Cell Cycle 11:24, 4492–4493; December 15, 2012; © 2012 Landes Bioscience

Telomere-binding proteins play roles in control of replication timing

Atsutoshi Tazumi and Hisao Masukata*

Department of Biological Sciences; Graduate School of Science; Osaka University; Toyonaka, Osaka, Japan

In the S phase of the eukaryotic cell cycle, DNA replication initiates at a number of fixed loci, called replication origins, to achieve faithful duplication of the large genome. However, replication origins do not fire simultaneously at the onset of S phase, but do so at distinct time points under a pre-fixed program that is common from yeast to metazoans.[1,2] In mammalian cells, the replication program is established within a specific time window, the timing decision point (TDP) in G_1 phase[3] and is thought to be coupled with gene expression during development.[4] Although chromatin structures and nuclear localizations are considered to play roles in such timing control, details of the mechanisms involved have been enigmatic.

The fission yeast *Schizosaccharomyces pombe* is a suitable model organism with which to study the mechanism of replication timing control, because all its early and late/dormant replication origins have been determined, and its chromatin structures have similarities to those in metazoans. Early and late origins are scattered along chromosome arm regions consisting of euchromatin. Among constitutive heterochromatin regions, the pericentromere and the silent *mat* locus replicate early, whereas sub-telomeres replicate later than any other chromosome regions.[5,6] We have shown previously that the heterochromatin protein HP1/Swi6 recruits Dbf4-dependent kinase (DDK) to pericentromeric and *mat* heterochromatin regions to promote early initiation.[7] However, the mechanisms responsible for timing control of early and late origins in euchromatin and sub-telomeres remain unknown.

To elucidate the mechanism of replication timing control in euchromatin regions, we examined whether a late origin is associated with an element that controls late replication timing by translocating a late origin fragment into the proximity of an early origin on the chromosome. By labeling newly synthesized DNA with BrdU, a heavy-density analog of thymidine and then carrying out classical CsCl density gradient centrifugation, we found that the fragment containing late origin *AT2088* repressed early replication of the proximal early origin, suggesting that the *AT2088* fragment contains an element that is functional for replication timing control (RTC).[8]

Using systematic deletions and base substitutions in the *AT2088* fragment, we identified the sequence element essential for RTC activity in the proximal region of the late origin. Disruption of the element at the native *AT2088* locus caused early replication from the locus, showing it is essential for the timing control. Interestingly, the element was found to contain two copies of a telomeric repeat-like sequence. The telomere-binding protein Taz1, a fission yeast counterpart of human TRF1/TRF2, is localized at the native *AT2088* locus, being dependent on telomeric repeats. Deletion of the *taz1*$^+$ gene indicates that Taz1 is required for late replication control of *AT2088*. Taz1 prevents loading of an initiation factor Sld3, which is dependent on DDK (**Fig. 1**). Moreover, genome-wide analyses of replication timing in wild-type and *taz1*Δ cells showed that 26 internal and 46 subtelomeric origins among 156 late/dormant origins are dependent on Taz1. CHIP-seq analysis showed that Taz1 is localized at 16 of the 26 internal late origins. In these cases, localization of Taz1 nearby late origins is required for timing control. In contrast, sub-telomeric origins, where Taz1 is not localized, are also regulated in a Taz1-dependent manner. Taz1 binding to chromosome ends appears to contribute to regulation of subtelomeric origins from a

