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Gaps and forks in DNA replication: Rediscovering old models

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The way in which cells are able to replicate DNA templates containing altered bases (sometimes referred to as postreplication repair- PRR) has been a topic of intense current interest in recent years. This was stimulated by the discovery in 1999 of the Y-family of DNA polymerases, which are able to carry out translesion synthesis (TLS) past damaged bases. However the first experiments giving insights into how damaged DNA is replicated predate the discovery of the Y-family polymerases by three decades [1]. The model proposed in this early work suggested that the replication forks proceed past the damage, leaving behind gaps that are subsequently repaired, whereas most currently proposed models envisage TLS taking place at the fork. Two recent papers provide support for the older models [2,3]. “Rediscovering old models” may sound like an inappropriate title for a Hot Topic, so pedantic readers may prefer to consider this as a “Reheated Topic”.

In their classic paper of 1968, Rupp and Howard-Flanders [1] showed that in *uvrA* strains of *Escherichia coli* (deficient in nucleotide excision repair), newly synthesised DNA strands in UV-irradiated cells were initially smaller than those in untreated controls. These small DNA pieces were subsequently converted into high molecular weight DNA. These results were interpreted as showing that the new strands contained gaps, which were presumed to be opposite the UV photoproducts (Figure 1A). These gaps were subsequently sealed, and a later paper by the same authors [4] showed that in the majority of cases this gap-filling process involved sister-strand recombinational exchanges. By this means the genetic information lost from the damage/gap site in one daughter duplex is regained from the sister duplex, which would be unlikely to be damaged at the same site. This mechanism, in today’s parlance, would fall into the category of damage avoidance. An important aspect of

this model is that the damage/gap is dealt with after the replication fork has moved away, and this gave rise to the term postreplication repair. In *E. coli* such a recombinational exchange process is the way in which the cell deals with the majority of UV lesions during replication. In a minority of cases, the lesion is replicated by TLS, requiring the UmuD,C-encoded DNA polymerase V.

Interpretation of the data in *E. coli* was relatively straightforward because the *E. coli* chromosome is replicated from only two replication forks diverging from a single origin. Interpretation of similar results from mammalian cells was much more difficult because the mammalian genome is replicated from multiple origins. This led to a great deal of controversy in the 1970's about the mechanism by which cells replicate damaged DNA. Little evidence could be obtained for sister strand exchanges as found in *E. coli* (eg [5]) and it was suggested that, although gaps may be left opposite damage in the lagging strands, this was unlikely to be the case for the leading strands (eg see [6]). Leading strands are normally considered to be synthesised continuously (in both prokaryotes and eukaryotes), and if gaps were left in the leading strands, this would entail replication restart beyond the lesions, which was considered unlikely. The way in which the fork overcame the blocks on the leading strand remained unresolved, and in fact there was little further research in this area for almost twenty years until the discovery of the TLS polymerases. The excitement generated by these polymerases and the new avenues of research that were opened by their discovery gave rise to new models, almost all of which envisaged the handover from replicative to TLS polymerase at the site where the fork was blocked, followed by handing back to the replicative polymerase once the block had been bypassed (Figure 1B). These models imply or assume that TLS occurs at the replication forks.

Two new papers have provided evidence that gaps are left in the daughter strands of UV-irradiated *Saccharomyces cerevisiae* in vivo[2], and that replication restart can indeed take place on the leading strand, using *E. coli* replication proteins in vitro[3]. Lopes et al used a NER-deficient *rad14* strain of *S. cerevisiae*, and employed electron microscopy to detect single-stranded regions of DNA (ssDNA) in replicating DNA molecules in UV-irradiated cells. They observed long single-stranded regions of DNA on one of the replicated strands immediately behind the replication fork. In some cases, they were able to identify whole replication bubbles and detected long regions of ssDNA on both arms of the bubble on opposite strands (Figure 2A). These findings were consistent with a model in which DNA damage stalled the progress of the leading strand, which became uncoupled from the lagging strand synthesis. Pages and Fuchs had reached similar conclusions previously from studies on *E. coli* [7]. Lopes et al also observed short ssDNA patches of up to 400 bp well behind the replication forks, up to 20 kb away (Figure 2). These discontinuities were found on both strands of the replicated DNA molecules, suggesting that synthesis not only of the lagging strand, but also of the blocked leading strand was reinitiated beyond the blocked site. These findings are completely consistent with the Rupp and Howard-Flanders model. However it should be noted that evidence that these discontinuities were indeed located opposite UV photoproducts was not provided. Lopes et al next examined replication structures in cells mutated in all the TLS polymerase genes *rad30*, *rev3* and *rev1*, or in a crucial recombination gene *rad52*. These mutations had minimal effect on ssDNA regions at the replication fork, but caused an increase in the frequency of discontinuities away from the fork. In the TLS mutant cells, more gaps were found relatively close to the fork (<5 kb), whereas in the *rad52* mutant, more

