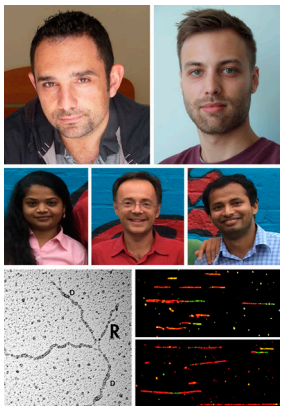
Text by Mitch Leslie
mitchleslie@comcast.net

A fresh start for stalled forks

Studies reveal mechanisms that reverse and then reactivate replication forks.



PHOTOS COURTESY OF LORENZA PENENGO (LOPES), CHRIS MOSER (ZELLWEGER), AND ALESSANDRO VINDIGNI (GOMATHINAYAGAM, VINDIGNI, AND THANGAVEL)

Focal Point

Two overlapping teams investigated how replication forks reverse and how replication recommences. In one study, Massimo Lopes (top left), Ralph Zellweger (top right), and colleagues (not pictured) show that the repair enzyme RAD51 is necessary for producing reversed forks like this one (bottom left); the newly synthesized strands are labeled "R." In the second study, a group including (center, left to right) Shivasankari Gomathinayagam, Alessandro Vindigni, Saravanabhavan Thangavel, and colleagues (not pictured) show that the nuclease DNA2 helps restart forks. In these images of DNA fibers (bottom right), the transition from red to green indicates resumption of replication, and fewer fibers restart in cells lacking DNA2 (bottom) than in controls (top).

Replication forks often run into obstacles, such as DNA damage or cross-links, that block their progress. Thangavel et al. (1) and Zellweger et al. (2) identify enzymes that enable cells to cope with these obstacles and resume replication.

When replication forks encounter a roadblock, they often perform a maneuver called fork reversal (3). The newly synthesized strands detach from their parental strands and adhere to each other. At the same time, the parental strands reconnect, partially zipping up the fork. As a result, the fork transforms into a structure, known as a reversed fork, that resembles a chicken's foot, with the newly synthesized strands forming the middle toe. Once the obstruction has been removed, the cell unravels this structure, allowing replication to proceed (4).

Fork reversal enables DNA replication to pause and then resume without chromosome breakage. But this process also permits cancer cells to survive DNA-damaging chemotherapy, so understanding how cells reverse and restart forks is important. Researchers have discovered that the DNA helicase RECQ1 spurs reversed forks to renew replication, but the mechanistic details remain unclear (5).

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Zellweger et al. started by asking what stimulates forks to reverse. The team tested a range of agents, from UV light to the anticancer drug doxorubicin, that injure DNA in different ways, including by blocking nucleotide synthesis and attacking individual bases. Nonlethal doses of all the agents triggered fork reversal in normal and cancer cells, the experiments showed. Up to 30% of forks reversed, or as many as 4,000 in the average cell.

The researchers found that the treatments interfered with coordinated DNA synthesis, creating long stretches of single-stranded DNA at so-called uncoupled forks. Single-stranded DNA also accumulates when cells use homologous recombination to fix double-strand breaks, and it attracts the enzyme RAD51, which helps repair these breaks. Zellweger et al. determined that RAD51 also gathered at reversed forks even if the DNA there wasn't broken. Moreover, if the scientists depleted RAD51, fewer reversed forks appeared in cells treated with the DNA-damaging agents, leaving more forks uncoupled.

Loss of RAD51 also boosted the number of double-strand breaks and increased fork speed upon DNA damage, suggesting that the protein protects forks by slowing fork progression and promoting reversal. By implicating a homologous recombination protein in fork remodeling and protection, the study shows that "the same pathway is presumably preventing the breaks, not just fixing them once they are formed," says senior author Massimo Lopes.

To understand how cells restart replication, Thangavel et al. probed how cells get rid of the newly synthesized strands, the middle toe in the reversed fork. The team discovered that the nuclease DNA2, which breaks down single-stranded DNA, is required for resumption of replication. They confirmed that DNA2 dissolved newly synthesized strands at forks and that cells lacking the enzyme were more likely to die.

DNA2 also helps repair double-strand breaks, but none of its partners in that task affected fork restart. However, DNA2 did need the assistance of the helicase WRN, Thangavel et al. determined. Without WRN, fewer stalled forks resumed replication.

When the researchers observed reversed forks with electron microscopy, they noticed that more of the middle prongs were double-stranded in cells lacking DNA2. They found that DNA2 breaks down one of the two newly synthesized strands. WRN's job is to open up the double helix of the fork's middle prong and allow DNA2 access to that strand. Two other proteins influence the process. RAD51 promotes DNA2's processing of reversed forks, presumably by helping to reverse the forks in the first place. In contrast, RECQ1 blocks DNA2, possibly to prevent the enzyme from destroying too much DNA and damaging the fork.

"We found an important mechanism of replication stress response," says senior author Alessandro Vindigni. DNA2 leaves behind a stretch of single-stranded DNA, and the researchers speculate that this stump might help restart the fork.

Both studies agree that RAD51 helps orchestrate fork reversal. A key question is whether other homologous recombination enzymes also take part. The two papers also suggest that targeting fork reversal might lead to improved cancer treatments.

1. Thangavel, S., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201406100.
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3. Atkinson, J., and P. McGlynn. 2009. *Nucleic Acids Res.* 37:3475–3492.
4. Petermann, E., and T. Helleday. 2010. *Nat. Rev. Mol. Cell Biol.* 11:683–687.
5. Berti, M., et al. 2013. *Nat. Struct. Mol. Biol.* 20:347–354.