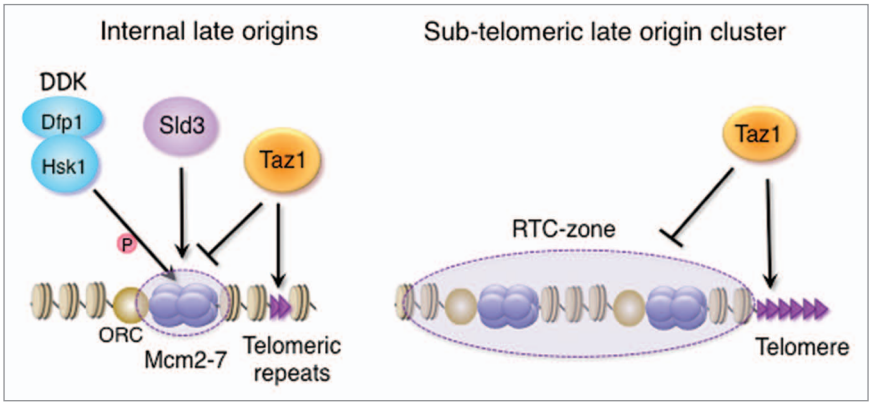

Figure 1. Control of replication timing by Taz1 at internal late origins and sub-telomeric origin cluster. Two copies of telomeric repeat located proximal to the internal late origins recruit Taz1, which prevents DDK-dependent Sld3 loading in early S phase. In sub-telomeres, clustered late origins are collectively regulated (RTC zone) by Taz1, which binds to the telomere.

*Correspondence to: Hisao Masukata; Email: masukata@bio.sci.osaka-u.ac.jp
Submitted: 10/27/12; Accepted: 10/30/12
http://dx.doi.org/10.4161/cc.22727
Comment on: Tazumi A, et al. Genes Dev 2012; 26:2050-62; PMID:22987637; http://dx.doi.org/10.1101/gad.194282.112.

long distance (**Fig. 1**). Furthermore, both Taz1-dependent and Taz1-independent origins require Rif1, which interacts with Taz1 at the telomere, consistent with a recent paper from Masai's group.[9] Rif1 may function in a process closer to that of replication initiation.

Taz1 and Rif1 seem to act as molecular linkers between regulation of telomere length and control of sub-telomeric replication timing. When a telomere becomes shorter, the decrease in telomere-bound Taz1 and Rif1 may cause early replication of sub-telomeric late origins, allowing telomerase to access the ends and elongate telomeric repeats. Our results show that fission yeast uses a set of telomeric sequences and the binding protein Taz1 for regulation of global replication timing. Because telomeric sequences and telomere-binding proteins are highly conserved across eukaryotes, the control mechanism may also be conserved.[10]

References

1. MacAlpine DM, et al. Genes Dev 2004; 18:3094-105; PMID:15601823; http://dx.doi.org/10.1101/gad.1246404.
2. Gilbert DM, et al. Cold Spring Harb Symp Quant Biol 2010; 75:143-53; PMID:21139067; http://dx.doi.org/10.1101/sqb.2010.75.011.
3. Dimitrova DS, et al. Mol Cell 1999; 4:983-93; PMID:10635323; http://dx.doi.org/10.1016/S1097-2765(00)80227-0.
4. Hiratani I, et al. Proc Natl Acad Sci USA 2004; 101:16861-6; PMID:15557005; http://dx.doi.org/10.1073/pnas.0406687101.
5. Kim SM, et al. Genes Dev 2003; 17:330-5; PMID:12569122; http://dx.doi.org/10.1101/gad.1046203.
6. Hayashi M, et al. EMBO J 2007; 26:1327-39; PMID:17304213; http://dx.doi.org/10.1038/sj.emboj.7601585.
7. Hayashi MT, et al. Nat Cell Biol 2009; 11:357-62; PMID:19182789; http://dx.doi.org/10.1038/ncb1845.
8. Tazumi A, et al. Genes Dev 2012; 26:2050-62; PMID:22987637; http://dx.doi.org/10.1101/gad.194282.112.
9. Hayano M, et al. Genes Dev 2012; 26:137-50; PMID:22279046; http://dx.doi.org/10.1101/gad.178491.111.
10. Yamazaki S, et al. EMBO J 2012; 31:3667-77; PMID:22850674; http://dx.doi.org/10.1038/emboj.2012.180.