BRCA1/BARD1 in mitotic spindle assembly account for chromosomal instability when its function is compromised, and how does this relate to the development of tissue-specific tumors? Given that breast and ovarian tumors are two of the most common forms of cancer and account for a substantial number of cancer-related deaths in women, answers to these questions are of vital interest to more than just cell biologists.

REFERENCES

Choudhury, A.D., Xu, H., Modi, A.P., Zhang, W., Ludwig, T., and Baer, R. (2005). J. Biol. Chem. *280*, 24669–24679.

Clarke, P.R., and Zhang, C. (2001). Trends Cell Biol. *11*, 366–371.

Hsu, L.C., and White, R.L. (1998). Proc. Natl. Acad. Sci. USA *95*, 12983–12988.

Joukov, V., Groen, A.C., Prokhorova, T., Gerson, R., White, E., Rodriguez, A., Walter, J.C., and Livingston, D.M. (2006). Cell, this issue.

Moore, W., Zhang, C., and Clarke, P.R. (2002). Curr. Biol. *12*, 1442–1447.

Sanderson, H.S., and Clarke, P.R. (2006). Curr. Biol. *16*, R466–R468.

Sankaran, S., and Parvin, J.D. (2006). J. Cell. Biochem. Published online June 30, 2006. 10.1002/jcb.21003.

Sankaran, S., Starita, L.M., Groen, A.C., Ko, M.J., and Parvin, J.D. (2005). Mol. Cell. Biol. *25*, 8656–8668.

Venkitaraman, A.R. (2002). Cell *108*, 171–182.

Yarden, R.I., Pardo-Reoyo, S., Sgagias, M., Cowan, K.H., and Brody, L.C. (2002). Nat. Genet. *30*, 285–289.

A SUMOry of DNA Replication: Synthesis, Damage, and Repair

Hannah L. Klein[1,*]

[1]Department of Biochemistry, New York University School of Medicine, New York, NY 10016, USA

*Contact: hannah.klein@med.nyu.edu

DOI 10.1016/j.cell.2006.10.019

Recombination at stalled replication forks is regulated at an early stage by sumoylation. In this issue of *Cell*, Branzei et al. (2006) show that the Ubc9/SUMO modification pathway controls the accumulation of cruciform structures at stalled forks.

All growing cells replicate their DNA during each cell cycle. Although there are multiple mechanisms to ensure fidelity by the replicative DNA polymerases, the process is still perilous. The template strands may contain lesions associated with the stalling of replication or may suffer more severe damage leading to the collapse of replication forks. These lesions associated with replication stalling are usually bypassed by translesion DNA polymerases that can accommodate modified bases in the template DNA. Another proposed mechanism for lesion bypass is template switching, a process in which the nascent sister strands pair and one is used as the template for synthesis of the stalled strand past the lesion (Figure 1).

Replication-fork reversal occurs when the checkpoint for DNA replication is defective. In the budding yeast *Saccharomyces cerevisiae*, fork stability requires the Mec1 and Rad53 checkpoint kinases (homologous to mammalian ATR and Chk2 kinases). When cells that have defective versions of these factors are subjected to conditions that lead to fork stalling, reversed forks are formed that resemble Holliday junctions (Sogo et al., 2002; Tercero and Diffley, 2001) (Figure 1). These structures are cruciform, containing four duplex strands of DNA that form during homologous recombination. However, the cruciform structures that form after fork stalling are considered pathological and are avoided by the presence of the checkpoint machinery. In extracts of eggs from the frog *Xenopus laevis*, the ATR checkpoint is required even during normal chromosomal DNA synthesis, presumably to stabilize the replication forks that stall under routine conditions and to aid the recruitment of repair factors (Shechter et al., 2004). Branzei et al. (2006) now report that sumoylation helps to prevent the accumulation of these cruciform structures at stalled forks.

When fork progression is retarded—for example, by limiting deoxyribonucleotides, introducing defective DNA polymerases, or reducing amounts of a replicative polymerase (Kokoska et al., 2000)—single-strand regions accumulate at the replication forks and recombination is increased in genetic assays. Single-strand DNA can be recombinogenic, as it is a target for the binding of Rad51 recombinase. It is also subject to breakage that can lead to the formation of recombinogenic double-strand breaks. The binding of Rad51 at rep-

