JCB

Spotlight

Replication timing kept in LINE

Rachel J. O'Neill[1,2] and Michael J. O'Neill[1,2]

[1]Institute for Systems Genomics and [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

Accurate and synchronous replication timing between chromosome homologues is essential for maintaining chromosome stability, yet how this is achieved has remained a mystery. In this issue, Platt et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201707082) identify antisense LINE (L1) transcripts within long noncoding RNAs as the critical factor in maintaining synchronous chromosome-wide replication timing.

The replication of chromosomes in eukaryotic organisms takes place in accord with a highly regulated temporal replication program, initiating within conserved replication domains that are defined by the 3D positioning of genomic regions within the cell nucleus (Rivera-Mulia and Gilbert, 2016). Several lines of evidence suggest an association between transcriptional activity of genomic regions and the relative timing of their replication. The topological positioning of these regions in the nucleus is also critical to replication timing. However, the cause/effect relationship of the functional and structural aspects of these phenomena remain controversial. In certain cases, structural rearrangement within a chromosome can delay the replication of the entire chromosome, leading to delayed condensation in mitosis and further destabilization of the genome. Through a combination of chromosome engineering and cytogenetic approaches over the past few years, Thayer and coworkers identified two loci that appear to play an essential role in chromosome-wide replication timing. These two loci, asynchronous replication and autosomal RNA (ASAR) on chromosome 6 (*ASAR6*; Donley et al., 2013) and *ASAR15* (Donley et al., 2015), express long noncoding RNAs (lncRNAs) that persist in the nucleus, forming a cloud surrounding chromosome 6 or chromosome 15, respectively. They have previously shown that *ASAR6* and *ASAR15* are expressed monoallelically and that deletion of either locus results in delayed replication timing (DRT) and delayed mitotic condensation (DMC) of the chromosome carrying the deleted *ASAR*. Thayer et al. (2012) have proposed that each chromosome in the human genome may contain a cis-acting locus that coordinates synchronous replication of homologues (Stoffregen et al., 2011). Although *ASAR6* and *ASAR15* are the only genes identified thus far with this capability, they share remarkably similar functional aspects with the *Xist* gene, which encodes a lncRNA necessary for X chromosome inactivation (XCI) in eutherian mammals (Lee et al., 1996).

Sex chromosome dosage compensation in mammals is achieved by transcriptional inactivation of one of the two X chromosomes in females. XCI occurs through monoallelic transcription of *Xist* RNA, which persists in a nuclear territory at the site of its transcription, forming a cloud around the X destined for inactivation. Deletion of the *Xist* gene not only disrupts XCI, it also leads to DRT/DMC of the X carrying the deletion and subsequent genome instability (Diaz-Perez et al., 2005). *Xist* RNA-mediated gene silencing occurs through recruitment and comigration with polycomb repressive complex 2 (PRC2), ultimately leading to accumulation of trimethylation of lysine 27 of histone 3 (H3K27me3) and spreading of heterochromatin across the inactive X (Pinter et al., 2012). Lyon (1998) proposed that long interspersed nuclear elements (LINEs) for which the X chromosome is highly enriched act as booster elements attracting *Xist* RNA; however, recent work suggests LINEs are anticorrelated with *Xist* RNA-binding sites (Simon et al., 2013). Nevertheless, *ASAR6*, *ASAR15*, and *Xist* not only share monoallelic expression, highly localized RNA cloud formation, and a role in replication timing, but they each harbor LINE sequences in their transcripts (Elisaphenko et al., 2008). In this issue, Platt et al. illuminate the critical role of specific LINE sequences in the coordination of replication timing of homologous chromosomes.

