A stitch in time: Replicate early and escape dosage compensation to express more

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The biological significance of conserved replication timing patterns in eukaryotic genomes remains a mystery. In this issue, Müller and Nieduszynski (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201701061) find that early replication is a requirement for the highest expression levels of certain genes.

Every time a cell divides, its genome needs to be fully duplicated. Eukaryotic cells contain large genomes that are replicated through the activity of hundreds to thousands of replication origins. It has been known for several decades that some genomic regions replicate reproducibly earlier than others, a property that applies both to yeast and to metazoan cells, although on different scales (Fig. 1 A; Rhind and Gilbert, 2013). In yeast such as *Saccharomyces cerevisiae*, early replication regions may range from 20 to 40 kb in length and depend on the activity of individual replication origins which display enhanced capacity of recruiting replication factors (Das et al., 2015). In mammalian cells, replication timing domains extend for hundreds of kilobases to megabases and contain several dozen replication origins, and their time of replication seems to arise from the distribution and stochastic activity of the origins located within these domains (Gindin et al., 2014). Thus, replication timing constitutes an emerging property of eukaryotic genomes, which emanates from the density and affinity for replication factors at origins. A striking feature of replication timing domains is that they are conserved between related species of yeast or between mammalian cell types (Yaffe et al., 2010; Müller and Nieduszynski, 2012). In addition, a large proportion of the mammalian genome is subject to developmentally regulated changes in replication timing that, to a certain extent, correlate with changes in transcriptional regulation (Hiratani et al., 2008). These observations suggest that the temporal order of genomic duplication should have physiological relevance. However, the biological significance of this difference in replication timing has not been elucidated. In this issue, Müller and Nieduszynski conduct a series of elegant experiments involving comparative genomics in phylogenetically diverse yeast species, as well as genetics, to investigate this long-standing question.

The authors start from the assumption that, if the temporal order of gene duplication is important for its function, its specific replication time should be evolutionarily conserved. To identify putative loci with replication timing constraints, they first analyze the replication timing profile of a selection of budding yeast species sufficiently divergent to break the synteny between genes and replication origins. From this, they assign a relative replication timing value to homologous genetic elements from each yeast species and evaluate the observed level of evolutionary conservation in replication time by comparison with a random model. Careful analysis of the evolutionary breakdown in genetic linkage between the elements with conserved replication times allows the authors to identify 185 ancestrally related genetic elements that have been subjected to evolutionary selective pressure for regulated replication timing. Among those, histone genes are the most significantly conserved group of protein-coding genes, replicating early in all species.

To test whether the level of expression of histone genes required during S phase was linked to their early doubling time, Müller and Nieduszynski (2017) beautifully exploited the exquisite temporal organization of *S. cerevisiae* replication, in which early replication regions depend on the activity of individual replication origins. By inactivating the origins of the early replicating region containing the paired genes *HTA1* and *HTB1* (two of the genes encoding for H2A and H2B, residing in chromosome 4), the replication time of the whole region was substantially delayed, resulting in a significant reduction in the expression of both genes. This suggests that early replication is a requirement to achieve appropriate histone mRNA levels during S phase. Importantly, they found that this reduction in transcript levels only affects *HTA1* and *HTB1* genes, but not any of the neighboring genes within the delayed region, exemplifying how discrete genetic entities can be responsible for the conservation of the replication timing of a whole domain.

These findings prompted the authors to make another unexpected discovery. Although gene dosage is transiently unbalanced during DNA replication (particularly between early and late-replicating genes), eukaryotic cells buffer these dosage differences by down-regulating gene transcription on newly replicated DNA to maintain expression homeostasis. It was recently found that, in *S. cerevisiae*, this dosage compensation mechanism occurs through the acetylation of H3K56 by Rett109/Asf1, resulting in reduced transcription efficiency on replicated DNA (Voichek et al., 2016). Now, Müller and Nieduszynski (2017) have found that histone genes, as well as early replicating cell cycle–regulated genes, were excluded from Rtt109-dependent repression. Thus, the early doubling time of these escapee genes will contribute to their maximum expression during S phase.

