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VIEWPOINT

Checking That Replication Breakdown Is Not Terminal

Antony M. Carr

Two new studies help to clarify the relationship between checkpoint proteins, recombination, and replication fork integrity.

DNA double-strand breaks (DSBs) are major pathological DNA structures in mitotic cells. Left unrepaired, these breaks can result in chromosome translocations and missegregation of genetic information, destabilizing the genome and potentially contributing to carcinogenesis. Spontaneous DSBs are caused by endogenous DNA damaging agents, but they also occur at stalled DNA replication forks in particular mutant backgrounds.

Replication-associated DSBs were first demonstrated in helicase-defective *Escherichia coli* (*1*). Surprisingly, DSB formation during replication requires enzymes involved in homologous recombination (HR). In *E. coli*, stalled replication forks are restarted by HR-mediated repair (*2*) (Fig. 1). Perhaps DSBs arise because processing of DNA structures at damaged forks by HR enzymes generates DNA intermediates that are susceptible to cleavage. This interpretation is consistent with a recent study showing that rescue of stalled replication forks in *E. coli* by the RecG helicase involves unwinding of both nascent strands at the fork and their subsequent annealing to form a four-stranded Holliday junction (HJ) (*3*). Inappropriate resolution of such a HJ would result in a DSB at a stalled fork.

HR proteins in yeast and other eukaryotes also play a role in maintaining replication fidelity and preventing the accumulation of DNA damage, including DSBs [e.g., (*4*, *5*)]. Eukaryotic replication checkpoint proteins were originally identified for their ability to prevent cell entry into mitosis during replication, but they also prevent genome instability through regulation of DNA repair within S phase (*7*). For example, in the fission yeast *Schizosaccharomyes pombe*, both recombination and replication checkpoint proteins are required for promotion of cell survival when DNA damage in S phase cannot be removed (*6*).

In this issue of *Science*, a new study by Sogo *et al.* (*8*) helps to clarify the relationship between checkpoint proteins, recombination, and replication fork integrity. The authors used electron microscopy to visualize stalled replication intermediates in the budding yeast *Saccharomyes cerevisiae* in the presence or absence of the replication checkpoint. In wild-type cells, these intermediates were largely bifurcating and double-stranded, with only limited regions of single-stranded (ss) DNA. In contrast, the replication intermediates isolated from checkpoint-defective (*rad53* mutant) cells showed extensive ssDNA regions and large numbers of reversed forks. This suggests that the replication checkpoint suppresses the formation of HJ-like replication intermediates. By implication, the absence of the checkpoint function may allow DSBs to occur through inappropriate processing of HJs.

In related work, also in this issue, Cha and Kleckner (*9*) demonstrate that DSBs occur in replication checkpoint–deficient *S. cerevisiae* cells during replicative stress. Specifically, these authors find that in the absence of Mec1 (a chromosome-bound signal transduction protein involved in DNA replication, repair, and recombination), DSBs occur late in S phase at specific genomic loci that correspond to slowly replicating regions in unstressed cells. These "replication slow zones" (RSZs) map between active replication origins, but deletion of the origins does not affect DSB formation, suggesting that RSZs are intrinsically susceptible to breakage during replication. The simplest interpretation is that RSZs exhibit slow replication because replication is more difficult in these regions, perhaps because DNA binding proteins need to be removed. Thus, RSZs are likely to experience additional difficulties when the supply of deoxynucleoside triphosphates (dNTPs) is depleted by the experimental conditions.

In both *E. coli* and *S. cerevisiae*, DSBs are induced when helicase activity is perturbed (*1*, *10*). Thus, checkpoint proteins may coordinate replication and recombination during replicative stress caused by global dNTP inhibition, localized DNA damage, or refractory chromatin architecture resulting from repetitive sequences or the binding of proteins that must be removed by specialized helicases. It is interesting that the replication checkpoint is not essential for viability in *S. pombe*, as it is in *S. cerevisiae.* This is because the two yeasts regulate ribonucleotide reductase (RNR) in different ways: Induction of *S. cerevisiae* RNR activity in S phase