discontinuities were found along the length of the replicated strands. These findings suggest that gaps are left opposite the damage and the cell first attempts to seal them using TLS, but if for some reason this is not successful, they remain for longer periods and are subsequently sealed by recombination.

To complement their electron microscopic studies, Lopes et al also analysed replication structures using 2-d gel electrophoresis to analyse fork progression [8]. Consistent with their electron microscopic studies they found that the forks could travel long distances past many UV lesions, albeit at a somewhat slower speed than in undamaged cells. In cells deficient in the TLS polymerases, replication fork progression was indistinguishable from that in cells capable of TLS. These data confirm that TLS is not necessary for progression of the fork, and by implication, that it occurs behind the fork.

One currently popular model for bypassing lesions involves regression of the stalled replication fork to allow annealing of the two daughter strands to form a so-called chicken-foot structure. This model was originally designated template strand switching, when it was first proposed [9]. The stalled daughter strand can then use its partner daughter strand as template to continue synthesis. Reversal of this structure will re-establish the replication forks with the damage having been bypassed in a damage avoidance process that does not involve physical exchanges [10]. Although found in hydroxyurea-treated *rad53* mutants in *S. cerevisiae* [11], there has been little evidence for these structures in UV-irradiated eukaryotic cells, and indeed Lopes et al only detected four reversed fork structures in 2100 forks examined [2]. These may have been pathological rather than productive structures.

The concept of gaps in the lagging strand opposite lesions has been readily acceptable because the lagging strand is in any case synthesised discontinuously with Okazaki fragments, so no major mechanistic change needs to be invoked to accommodate this concept. However gaps in the leading strand had been considered somewhat heretical, as they would require re-establishment of the replication machinery, a process that is normally thought to take place on the leading strand only at replication origins.

Using an *in vitro* approach with purified *E. coli* proteins, the work by Heller and Marians addresses the issue of restarting DNA synthesis downstream of a blocked nascent leading strand [3]. Recent *in vivo* investigations in *E. coli* have revealed that leading and lagging strand synthesis can be uncoupled by a single blocking lesion placed in either strand of a plasmid [7]. The size of the gap generated with the leading strand block was estimated to be > 1 kb, but the small size of the plasmid precluded the detection of any potential downstream repriming events. During normal DNA synthesis, lagging strand repriming occurs at every Okazaki fragment, via the DnaG primase that is recruited by the DnaB helicase moving along the lagging strand. In contrast, no mechanism for leading strand repriming has so far been proposed. In their paper, Heller and Marians used a 6.9 kb linear forked template and a leading strand primer that was blocked with 2'3'dideoxyCMP at its 3' end. As expected, this primer could not be extended. However in the presence of DnaG primase, leading strand synthesis was achieved. The data suggested that the single DnaB replication fork helicase loaded on the lagging strand could recruit DnaG primase molecules to reprime not only on the lagging strand but also on the leading strand (Figure 2B). The recent finding that multiple primase monomers can bind a DnaB hexamer [12]

supports the idea that a single helicase can mediate the recruitment of more than one DnaG molecule, thus possibly mediating both lagging and leading strand priming events.