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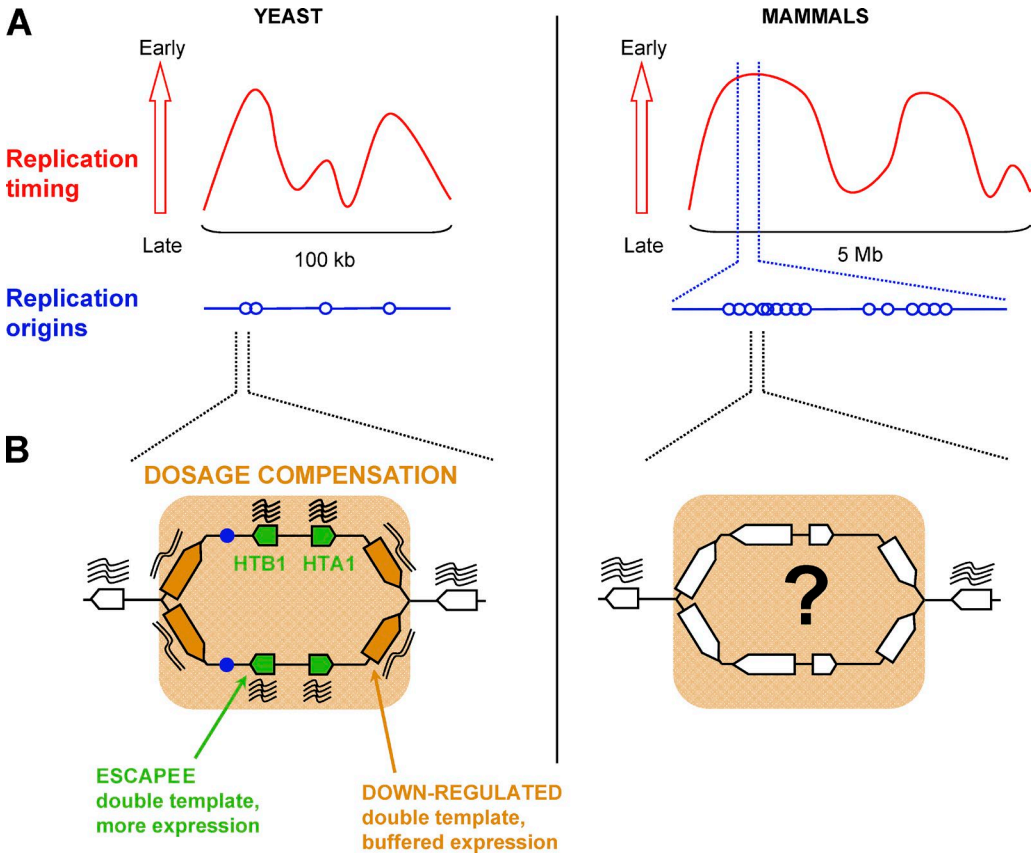

Figure 1. **DNA replication timing regulates gene expression level.** (A) Illustration of replication timing profiles from a portion of the genome of yeast (left) versus mammalian cells (right). The profiles look qualitatively similar, but the scales and the organization in terms of replication origins and activity are different (see text). (B) The work of Müller and Nieduszynski in yeast reveals that loci of conserved early replication timing, such as histone genes (exemplified by *HTB1* and *HTA1*), require an early doubling time to achieve its maximal expression levels. They do so by escaping (green arrow shapes) the dosage compensation mechanisms occurring immediately after replication (orange shadow) that ensure expression homeostasis during S phase (orange arrow shapes). Whether a similar mechanism for regulating gene expression in a replication-dependent manner occurs in mammalian cells is currently unknown (question mark).

Because Rtt109 acetylates newly synthesized histones before their incorporation into DNA (Han et al., 2007), genes escaping from dosage compensation might somehow be refractory to the inhibitory effect of this mark on transcription. Investigating the mechanism by which individual genes escape dosage compensation while all their surrounding genes do not constitutes an interesting new avenue of research. Besides the molecular details of such an exciting process, this novel finding that certain genes are excluded from dosage compensation mechanisms implies that yeast cells can use gene dosage differences as a way to regulate gene expression in a replication-dependent manner. If these findings can be extrapolated to the more complex scenario of mammalian cells (where replication timing domains span megabase-sized regions containing dozens of genes and replication origins), escaping dosage compensation might constitute a potential new way of regulating gene expression (Fig. 1 B).

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