Platt et al. (2018) use two model systems to explore the human *ASAR* control elements: mouse chromosomes engineered to contain an ectopic human *ASAR6* locus on mouse chromosome 3 (Mmu3) and human cells in which regions of *ASAR6* on each chromosome 6 (Hsa6) homologue are differentially targeted for silencing. A single bacterial artificial chromosome (BAC) transgene containing the human *ASAR6* locus was inserted into one copy of Mmu3, resulting in DRT of the *ASAR6*-expressing mouse chromosome. In other words, ectopic expression of human *ASAR6* facilitated asynchronous replication timing of the single mouse chromosome in which it resides. When a 29-KB portion of the BAC transgene was deleted, replication timing returned to synchrony, narrowing the search for the controlling locus to this 29-KB segment of the *ASAR6* lncRNA. Transgenes were derived from different regions within this 29-KB region, and each was tested for the ability to cause chromosome-wide DRT. Of six transgenes examined, only those containing regions of a specific LINE element, L1PA2, could impart DRT and DMC of its surrounding chromosome. In fact, the critical region was further refined to a 1.5-KB window that included 1.2 KB of the 3′ end of the L1PA2 and ~360 bp downstream of the element's 3′UTR. Notably, this L1 is found as an antisense transcript within the larger *ASAR6* lncRNA.

Further validating that the antisense L1PA2 is the critical control element, when locked nucleic acid–GaperRs targeting the L1PA2 antisense transcript were deployed, replication

Correspondence to Rachel J. O'Neill: Rachel.oneill@uconn.edu

© 2018 O'Neill and O'Neill This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).

Downloaded from http://rupress.org/jcb/article-pdf/217/2/441/1616803/jcb_201712173.pdf by guest on 07 February 2026

JOURNAL OF CELL BIOLOGY

The Rockefeller University Press
J. Cell Biol. Vol. 217 No. 2 441–443
https://doi.org/10.1083/jcb.201712173

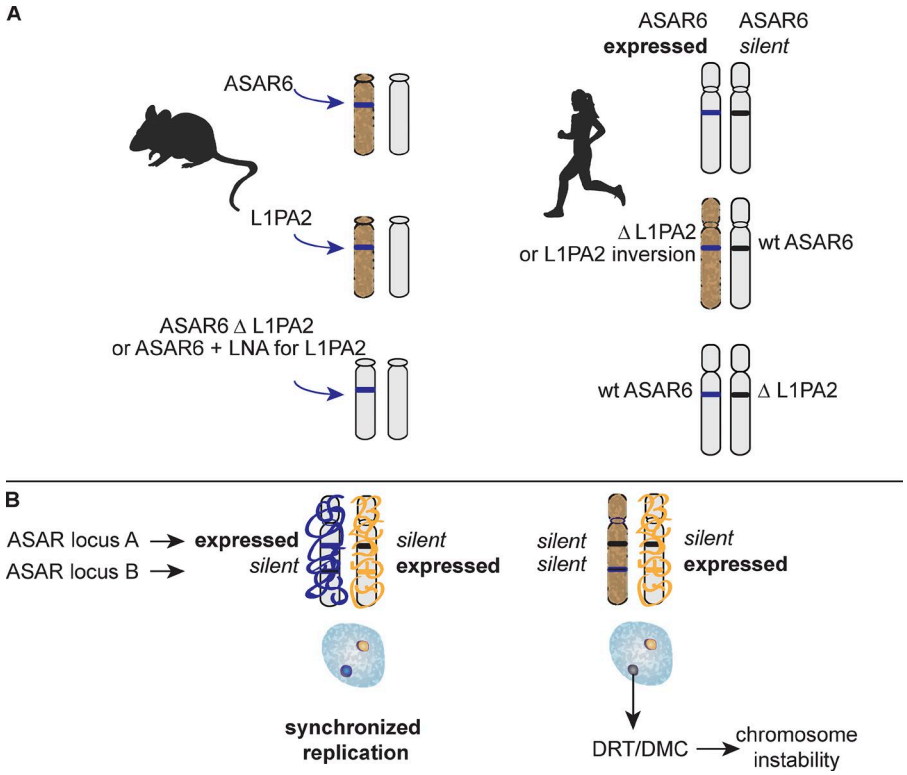

Figure 1. **ASARs in replication timing. (A)** Overview of the effects of various transgene constructs of human *ASAR6* on mouse chromosome 3 (left) and the effect of modifications to the native ASAR6 locus on human chromosome 6 (right). Delayed replication is indicated by a colored and mottled chromosome. **(B)** Model of chromosome-wide control of replication timing via noncoding RNAs expressed from the *ASAR* loci. Each homologue contains two different *ASAR* loci (locus A and locus B), with only one of each pair expressed monoallelically from each homologue. Noncoding RNAs from the expressed *ASAR* (color-coded squiggles matching the expressed locus) form a cloud around the chromosome in cis (transcripts are illustrated on each homologue [top] and in a representative interphase cell [bottom]). Loss of one expressed *ASAR* leads to loss of its noncoding RNA cloud, a DRT/DMC phenotype, and chromosome instability.

