Indian J.L.Sci.2(1) : 153-157, 2012

Review

RIF1: THE NOVEL REPLICATION REGULATOR IN YEAST AND MAMMALS

DHIRENDRA KUMAR SINGH[1]

School of Environmental Studies,National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Kochi-Kerala

[a]E-mail: dhirendrabhadauria03@gmail.com

Rif1 (Rap1-interacting factor 1) Rif1, as a novel global determinant of the mammalian DNA replication, and provide a link between higher order chromatin assembly and proper cell-cycle progression. Rif1 originally identified as a telomere-binding factor in yeast, is a critical determinant of the replication timing programme in human cells .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. Study now show, human Rif1 also affects replication patterns. Human Rif1 is associated with nuclear scaffold structures during interphase and binds chromatin tightly after mitosis, concomitant with the replication timing decision point. RNA interference of Rif1 decreases the efficiency of homology directed repair (HDR). Rif1 tightly binds to nuclear-insoluble structures at late M to early G1 and regulates chromatin-loop sizes. Thus, Rif1 establishes the mid S replication domains that are restrained from being activated at early-S-phase. Rif1 plays crucial roles in determining the replication timing domain structures in human cells through regulating higher-order chromatin architecture.

KEYWORDS : Rif1, Heterochromatin, DNA replication, Homologous recombination, Checkpoint, Taz1, Rap1, ATR, cdc25

Abbreviations

(a) DSBs, double-strand breaks;
(b) HR, homologous recombination;
(c) RNAi , RNA interference;
(d) HP1, heterochromatin protein 1;
(e) HC, heterochromatin;
(f) cHC, constitutive heterochromatin;
(g) RAP1 repressor activator protein 1;
(h) ATR, Ataxia Telangiectasia and Rad3-related kinase;
(i) ACS, ARS consensus sequence;
(j) ARS, Autonomously replicating sequence;

Why do some replication origins fire early while others fire late? This fascinating study by Hayano et al.,(2012) brings us much closer to answering this important question. The results show that Rif1 a fission yeast telomere-associated protein binds to chromosome arms and is essential for the early replication of some origins and the late replication of others. Replication of genomic DNA occurs only once in a cell cycle, and its accuracy ensures genome integrity. As in prokaryotes, eukaryotic DNA replication is also believed to initiate at specific genomic loci called replication origins (ori). Origin of replication in budding yeast are known to be associated with a specific 11-base-pair (bp) 5'-T/ATTTAYRTTTT/A-3' sequence(where Y is pyrimidine and R is purine) termed ACS [(ARS-autonomous replicating consensus sequence). Studies in yeasts showed that chromatin structures (histone modification) near the origins can affect the firing efficiency. The checkpoint(G1, G2 and metaphase) also regulates the sites and timing of firing. Dormant or late origins are activated in the absence of checkpoint regulators - cyclins and cdk . The checkpoint represses late origins also in mammalian cells. However, factors that determine the genome-wide replication programme or replication domain structures are still elusive. The mechanisms of dramatic reorganization of replication domains during differentiation are not clear. Study show that Rif1 was originally identified in budding yeast as a Rap1 interacting protein may selectively bind to not only telomeres, but also arm regions, and may regulate the sites and timing of origin firing throughout chromosomes in fission yeast. In mammalian cell it is identified that Rif1 acts as a double-strand break (DSBs) response factor. In addition, Rif1 had a very important role during fork re-start downstream of ATR - Ataxia telangiectasia and Rad3-related kinase (Buonomo et al.,2009). Through studying this key regulator, we can therefore provide an insight into the links between replication-timing control, chromatin organization and genome stability.

Rif 1 as Replication Time Regulator

Shortly after cell division (after cytokinesis), cells organize their genomes into large (>1 Mb) 'replication domains' containing multiple replication initiation sites (Initiation of DNA replication occurs at multiple genomic

[1]**Corresponding author**

loci),that replicate at the same time, share distinct chromatin conformations and localize to discrete chromatin-interaction compartments. Replication domain (Takebayashi et al.,2012) organization occurs during the G1 phase of the cell cycle at the so-called timing decision point, coincident with chromosome positioning. The DNA replication programme has been shown to be regulated by the local chromatin environment. Although progress has been made in establishing that chromatin modifications impact the activation of replication origins, 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. Histone modifying enzymes and transcription factors can indeed alter replication timing of some mammalian chromosomal regions; and inhibition of histone acetylation can alter the replication program and induce replication stress. 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 the study, 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 (Prieur et al., 2011) .

In S. cerevisiae, Rif1 gene transcription displays periodic fluctuation through the cell cycle, peaking during S phase. In addition, Rif1p protein association with telomeric chromatin changes during the cell cycle, being minimal at the beginning of S and G1 phase and maximal at mid/late S and G2 phase, and decreasing around mitosis 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.

Studied that 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.. 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. Two papers Yamazaki et al., 2012, Cornacchia et al. ,2012, strongly support the hypothesis that Rif1 establishes the timing program by delaying the replication of specific groups of chromatin regions and also by affecting higher order chromatin structure. Both studies report an attachment of Rif1 protein to a nuclear scaffold structure during interphase.