These data provide a mechanism for restarting DNA synthesis beyond a lesion on the leading strand. However they may also have implications for DNA replication in undamaged cells. It was initially proposed from results involving alkaline sucrose gradient centrifugation of nascent DNA fragments labelled *in vivo* by short pulse-labeling, that both nascent lagging and leading strands were made discontinuously [13-15]. However reconstitution of replication forks clearly showed that leading strand synthesis is continuous *in vitro* and this has now become accepted dogma [16]. This issue has recently been re-addressed by Amado and Kuzminov, who, using a temperature-sensitive ligase mutant of *E. coli*, showed that essentially all newly synthesised DNA was synthesised in small pieces [17]. This strongly suggests that both leading and lagging strands can be synthesised discontinuously *in vivo*, contrary to accepted dogma, but supporting the original model [13]. 1968 was a year of widespread revolution in the political arena. It seems that in the same year, revolutionary models based on findings in the area of DNA replication, may, almost four decades later, be proven correct.

References

- [1] W.D. Rupp and P. Howard-Flanders, Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation, J. Mol. Biol. 31 (1968) 291-304.
- [2] M. Lopes, M. Foiani and J.M. Sogo, Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions, Mol Cell 21 (2006) 15-27.
- [3] R.C. Heller and K.J. Marians, Replication fork reactivation downstream of a blocked nascent leading strand, Nature 439 (2006) 557-562.
- [4] W.D. Rupp, C.E. Wilde, D.L. Reno and P. Howard-Flanders, Exchanges between DNA strands in ultraviolet irradiated *Escherichia coli*, J. Mol. Biol. 61 (1971) 25-44.
- [5] A.R. Lehmann, Postreplication repair of DNA in ultraviolet-irradiated mammalian cells, J. Mol. Biol. 66 (1972) 319-337.
- [6] R. Meneghini, Gaps in DNA synthesized by ultraviolet light-irradiated WI38 human cells, Biochim Biophys Acta 425 (1976) 419-427.
- [7] V. Pages and R.P. Fuchs, Uncoupling of leading- and lagging-strand DNA replication during lesion bypass in vivo, Science 300 (2003) 1300-1303.
- [8] B.J. Brewer and W.L. Fangman, The localization of replication origins on ARS plasmids in *S. cerevisiae*, Cell 51 (1987) 463-471.
- [9] N.P. Higgins, K. Kato and B. Strauss, A model for replication repair in mammalian cells, J Mol Biol 101 (1976) 417-425.
- [10] P. McGlynn and R.G. Lloyd, Recombinational repair and restart of damaged replication forks, Nat Rev Mol Cell Biol 3 (2002) 859-870.

- [11] J.M. Sogo, M. Lopes and M. Foiani, Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects., *Science* 279 (2002) 599-602.
- [12] A.V. Mitkova, S.M. Khopde and S.B. Biswas, Mechanism and stoichiometry of interaction of DnaG primase with DnaB helicase of *Escherichia coli* in RNA primer synthesis, *J Biol Chem* 278 (2003) 52253-52261.
- [13] R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto and A. Sugino, Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains, *Proc Natl Acad Sci U S A* 59 (1968) 598-605.
- [14] T. Ogawa and T. Okazaki, Discontinuous DNA replication, *Annu Rev Biochem* 49 (1980) 421-457.
- [15] T.C. Wang, Discontinuous or semi-discontinuous DNA replication in *Escherichia coli*?, *Bioessays* 27 (2005) 633-636.
- [16] C.A. Wu, E.L. Zechner and K.J. Marians, Coordinated leading- and lagging-strand synthesis at the *Escherichia coli* DNA replication fork. I. Multiple effectors act to modulate Okazaki fragment size, *J Biol Chem* 267 (1992) 4030-4044.
- [17] L. Amado and A. Kuzminov, The replication intermediates in *Escherichia coli* are not the product of DNA processing or uracil excision, *J Biol Chem* (2006).

Figure Legends

Figure 1: Models for replication of damaged DNA. The arrows indicate the fork direction. For simplicity only the replication of the leading strand is shown.