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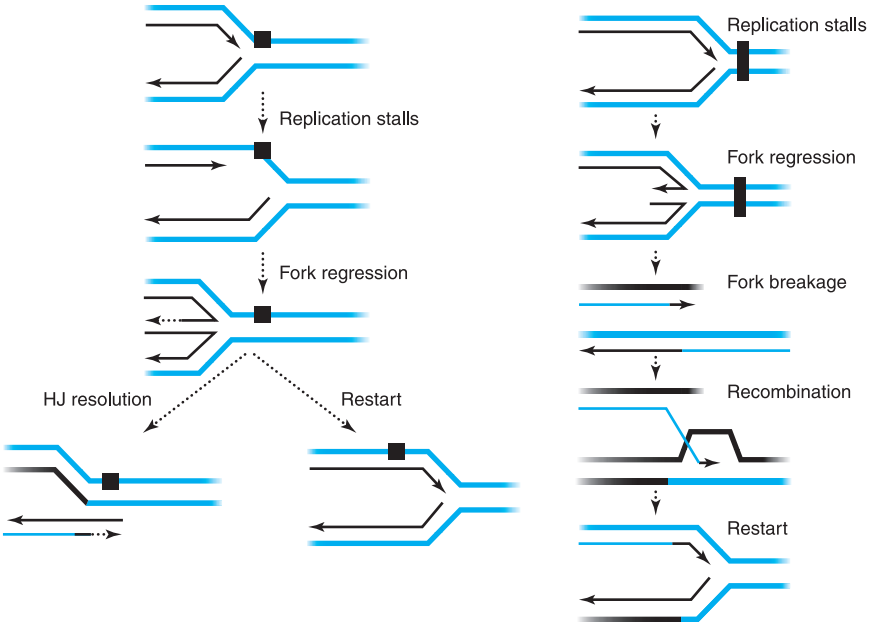

Fig. 1. Models for replication restart by recombination. (**Left**) Holliday junction (HJ) formation by fork regression allows bypass of unreplicatable DNA damage. (**Right**) A stalled fork can be restored after breakage by D-loop formation and strand invasion.

requires the replication checkpoint proteins Mec1 and Rad53 (*11*), whereas in *S. pombe* it does not. Thus, ablation of *S. cerevisiae* Mec1 removes checkpoint function and depletes dNTP pools. This results in aberrant replication intermediates (*8*) and the accumulation of DSBs (*9*).

ATR, the Mec1 homolog in mammalian cells, is an essential protein. Although a role in RNR regulation may partially explain this, it is also likely that, because chromatin architecture is more complicated in mammalian cells than in yeasts, the coordination of replication and recombination is more important for survival, because fork stalling occurs more frequently in unstressed cells. Again, analogy with the *E. coli* system is informative. Generation of a DSB by resolution of HJs formed at the replication fork is a potentially dangerous act. Studies of *E. coli* suggest that the combined activities of a nuclease (RecBCD) and a helicase (RuvAB) process HJs formed from stalled forks to reestablish the fork structure and to promote replication restart without chromosome breakage. However, there may also be situations in which HJ cleavage (RuvABC), and thus fork breakage, followed by RecBCD- and RecA-mediated recombination is the only effective way to restart replication. Thus, activities that determine the fate of a HJ formed from a fork might have critical roles in determining the frequency of DSB formation (to promote restart), and their misregulation may influence the frequency of genomic rearrangements.

If limited DNA processing by HR proteins is necessary in eukaryotic S phase to restart stalled replication, then this process may be coordinated by ATR-dependent checkpoints. In the absence of ATR, inappropriate production and/or resolution of HR-dependent DNA structures could induce DSBs. Perhaps checkpoint proteins inhibit the activity of certain HR enzymes at stalled replication sites while promoting the activity of others. Of interest in this context are the recent reports of checkpoint proteins binding to (*12*) and phosphorylating (*13*) HR proteins.

It will be important to ascertain the requirements for DNA double-strand breakage at replication stall sites in eukaryotes. The processing of the pathological structures observed by Sogo *et al*. by various nucleases could give rise to the DSBs observed by Cha and Kleckner in the absence of the replication checkpoint. It will also be of interest to investigate which specific aspects of HR are regulated by ATR-dependent checkpoints to control or suppress recombination and maintain genome stability.

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