Earlier studies demonstrating that mitotic remodelling 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. Since Rif1-depleted cells also exhibit slow replication, possibly due to a DNA repair defect, 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 programme 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

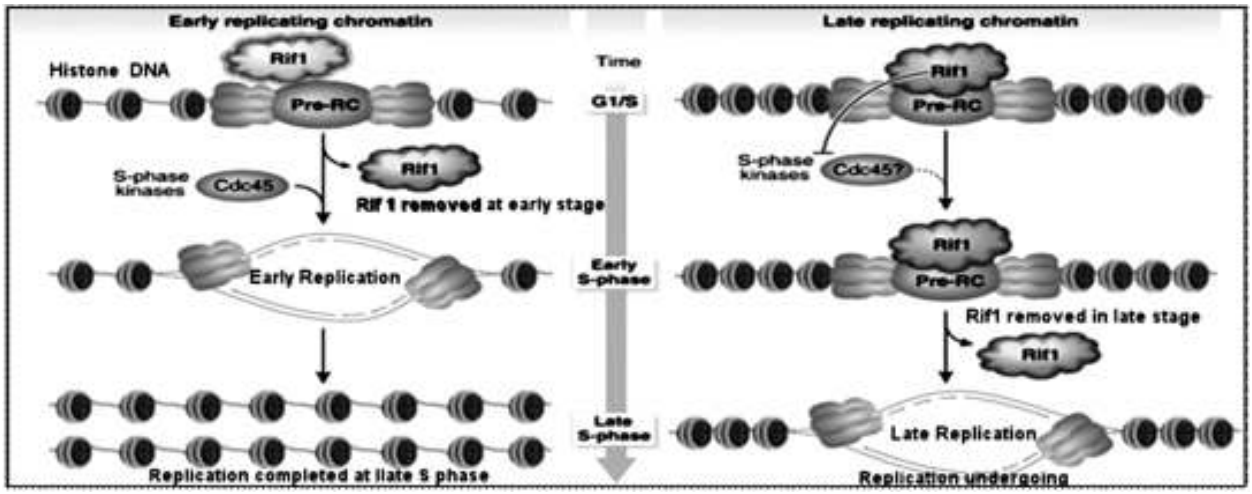

Figure 1: Rif1 binds chromatin during the G1 phase of the cell cycle. Rif1 binding occurs concomitant with the binding of the pre-replication complex (purple) and recruitment of replicative helicase components (blue), but the two binding events are independent of each other

components of the replication machinery (e.g., Cdc45) to prevent premature replication (Figure1). 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 initiation complex is sufficient to advance replication time. 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. Since distinct DNA elements can affect replication timing of individual loci and can alter the replication timing of whole chromosome.

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.

Rif1 regulators of replication in telomeres

Chromosome ends assemble specialized nucleoprotein structures, known as telomeres, that prevent fusion and attrition of chromosome ends. Telomeric DNA consists of simple sequence repeats, TTAGGG in vertebrates and closely related sequences in most other eukaryotes (e.g. TTAC(A)G16 in fission yeast), terminating in a 3' overhang of the G-rich strand. The telomere repeats recruit sequence-specific binding proteins and proteins that bind to telomeres through proteinprotein interactions. Together, these constituents form the telomere 'cap', the structure that inhibits inappropriate recombinational, nucleolytic and end-joining activities, all of which profoundly disrupt genomic integrity if allowed at telomeres. In addition, telomeres recruit and control telomerase, the ribonucleoprotein enzyme that synthesizes telomeric DNA repeats.

The fission yeast ortholog of human TRF1/2, Taz1 binds telomeric DNA (Figure,2) and regulates numerous aspects of telomere function (Hayano et al.,2012) . In fission yeast, Rif1 binds to the dsDNA in telomeres through interaction with Taz1 (Chikashige and Hiraoka , 2001). Rif1 is required for telomere maintenance in a Taz1-dependent manner (Kanoh and Ishikawa, 2001), Although its role in telomere maintenance is minor, and