A: Gaps behind the fork. Newly replicated DNA (thin line) is made discontinuously, gaps being left opposite lesions in the parental strand (thick line). The gap-filling reaction (orange patches), which involves specialized and replicative DNA polymerases or sister-strand exchanges, occurs behind the advancing replication fork.

B: TLS at the fork: In this model, the fork stalls at each lesion, specialized DNA polymerases synthesize a short TLS patch (orange patch), replication resumes, the same process occurs at the next lesion.

Figure 2: Gaps in leading and lagging strands

A: Schematic drawing of replication intermediates in UV-irradiated *S. cerevisiae* cells, as observed by EM (Lopes et.al., 2006). The grey arrows show gaps observed in both the leading and lagging strands, possibly opposite UV lesions. The dashed arrows indicate the positions of the forks. **B:** A single replicative DnaB helicase (red hexamer) opens the double helix by sliding along the lagging strand. It can recruit two DnaG primases (green crescent) that mediate priming of both the lagging strand (as in normal DNA synthesis) and occasionally the leading strand when a gap is formed as a consequence of a template leading strand block (according to Heller and Mariani, 2006).

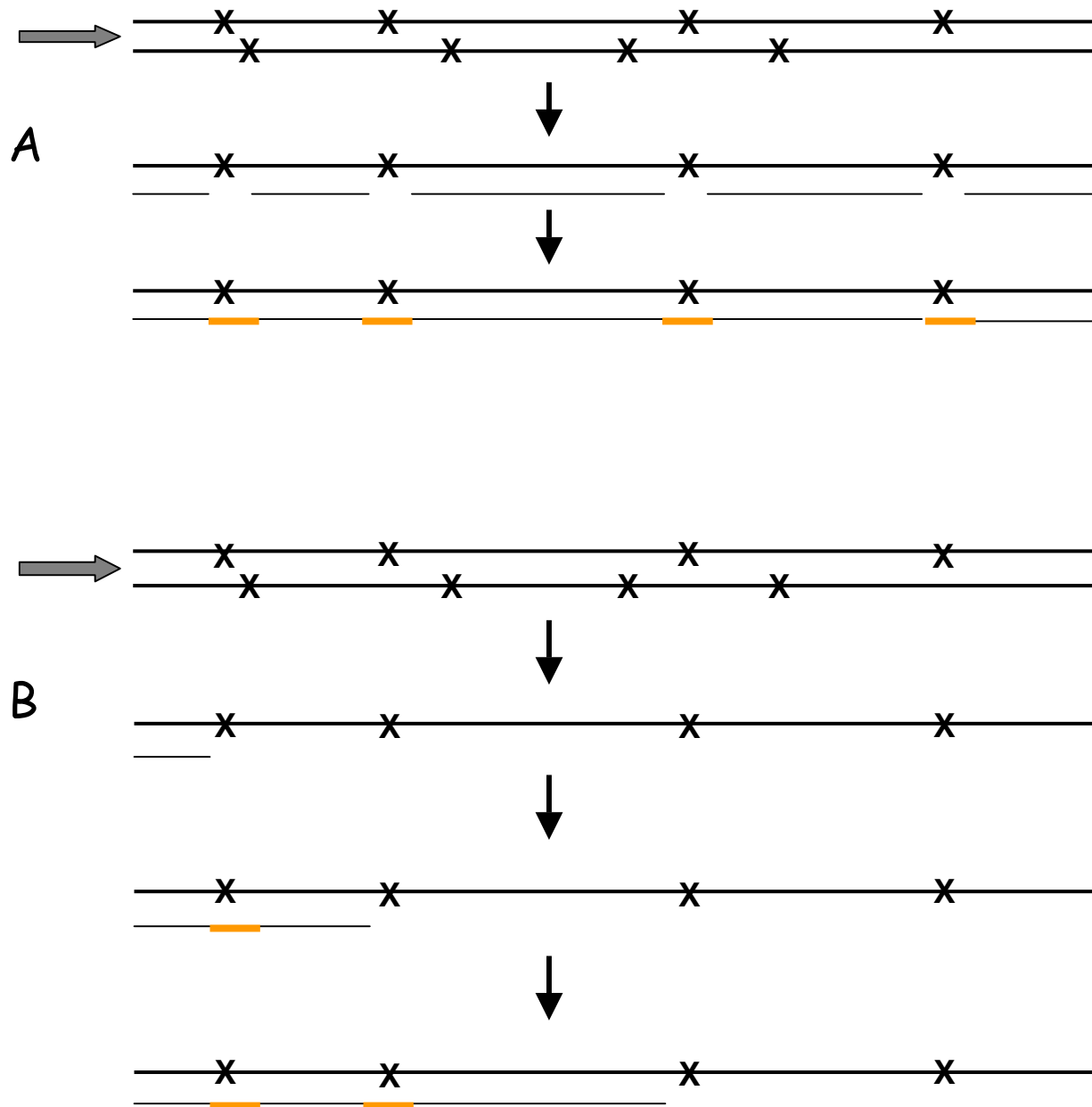


Figure 1

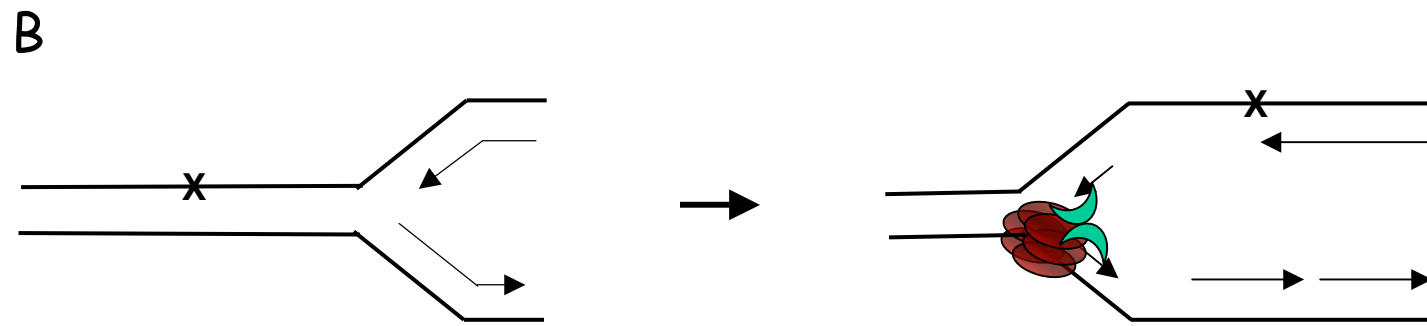
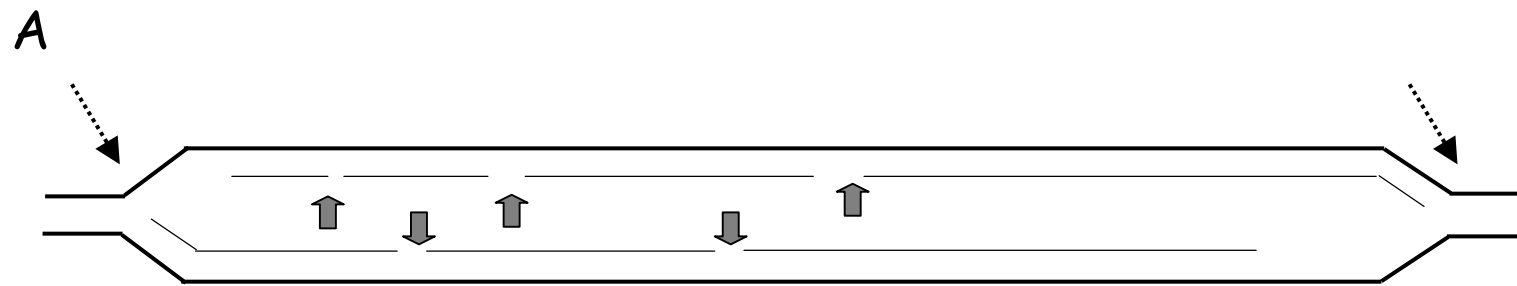


Figure 2