nonstress conditions. The authors show that the association of SRp38 with 14-3-3 proteins, a family of highly conserved phospho-serine/threonine binding proteins, protects SRp38 from dephosphorylation under nonstress conditions. Importantly, this complex dissociates upon heat shock.

As with every new exciting finding, some important issues are still left to be addressed. One intriguing question remaining is whether the mechanism underlying splicing arrest during mitosis is similar to that described for heat shock. Furthermore, SRp38 protein expression is nearly absent in some tissues (e.g., heart, kidney, and liver [Shin and Manley, 2002]) raising the question of how these cell types regulate mRNA splicing in response to heat shock.

The most remarkable finding of the work from Shi and Manley (2007) is that the specificity of SRp38 dephosphorylation following heat shock is mainly due to the differential activity of SR protein kinases on different SR proteins. This work has now established a complex pathway that controls SRp38 phosphorylation status and regulates its activity with high specificity in response to heat shock.

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Reversal of Fortune: Rad5 to the Rescue

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In a recent issue of *Molecular Cell*, Blastyák et al. (2007) show that the yeast Rad5 protein can promote error-free template switching and replication past a DNA lesion via a novel DNA unwinding reaction that also pairs nascent and parental strands.

DNA lesions are usually repaired by a templated process. The nucleotide excision repair, base excision repair, and mismatch repair pathways follow the paradigm of excision of a single-stranded DNA fragment flanking the damaged site followed by repair synthesis using the undamaged complementary single strand as a template. Repair of double-strand breaks by homologous recombination is also templated, using strand invasion from the processed double-strand break end into homologous duplex DNA to initiate gap repair synthesis using the intact strands of the invaded duplex as a template. In contrast, DNA lesions on the template strands stall replication forks and do not have a natural template for repair as the damaged template strand becomes single stranded during replication. To continue replication two modes of repair can occur, which have been termed postreplication repair and are promoted by a group of proteins that modify the PCNA clamp at the stalled replication fork (Hoege et al., 2002).

When the replication fork stalls at a lesion on the template strand, it is thought that leading and lagging strand synthesis become uncoupled and synthesis continues only on the undamaged template strand (Pages and Fuchs, 2003). If this is the lagging strand template, the gap that occurs can be filled in by synthesis from the adjacent Okazaki fragment. However, if the damage is on the template for the leading strand, then a gap will be formed that cannot be repaired by fill-in synthesis using the replicative DNA polymerases. Excision of the damaged region on the template strand would create a double-strand break that would destroy the replication fork. To avoid this, an alternative mode of repair is used which, strictly speaking, is in fact not a repair event. Instead it is a damage tolerance event that gets around or bypasses the damaged region. What is really repaired is the gap at or behind the replication fork, while the lesion in the template strand remains. One method of achieving this is through the translesion synthesis DNA polymerases Polζ and Polη,