timing for the mouse chromosome containing the human *ASAR6* locus once again returned to synchrony. Indeed, small transgenes containing only the L1PA2 element driven by a cytomegalovirus promoter were used to confirm that the antisense orientation of the L1 transcript is critical; sense L1PA2 transgenes have no impact on replication timing, whereas antisense L1PA2 transgenes recapitulate chromosome-wide DRT/DMC in cis. Platt et al. (2018) also show that an *ASAR15* transgene causes DRT/DMC in cis in mouse cells and contains ~1.8 KB of the 3′ end of a truncated L1PA2 in the antisense orientation with respect to the *ASAR15* lncRNA, further linking LINE1s to chromosome-wide replication control.

Platt et al. (2018) include experiments in human cells that afforded the opportunity to manipulate the *ASAR6* loci to test for cis effects with respect to their inherent monoallelic expression. In human HTD114 cells, the expressed allele of *ASAR6* is located on a chromosome 6 distinguished from its homologue by a larger centromere, facilitating identification of expressed and silent *ASAR6* alleles in situ. After CRISPR/Cas9 targeting of the *ASAR6* L1PA2 critical sequences, cells were screened for deletion of the L1PA2 in either the expressed or silent allele of *ASAR6*, and replication timing for both homologues was assessed. Deletion of the L1PA2 within *ASAR6* of the expressed allele showed DRT/DMC, but deletion of the L1PA2 within *ASAR6* of the silent allele had no impact on replication timing. An inversion of the L1PA2 rather than a deletion did not affect lncRNA production but did result in the same DRT/DMC phenotype as the L1PA2 deletion, indicating that expression and orientation are both requisite for control of chromosome-wide replication synchrony in cis.

This study includes what appears at face value to be contradictory data: the insertion of a human *ASAR* onto a mouse chromosome disrupts synchronized replication timing of the mouse chromosome pair. Platt et al. (2018) suggest the human *ASAR* transgene overrides the mouse *ASARs* presumed to provide replication timing control of this chromosome pair because it is unbalanced. The elegant model proposed by Platt et al. (2018) assumes that each chromosome pair carries not one but two *ASAR* loci that act reciprocally to balance replication timing between two homologues. In this model (Fig. 1), only one of the two *ASAR* loci (A or B) is expressed from each homologue (i.e., monoallelically). This expressed *ASAR* produces a noncoding RNA that coats its chromosome in cis, possibly providing a 3D territory to maintain equilibrium in replication timing with its homologous chromosome, also controlled by an opposing *ASAR*. In the case of the mouse chromosomes carrying a single human *ASAR* locus, the single *ASAR* is no longer balanced by another locus, rendering the chromosome from which it is expressed subject to delayed replication. One can speculate that *ASAR* loci can emerge that displace paired loci. When one of the two loci are disrupted, a DRT/DMC phenotype is observed for the chromosome that has lost its *ASAR* RNA coat. The consequences of this phenotype can be catastrophic, with the sequestration of the affected chromosome into micronuclei and chromosome pulverization as possible outcomes.

The new work by Platt et al. (2018) further solidifies the *ASAR* loci as among the control elements that each chromosome possesses for faithful segregation, acting as inactivation/stability centers (Thayer, 2012). What is remarkable in this study is the finding that a recently evolved mobile element is the center of *ASAR* activity, at least on two human chromosomes. It is surprising that LINE elements within a subclass that are primate specific are found to act as the nascent inactivation/stability centers given the apparent necessity for synchronous replication timing of homologues. This would imply that each chromosome would have a pair of *ASARs* and that each species may contain species-specific inactivation/stability centers. The next phase of this research is primed to define the

Downloaded from http://rupress.org/jcb/article-pdf/217/2/441/1616803/jcb_201712173.pdf by guest on 07 February 2026

way in which retroelement transcripts coat a chromosome and recruit specific histone marks and why antisense transcripts are a preferred form of noncoding RNA for replication timing control. In addition, how two *ASARs* interact with one another on a single chromosome, resulting in the monoallelic expression of one *ASAR* per homologue, is unknown, as are the identities of the remaining inactivation/stability centers across the human karyotype. Lastly, the recruitment of recently evolved retroelements as *ASARs* implies rapid evolution and perhaps recurrent recruitment of new mobile elements as *ASARs*. Such turnover evokes the regimes of intragenomic conflict that may underlie the rapid evolution of another element critical to faithful segregation, the centromere.

