

## Replication checkpoint-mediated symmetric DNA synthesis: beginning to understand mechanism

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Beginning in the 1950's, Arthur Kornberg's research on DNA polymerases, followed by the efforts of many others, provided a seminal understanding of catalytic DNA synthesis [1], a fundamental process enabling the anti-parallel strands of the DNA double helix to be copied by DNA polymerases with a single polarity. A leading strand, copied in a relatively continuous manner, and a lagging strand, copied in a discontinuous manner as a series of Okazaki fragments, resolved the issue. Building upon this work, Bruce Alberts proposed the "trombone model" over 35 years ago to visualize the apparent coordination between leading- and lagging-strand synthesis at the replication fork [2].





Although the emerging story of coordinated semi-conservative DNA replication is satisfying, it is now evident that during conditions of replication stress (invoked by nucleotide depletion, DNA damage, secondary DNA structure, oncogene activation, *etc.*), the scenario is more complex than previously thought. Fork dynamics and the cellular response dictate sophisticated mechanisms to ensure fork integrity, resumption of DNA synthesis, and ultimately genomic stability. During replication stress, the DNA replication checkpoint response mediated by apical kinases (*S. cerevisiae* Mec1, human ATR) and effector kinases (*S. cerevisiae* Rad53, human CHK1) adjusts cell cycle transitions (including entry into mitosis), suppresses firing of new replication origins, elevates the deoxynucleotide (dNTP) pool, and modulates gene gating proposed to relieve topological stress [3]. In addition, replication stress may directly affect replication fork DNA architecture through remodeling events and by altering interactions of proteins that stably or transiently associate with the replisome [3]. These aspects of fork dynamics are still not well understood.

A very recent study by Zhiguo Zhang of Columbia University and colleagues, appearing in the October 19, 2017 issue of *Molecular Cell*, has provided a fresh perspective on how the DNA replication checkpoint pathway plays a role in the coordination of leading- and lagging-strand synthesis during hydroxyurea (HU)-induced replication stress which depletes the dNTP pool [4]. Using a clever experimental strategy to measure nascent leading and lagging strands in wild-type and *rad53-1*

mutant checkpoint defective yeast cells, the authors convincingly show that a deficiency in the replication checkpoint results in asymmetric DNA synthesis with extended DNA synthesis complementary to the lagging-strand template, but *not* the leading-strand template. Concurrently, the exposed single-stranded DNA present in the leading-strand template is coated by Replication Protein A (RPA), which normally provides an early signal for the replication checkpoint. In *rad53-1* checkpoint defective cells, the replication fork helicase known as minichromosome maintenance protein complex (MCM) and associated DNA polymerase epsilon ( $\epsilon$ ) move ahead of the newly synthesized DNA (without continuing to catalyze polynucleotide synthesis), which is likely to further contribute to fork asymmetry under the condition of HU-induced replication stress. Finally, the authors provide evidence that increasing the dNTP level in the HU-treated *rad53-1* mutant cells effectively suppressed the uncoordinated leading- and lagging-strand synthesis.

The significance of this work lies in the demonstration that eukaryotic cells rely on a checkpoint mechanism to prevent uncontrolled asymmetric DNA synthesis at the fork, presumably by inhibiting MCM-catalyzed unwinding of the parental duplex. However, the suppressive effect of activated Rad53 on MCM helicase activity was not formally shown in the study. Nonetheless, this work provides new insight to a cellular pathway whereby replication fork catastrophe is avoided and genomic integrity is maintained, even under adverse conditions of compromised DNA synthesis. However, like many innovations, this study raises some important unanswered questions pertaining to the mechanism whereby eukaryotic cells ensure coordinated leading- and lagging-strand synthesis during replication stress (Figure 1).

Although there has been a plethora of studies on the S-phase checkpoint and pathways to maintain the replication fork and ensure chromosomal stability, truly mechanistic insights have been lagging (so to speak) in the field. This latest advance by Gan et al. [4] should inspire new efforts and innovative experimental strategies to characterize the precise molecular pathways responsible for promoting coordinated DNA synthesis of the leading- and

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What are the phosphorylation targets of Rad53 (CHK1) and Mec1 (ATR) kinases during replication stress induced by dNTP depletion that affect fork symmetry?
Are the same kinase targets and mechanisms to ensure fork symmetry invoked by other forms of replication stress ( <i>e.g.</i> , DNA damage, secondary DNA structure, <i>etc.</i> ) compared to those elicited by HU-induced dNTP depletion? Are these conserved in unicellular fungi and multicellular mammals?
Does Rad53/CHK1 kinase, either directly by MCM phosphorylation ( <i>e.g.</i> , <sup>[5]</sup> ) or indirectly by a signal transduction pathway, slow the unwinding rate of MCM helicase complex and/or alter pol $\epsilon$ interaction with the leading-strand template at the fork in cells undergoing replication stress?
Does CHK1-mediated phosphorylation of the Bloom's syndrome (BS) helicase BLM <sup>[6]</sup> , an ortholog of <i>S. cerevisiae</i> SGS1 known to associate with stalled forks, contribute to fork symmetry in cells undergoing replication stress?
Does the pyrimidine pool imbalance in BS patient cells induced by a cytidine deaminase deficiency <sup>[7]</sup> contribute to genomic instability prevalent in BS via perturbation of the coordination between leading- and lagging-strand synthesis?

**Figure 1.** A recent advance by Gan et al. [4] on the role of the replication checkpoint in coordinating leading- and lagging-strand synthesis during replication stress raises new and provocative questions in the field.

lagging-strands during replication *in vivo*, a process so fundamental to DNA biology but still difficult to fully comprehend.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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