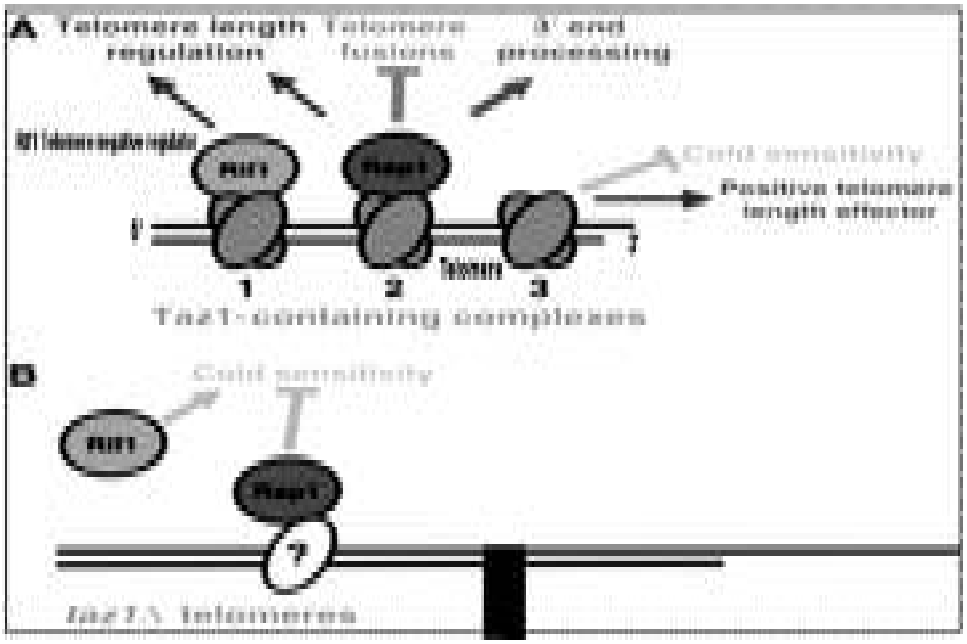

Figure 2: Summary of genetic analysis of Rap1, Rif1 and Taz1. (A) Taz1-containing telomere complexes. Taz1 organizes at least three functional telomere complexes

Rif1 shows diffuse nuclear localization even in Taz1. Rif1 as the first key component of the molecular machinery that determines the replication timing programme in mammalian cells. As for Rap1 (Pardo and Marcand , 2005) , Rif1 deletion causes telomere hyper-elongation, suggesting that Rif1 is a telomerase negative regulator. The mechanism by which Rif1 participates in telomere homoeostasis remains unclear. However, it was recently published that budding yeast Rif1 regulates telomere replication timing by temporally restricting telomerase access. In fission yeast, Rif1 does not bind Rap1, but still interacts with telomeres and participates in their length regulation.

In human cells, Rif1 localizes to midzone microtubules between dividing chromosomes during anaphase, and to chromosomes during telophase. This localization is compatible with a role for Rif1 in controlling resolution of entwined chromosomes by topoisomerase II. Alternatively, Rif1 may act indirectly by controlling transcription of telomere maintenance genes.

CONCLUSION

Now it is clear that Rif1 play a role in replication timing, protect and maintain telomers by binding with telomeric proteins Taz1 and Rap1.It is also shown that mammalian Rif1 contributes to replication stress survival and homology-directed repair mechanism. Rif1 deficiency leads to failure in embryonic development, and conditional deletion of Rif1 from mouse embryo fibroblasts affects S-phase progression, rendering cells hypersensitive to replication poisons. In cancer cells it is also shown depletion of Rif1 by RNAi impaired cell growth.

ACKNOWLEDGMENTS

I would like to thank all the members of NCAAH, in particular Prof. I.S Bright Singh, Dr.Valsamma Joseph, Dr. T.P Sajeevan, and Jayesh P. for their guidance. I am also grateful to Mantosh Kumar, Neelanchal Vaid , Snigdha Gupta, Ayesha, (M.Tech in Marine Biotechnology at NCAAH), and Dheeraj Singh (M.Tech Biotechnology at BITS Ranchi) for comments and discussion.

REFERENCES

Buonomo S.B., Wu Y., Ferguson D.and de Lange T.,2009. Mammalian Rif1 contributes to replication stress survival and homology- directed repair. J Cell Biol., **187**: 385-398.

Cornacchia D., Quivy J.P., Foti R.,Tili F.,Santarella-Mellwig R. Anthony C. and Almouzni G. ,2012. Mouse Rif1 is a key regulator of the replication-timing programme in mammaliancells. EMBO, J. **31**: 3678-3690.

Chikashige Y.and Hiraoka Y (2001) Telomere binding of the Rap1 protein is required for meiosis in fission yeast. Curr Biol., **11**: 1618-1623.

Hayano M., Kanoh Y., Matsumoto S. Renard-Guillet C., Shirahige K., Masai H. ,2012. Rif1 is a global regulator of timing of replication origin firing in fission yeast. Genes Dev., **26**: 137-150.

Kanoh J.and Ishikawa F. ,2001. spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. Curr Biol .,**11**: 1624-1630.

Pardo B. and Marcand S. 2005. Rap1 prevents telomere fusions by non-homologous end-joining. EMBO J.Prieur A., Besnard E., Babled A., Lemaitre J.M.,2011. p53 and p16 (INK4A) independent induction of senescence by chromatin-dependent alteration of S-phase progression. Nat Commun ,**2**: 473.

Takebayashi S.I., Dileep V., Ryba T., Dennis J.H. and Gilbert D.M.,2012. Chromatin-interaction compartment switch at developmentally regulated chromosomal domains reveals an unusual principle of chromatin folding. Proc Natl Acad Sci USA ,109: 12574-12579.

Yamazaki S., Ishii A., Kanoh Y., Oda M., Nishito Y. and Masai H.,2012.Rif1 regulates the replication timing domains on the human genome. EMBO, J. **31**: 3667-3677.