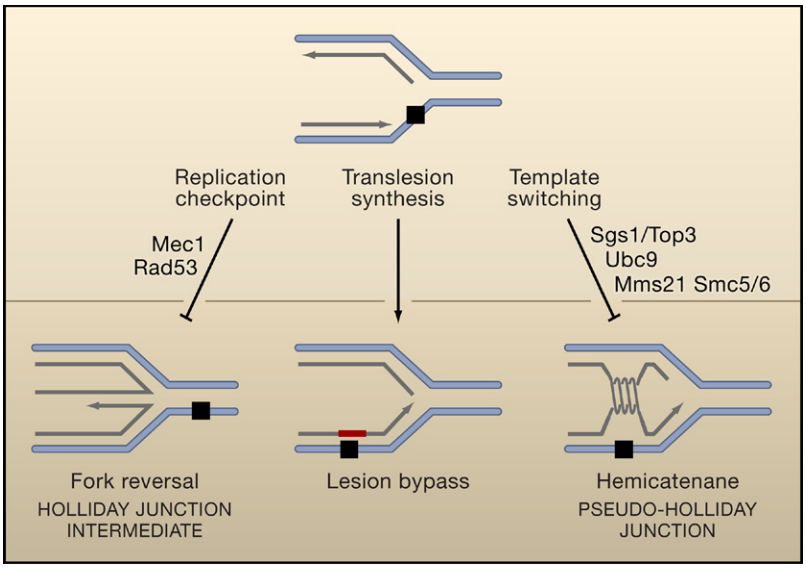

Figure 1. Formation of Recombination-like Intermediates at Stalled Replication Forks
A lesion on the template strand is shown as a black rectangle. The DNA replication checkpoint prevents fork reversal (left), which would form a four-way junction that resembles a Holliday junction. Lesion bypass (red line) can occur by translesion polymerase synthesis (center), which does not produce a recombination-like intermediate. Template switching (right) can lead to the accumulation of pseudo-Holliday junctions if the proposed hemicatenane intermediate is not dissolved. The SUMO-conjugating enzyme Ubc9, the SUMO ligase Mms21 and the DNA helicase Sgs1 in conjunction with the topoisomerase Top3 are proposed to promote the dissolution of pseudo-Holliday junctions.

lication forks is counteracted by the UvrD-like DNA helicase Srs2 through an interaction with SUMO-modified PCNA (proliferating cell nuclear antigen) (Papouli et al., 2005; Pfander et al., 2005). The recruitment of Srs2 by SUMO-modified PCNA serves to regulate the initiation of recombination at stalled forks and to promote bypass synthesis by translesion DNA polymerases. It has been shown that the sumoylation of PCNA requires the E3 factors Siz1 and Siz2.

The Foiani group has previously shown that the RecQ-like DNA helicase Sgs1 prevents the formation of cruciform structures at replication forks that encounter damaged template strands (Liberi et al., 2005). The human BLM DNA helicase, a member of the RecQ family, can dissolve Holliday junctions in vitro (Wu and Hickson, 2003) in conjunction with the topoisomerase hTOPO IIIα. BLM is mutated in Bloom's syndrome, a disorder characterized by a high frequency of chromosome breaks and rearrangements and sister-chromatid recombination. It can be inferred that Sgs1 (in conjunction with the yeast Top3 topoisomerase) has the same activity as BLM and that the cruciform structures represent unresolved true or pseudo-Holliday junctions. This idea is also supported by the findings that Bloom's syndrome is characterized by increased sister-chromatid exchange and that yeast *sgs1* mutants have increased crossover frequencies (Ira et al., 2003). One can conclude from these observations that, when recombination occurs, it is regulated at the final stages of resolution to prevent or limit crossovers.

Branzei et al. (2006) now show that formation of cruciform structures at damaged replication forks is also regulated by SUMO modification. They extend their observations to suggest that this SUMO modification normally controls replication termination and promotes template switching when encountering a fork-stalling lesion. Such insights were obtained when the investigators tested a temperature-conditional allele of the *UBC9* gene of *S. cerevisiae* called *ubc9-1*. *UBC9* encodes a SUMO-conjugating enzyme, and studies suggest that mouse Ubc9 can sumoylate target proteins without a SUMO ligase. The *ubc9-1* mutant completes DNA replication at the restrictive temperature but accumulates DNA lesions and requires homologous recombination functions for viability. Examination of DNA structures in the mutant showed that, under damage conditions, cruciform structures accumulated. This phenotype was similar to that of yeast *sgs1* mutants but dissimilar to *srs2* mutants. Similarly, mutants that affect PCNA sumoylation, *siz1* and *siz2*, did not accumulate cruciform structures. This suggests either that cruciform structures reflect a late stage of recombination intermediates at replication forks or that they might be formed from a reaction that does not involve a strand invasion. The Mms21 SUMO ligase, which is not required for PCNA sumoylation, was required to counteract cruciform accumulation at stalled forks.

The targets of Ubc9 and Mms21 involved in the prevention of cruciform-structure formation at damaged forks are not known. One obvious candidate is the Sgs1 helicase. Sgs1 is sumoylated, and this could affect its recruitment to the hemicatenane structures (in which the two DNA molecules are linked by single-strand intertwining) (Figure 1). Additionally, Sgs1 sumoylation could also affect its association with other proteins or its activity. Branzei et al. (2006) find that Sgs1 interacts with Ubc9 and that Sgs1 sumoylation is dependent on Ubc9 activity, but not on Mms21 activity. Mms21 has other targets, including Smc5 of the Smc5/6 complex, which is required for DNA repair through sister-chromatid recombination. The Smc5/6 complex has been proposed to prevent accumulation of branched structures at collapsed replication forks (Lindroos et al., 2006). Because both *UBC9* and *MMS21* are essential genes, it is difficult to determine whether they act

in separate pathways or processes to prevent cruciform accumulation. However, the homolog of *SGS1* in the fission yeast *Schizosaccharomyces pombe*, *rqh1*, genetically interacts with genes encoding the Smc5/6 complex, suggesting that the Smc5/6 complex, which includes the Mms21 subunit, is needed for the resolution or dissolution of these cruciform structures.

