

Template Switching: From Replication Fork Repair to Genome Rearrangements

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Genome rearrangements are a hallmark of human genomic disorders and occur largely through recombination mechanisms. In this issue, Lee et al. (2007) show that the complex nonrecurrent rearrangements observed in the dysmyelinating disorder Pelizaeus-Merzbacher disease (PMD) are likely to be caused by a replication mechanism involving template switching.

Genomic disorders are a group of human genetic diseases characterized by genomic rearrangements consisting of deletions, duplications, and inversions of specific genomic segments. These rearrangement events are triggered by architectural features of the genome and usually result in a change in copy number of disease-specific genes for which dosage is critical. Over 5% of the human genome contains duplicated segments and repetitive elements that make it susceptible to rearrangements. Nonallelic homologous recombination (NAHR) between low-copy repeats, duplicated segments, or sometimes repetitive sequences that flank the rearranged genomic segment is a primary mechanism that accounts for genomic disorders characterized by recurrent genomic rearrangements. The architecture of the genomic DNA can determine the mechanism for NAHR associated with the rearrangement. Thus, recombination between low-copy repeats in direct orientation results in duplication and deletion, whereas recombination between inverted low-copy repeats causes inversion. Sequence analyses of junction fragments of rearranged regions have suggested that pathways for homologous recombination involving either double-strand break repair or synthesis-dependent strand-annealing (Paques and Haber, 1999) take part in such rearrangements. These studies have also revealed

the presence of low-copy repeats with remarkable sequence identity flanking the genomic segments that undergo recurrent recombination (Inoue and Lupski, 2002). Furthermore, the identification of unique junction fragments of identical size in different patients afflicted by the same disease points to precise and recurrent recombination events. Recombination by nonhomologous end joining (NHEJ) has also been proposed to underlie the rearrangements observed in several genomic disorders, where the regions flanking the deleted genomic interval showed no homologous sequences, and/or the deletion breakpoints of a rearrangement were mapped to different locations, with no common breakpoint observed (Inoue et al., 2002). However, a number of complex rearrangements associated with diseases are not readily explained by mechanisms involving either NAHR or NHEJ.

Lee et al. (2007) now characterize the nonrecurrent genomic rearrangements associated with the dysmyelinating disorder Pelizaeus-Merzbacher disease (PMD) and show that they most likely occur during replication. They propose a replication slippage mechanism, with the replication fork skipping backward or forward at those genomic regions that are susceptible to rearrangement and have complex genomic architecture. The authors performed comparative genomic hybridization

and breakpoint sequence analyses of PMD-associated nonrecurrent duplications from 17 patients. Duplication of the genomic segment that contains the entire dosage-sensitive proteolipid protein 1 (*PLP1*) gene is responsible for PMD in the majority of patients, although nonrecurrent *PLP1* deletion or point mutations also occur in a minority of cases (Inoue and Lupski, 2002). Lee et al. (2007) find that the nonrecurrent rearrangements occurring in PMD patients were often more complex than simple tandem duplications. Interestingly, the authors observed interrupted duplications in which stretches of DNA of normal copy number were punctuated by stretches of DNA that were amplified two or three times. These results are consistent with these rearrangements being produced during replication, and the authors term this mechanism *Fork Stalling and Template Switching* (FoSTeS).

In both prokaryotes and eukaryotes, replication forks often arrest in response to low levels of deoxyribonucleotides or reducing amounts of defective DNA polymerases, or when the replication fork encounters complex DNA structures or protein-DNA complexes (see Goldfless et al., 2006; Lemoine et al., 2005); additional factors are then required to restart or repair replication forks. Consistent with these observations, Lee et al. find that the proposed FoSTeS events occur