Acknowledgments

R.J. O'Neill is supported by National Science Foundation grants 1613806 and 1643825.

The authors declare no competing financial interests.

References

Diaz-Perez, S., Y. Ouyang, V. Perez, R. Cisneros, M. Regelson, and Y. Marahrens. 2005. The element(s) at the nontranscribed Xist locus of the active X chromosome controls chromosomal replication timing in the mouse. *Genetics.* 171:663–672. https://doi.org/10.1534/genetics.105.043026

Donley, N., E.P. Stoffregen, L. Smith, C. Montagna, and M.J. Thayer. 2013. Asynchronous replication, mono-allelic expression, and long range Cis-effects of ASAR6. *PLoS Genet.* 9:e1003423. https://doi.org/10.1371/journal.pgen.1003423

Donley, N., L. Smith, and M.J. Thayer. 2015. ASAR15, A cis-acting locus that controls chromosome-wide replication timing and stability of human chromosome 15. *PLoS Genet.* 11:e1004923. https://doi.org/10.1371/journal.pgen.1004923

Elisaphenko, E.A., N.N. Kolesnikov, A.I. Shevchenko, I.B. Rogozin, T.B. Nesterova, N. Brockdorff, and S.M. Zakian. 2008. A dual origin of the Xist gene from a protein-coding gene and a set of transposable elements. *PLoS One*. 3:e2521. https://doi.org/10.1371/journal.pone.000252118575625

Lee, J.T., W.M. Strauss, J.A. Dausman, and R. Jaenisch. 1996. A 450 kb transgene displays properties of the mammalian X-inactivation center. *Cell.* 86:83–94. https://doi.org/10.1016/S0092-8674(00)80079-3

Lyon, M.F. 1998. X-chromosome inactivation: a repeat hypothesis. *Cytogenet. Cell Genet.* 80:133–137. https://doi.org/10.1159/000014969

Pinter, S.F., R.I. Sadreyev, E. Yildirim, Y. Jeon, T.K. Ohsumi, M. Borowsky, and J.T. Lee. 2012. Spreading of X chromosome inactivation via a hierarchy of defined Polycomb stations. *Genome Res.* 22:1864–1876. https://doi.org/10.1101/gr.133751.111

Platt, E.J., L. Smith, and M.J. Thayer. 2018. L1 retrotransposon antisense RNA within ASAR lncRNAs controls chromosome-wide replication timing. *J. Cell Biol.* https://doi.org/10.1083/jcb.201707082

Rivera-Mulia, J.C., and D.M. Gilbert. 2016. Replication timing and transcriptional control: beyond cause and effect-part III. *Curr. Opin. Cell Biol.* 40:168–178. https://doi.org/10.1016/j.ceb.2016.03.022

Simon, M.D., S.F. Pinter, R. Fang, K. Sarma, M. Rutenberg-Schoenberg, S.K. Bowman, B.A. Kesner, V.K. Maier, R.E. Kingston, and J.T. Lee. 2013. High-resolution Xist binding maps reveal two-step spreading during X-chromosome inactivation. *Nature.* 504:465–469. https://doi.org/10.1038/nature12719

Stoffregen, E.P., N. Donley, D. Stauffer, L. Smith, and M.J. Thayer. 2011. An autosomal locus that controls chromosome-wide replication timing and mono-allelic expression. *Hum. Mol. Genet.* 20:2366–2378. https://doi.org/10.1093/hmg/ddr138

Thayer, M.J. 2012. Mammalian chromosomes contain cis-acting elements that control replication timing, mitotic condensation, and stability of entire chromosomes. *BioEssays.* 34:760–770. https://doi.org/10.1002/bies.201200035

Downloaded from http://rupress.org/jcb/article-pdf/217/2/441/1616803/jcb_201712173.pdf by guest on 07 February 2026