Although the formation of cruciform structures at damaged forks is dependent on the recombinase Rad51, this does not necessarily mean that they are formed by homologous recombination. Rad51 could stabilize a proposed hemicatenane intermediate of template switching by binding to the paired nascent strands. This would stabilize the pseudo-Holliday junctions and lead to an accumulation of the cruciform structures seen on two-dimensional gels. Thus, recombination-like intermediates would accumulate, promoted by a homologous recombination factor, without the actual occurrence of a double-strand break and strand invasion. If the pseudo-Holliday junction intermediate is not dissolved by the Sgs1/Top3 helicase-topoisomerase complex, it could be a target for nicking by structure-specific endonucleases that could form double-strand breaks and promote recombination. This, then, could be the real pathological consequence of damaged forks: the provocation of recombination during replication. Indeed, the other pathological structure suggested to occur at forks is the reversed-fork four-way structure, which resembles a Holliday junction. These are observed when the replication checkpoint does not stabilize a stalled fork. This, too, is a case where a recombination intermediate forms without homologous recombination.

Why would it be necessary to regulate Sgs1 activity through sumoylation? The authors note that the DNA structures occurring during the termination of replication have some similarity to the proposed hemicatenane intermediate for template switching. Errors in termination, which might occur in *ubc9* or *sgs1* mutants under non-damaging conditions, could lead to increased DNA breaks and genomic rearrangements. Sgs1 sumoylation might direct its activity to structures that form during replication termination through association with some component of the replication apparatus but would not involve Sgs1 in the resolution of true homologous recombination intermediates found during G2. Although most mitotic homologous recombination events are noncrossover events and occur by synthesis-dependent strand annealing, true reciprocal crossovers do occur in mitotic cells and are presumably the result of resolution of Holliday junctions by endonucleases. Therefore, it will be interesting to determine whether the Smc5/6 complex or Sgs1 is involved in replication termination.

The implications of this study can be extended to meiotic recombination and chromosome segregation. Significant chromosome missegregation occurs at meiosis I in *sgs1* mutants due to excess crossing-over, particularly near the centromeric regions, which are usually devoid of crossovers (Rockmill et al., 2006). The recent study by Rockmill et al. also suggests that crossovers are linked to local loss of sister-chromatid cohesion and that loss of cohesion near the centromeres might lead to separation of centromeres in meiosis I and precocious sister-chromatid separation, with ensuing chromosome missegregation. Unregulated crossover recombination in mitosis might also lead to chromosome missegregation in a similar fashion. *SGS1* and *BLM* mutant cells are characterized by increased chromosome loss. Thus, factors that act on Holliday junction-like intermediates must be regulated to dissolve these structures and prevent crossovers. How sumoylation might affect Sgs1 activity in meiotic recombination is not known, but the *Drosophila UBC9* homolog regulates chromosome disjunction in meiosis I, and the *S. cerevisiae UBC9* gene affects chromosome synapsis (the close pairing of homologous chromosomes during meiosis). It is possible that other Ubc9 targets that function in chromosome pairing and recombination might also regulate chromosome synapsis.

REFERENCES

Branzei, D., Sollier, S., Liberi, G., Zhao, X., Maeda, D., Seki, M., Enomoto, T., Ohta, K., and Foiani, M. (2006). Cell, this issue.

Ira, G., Malkova, A., Liberi, G., Foiani, M., and Haber, J.E. (2003). Cell *115*, 401–411.

Kokoska, R.J., Stefanovic, L., DeMai, J., and Petes, T.D. (2000). Mol. Cell. Biol. *20*, 7490–7504.

Liberi, G., Maffioletti, G., Lucca, C., Chiolo, I., Baryshnikova, A., Cotta-Ramusino, C., Lopes, M., Pellicioli, A., Haber, J.E., and Foiani, M. (2005). Genes Dev. *19*, 339–350.

Lindroos, H.B., Strom, L., Itoh, T., Katou, Y., Shirahige, K., and Sjogren, C. (2006). Mol. Cell *22*, 755–767.

Papouli, E., Chen, S., Davies, A.A., Huttner, D., Krejci, L., Sung, P., and Ulrich, H.D. (2005). Mol. Cell *19*, 123–133.

Pfander, B., Moldovan, G.L., Sacher, M., Hoege, C., and Jentsch, S. (2005). Nature *436*, 428–433.

Rockmill, B.M., Voelkel-Meiman, K., and Roeder, G.S. (2006). Genetics. Published online October 8, 2006. 10.1534/genetics.106.058933.

Shechter, D., Costanzo, V., and Gautier, J. (2004). Nat. Cell Biol. *6*, 648–655.

Sogo, J.M., Lopes, M., and Foiani, M. (2002). Science *297*, 599–602.

Tercero, J.A., and Diffley, J.F. (2001). Nature *412*, 553–557.

Wu, L., and Hickson, I.D. (2003). Nature *426*, 870–874.