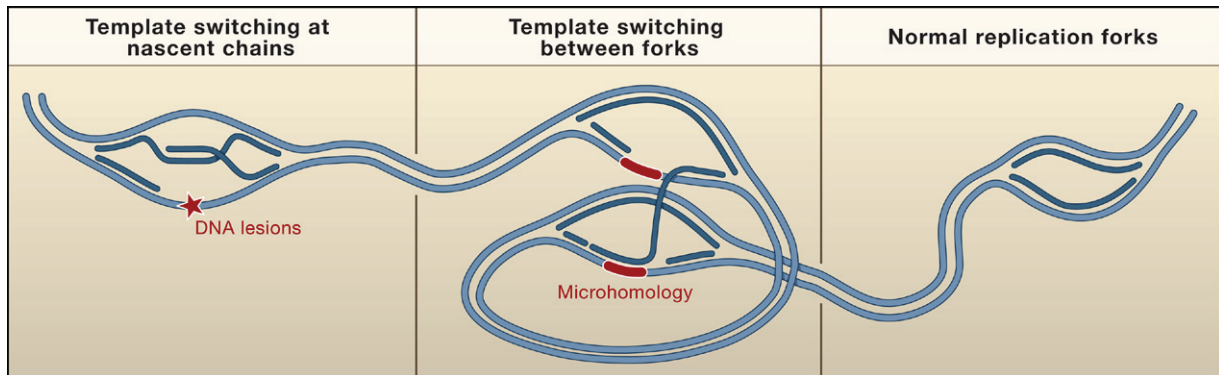


Figure 1. Fork Repair and Chromosomal Rearrangements by Template Switching

Replication forks encountering DNA lesions (red star) on the template strand can engage the nascent chains into template switching to bypass the DNA lesion. Forks encountering low-copy repeats or repetitive elements are prone to stalling and, occasionally, switch templates in the presence of a nearby template at another fork, thus generating chromosomal rearrangements. This process might require regions of microhomology (red bars).

preferentially in regions of complex genomic architecture. Indeed, the region surrounding the *PLP1* gene that is frequently duplicated in PMD patients contains abundant low-copy repeats with high sequence identity and in various orientations, which could favor replication fork stalling and slippage and, consequently, genome rearrangements. Replication fork slippage has been previously proposed to account for rearrangements among repeated DNA sequences in bacteria, yeast, and humans (see Goldfless et al., 2006 and references therein), and evidence suggests that mechanisms other than homologous recombination are at least in part responsible for such rearrangements. Occasionally, prolonged replication stalling may induce fork collapse and DNA double-strand breaks, followed by repair attempts via break-induced replication with multiple rounds of strand invasion, DNA synthesis, and dissociation. This could similarly lead to chromosome rearrangements if dissociation and strand invasion occur within dispersed repeated sequences (Narayanan et al., 2006; Smith et al., 2007).

Previous studies in *E. coli* have revealed a class of rearrangements that are independent of RecA-mediated recombination but dependent on DnaK, a chaperone also required to remodel the replisome to permit replication fork repair (Goldfless

et al., 2006). Genetic studies have shown that *dnaK* specifically affects a replication misalignment pathway promoted by hairpin structures on the lagging template that is likely responsible for producing tandem repeat rearrangements. These features of DnaK are similar to the eukaryotic Rad5/Rad18 postreplication repair proteins that are required for gap-filling repair presumably through a template-switch mechanism. Interestingly, like DnaK, yeast Rad5 increases the instability of simple repeated sequences (Johnson et al., 1992). The template-switching mechanism occurring between two nascent chains within the same replication fork and involving sister chromatid pairings (Goldfless et al., 2006) predicts the formation of cruciform DNA intermediates (Figure 1). These DNA structures have been observed in yeast during replication of damaged templates, and defects in their resolution may cause genomic instability by provoking unscheduled recombination events (Branzei et al., 2006 and references therein).

The more complex amplifications observed by Lee et al. (2007) in their new study could be explained by long-distance template-switching models between different replication forks (Figure 1). These pairing events could be facilitated by the genomic architecture that might bring into proximity highly similar DNA seg-

ments or repetitive sequences that normally lie far apart. Evidence for long-distance template switching has been previously suggested by studies in *E. coli* (Slack et al., 2006). Unlike the DNA double-strand break-induced genome rearrangement model involving NAHR or simple NHEJ, the long-distance template-switch model for genome amplifications suggests a single-strand DNA lesion as the initiating trigger (Lee et al., 2007; Slack et al., 2006). Indeed, the studies done in *E. coli* provide evidence that 3'-single-strand DNA ends act as intermediates in this process, and that lagging-strand templates are involved (Slack et al., 2006). In *E. coli*, most of these amplifications, proposed to occur by long-distance template switching, are stress induced and not spontaneous and therefore could underlie adaptive evolution.

The FoSTeS replication-based mechanism proposed by Lee et al. could be responsible for other non-recurrent disease-causing genomic rearrangements. Given the deleterious consequences of such events for genomic stability, it will be important to understand if cells are endowed with mechanisms controlling these template-switch events under normal conditions or whether these mechanisms are induced under circumstances that require adaptability or long-term evolutionary changes in the genome.

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