns of DNA replication. Similarly to the observations of Yamazaki *et al* (2012), this showed that mouse Rif1 exhibits a focal distribution. Rif1 foci do not localize to sites of active replication but rather appear to precede and anticipate replication foci. Consistent with this, Rif1 depletion in murine cells also results in abnormal replication foci patterns. Together the two papers therefore

strongly support the hypothesis that Rif1 establishes the timing program by delaying the replication of specific groups of chromatin regions.

Combined observations from both papers suggest that Rif1 affects higher order chromatin structure. Both studies report an attachment of Rif1 protein to a nuclear scaffold structure during interphase. In the hands of Yamazaki *et al* (2012) Rif1 depletion resulted in larger chromatin loops, and Cornacchia *et al* (2012) found that it altered chromatin assembly patterns. Notably, earlier studies demonstrating that mitotic remodeling of chromatin loops modulates the efficiency of DNA replication might suggest that higher order chromatin structure acts upstream of replication timing, but other studies support an association between replication fork speed during S phase and the size of chromatin loops in the next G1 phase (Courbet *et al*, 2008 and references therein). Since Rif1-depleted cells also exhibit slow replication, possibly due to a DNA repair defect (Xu *et al*, 2010), it is possible that Rif1 primarily affects replication, and that the size of chromatin loops is a downstream consequence of the replication phenotype. Regardless of the primary defect, Rif1 can clearly provide a bridge between the spatial and temporal features of the replication program and higher order chromatin structure.

How does Rif1 link the nuclear scaffold, chromatin assembly and replication timing? DNA replication starts when components of pre-replication complexes, which bind chromatin right after mitosis, are phosphorylated by S phase-specific kinases to recruit additional proteins and trigger helicase activity. One attractive possibility is that Rif1 binds nuclear scaffolds and reduces chromatin accessibility of later replicating chromatin domains, thereby delaying their interactions with components of the replication machinery (e.g., Cdc45) to prevent premature replication (Figure 1). This hypothesis is supported by the observed higher abundance of Cdc45 on Rif1-depleted chromatin, and is consistent with recent studies in yeast suggesting that overexpressing components of the pre-initiation complex is sufficient to advance replication time (Mantiero *et al*, 2011; Tanaka *et al*, 2011). Further insights into the molecular basis of Rif1 actions might be obtained by determining if, how and when Rif1 interacts with components of pre-replication complexes that modulate DNA replication, as well as with proteins such as cohesin, which colocalizes with replication initiation sites and affects chromatin loop size (Guillou *et al*, 2010). Since distinct DNA elements can affect replication timing of individual loci (Fu *et al*, 2006) and can alter the replication timing of whole chromosomes (Stoffregen *et al*, 2011), it will be interesting to ask if Rif1 binds those elements, as well as matrix attachment sites, locus control regions and replication initiation sites. Detailed, genome-level mapping of Rif1 association with distinct structures on chromatin might also help characterize the behaviour of those recently described chromatin components that apparently deviate from the general rule linking early replication with chromatin condensation (Takebayashi *et al*, 2012).

Although the advantage of a consistent replication program is not immediately evident, changes in replication timing are frequent in blood cancers and accompany several inherited disorders, and the temporal order of chromosome replication affects somatic mutation rates and determines the frequency of cancer-related somatic copy number alterations.

Chromosomal translocations associated with chromosome-wide replication delays and gross chromosomal rearrangements are found frequently in cancers. Understanding the determinants of replication timing and their interactions with the replication machinery using Rif1 as a tool can therefore open an interesting and productive avenue for further investigation.

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Conflict of interest

The author declares that she has no conflict of interest.

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