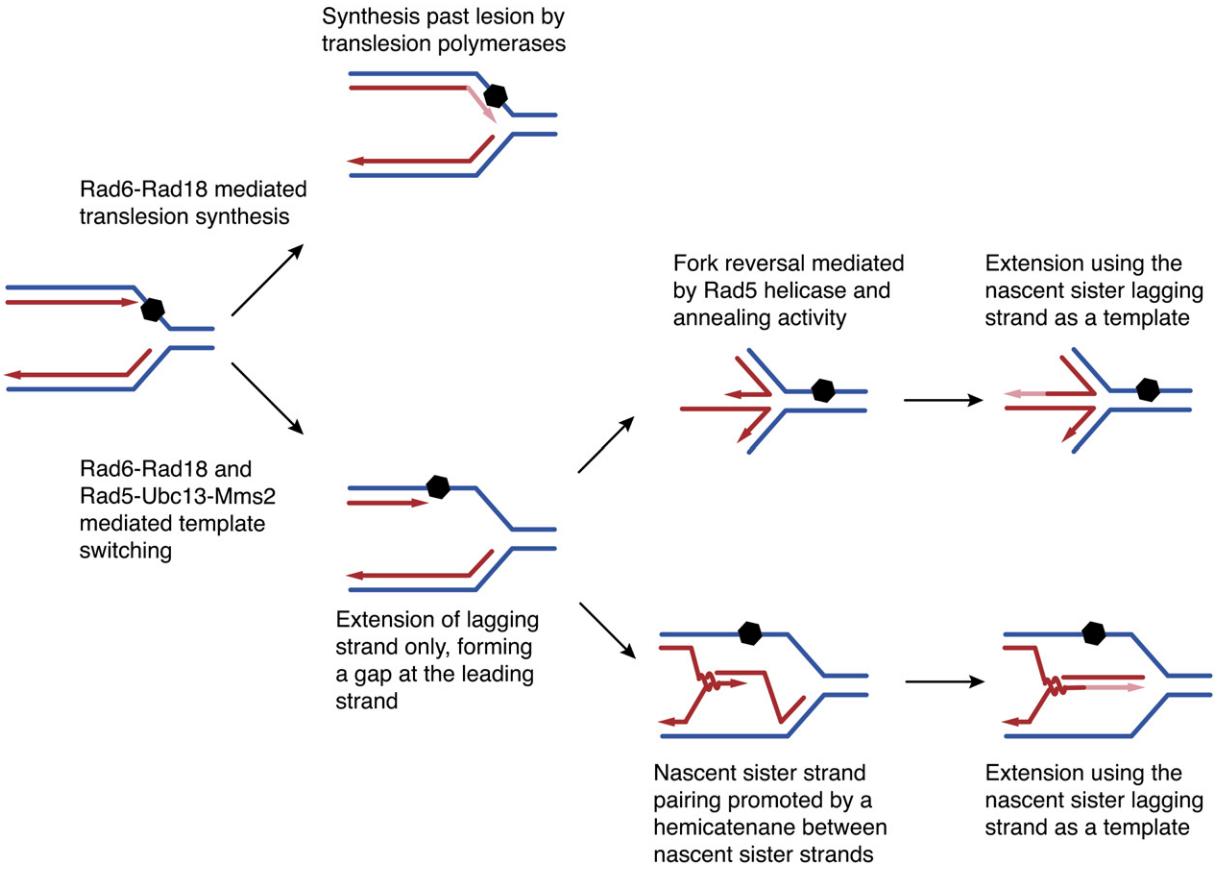

Figure 1. DNA Damage Tolerance and Rad5 in Template Switching
When DNA replication is stalled by a lesion in the template strand, Rad6-Rad18 can promote synthesis of the stalled nascent strand past the lesion by Rad6-Rad18 monoubiquitination of PCNA and recruitment of translesion polymerases to the stalled site. PCNA can be further modified by polyubiquitination through Rad5-Ubc13 and Mms2 to promote lesion bypass through template switching. In this figure, the lagging strand is extended, leaving a gap at the lesion on the leading strand. Rad5 is postulated to promote replication fork reversal through combined helicase and annealing activities to form a four-way junction with paired nascent strand and template strands. The nascent lagging strand can now serve as a template for extension of the leading strand. Eventually the reversed fork must be regressed through a reversed branch migration reaction to reform the three-way junction fork and complete replication. Alternatively, the nascent sister strands may be held together by hemicatenanes, which may be acted on by Rad5. The hemicatenane structure can then be extended using the lagging strand as template for the leading strand. Again, the nascent strands must be repaired with the template strands to continue replication.

which can replicate past damaged bases due to a more relaxed and open structure. Repair via Polζ is termed error prone as the polymerase tends to insert incorrect bases opposite damaged bases, while repair via Polη is referred to as error free as this polymerase is more accurate when bypassing UV adducts. Recruitment of the translesion polymerases to stalled replication forks is promoted by ubiquitin modification of PCNA through the Rad6 ubiquitin-conjugating enzyme and the Rad18 ubiquitin ligase that is also a DNA-binding protein.

The second method for replication past a lesion on the template strand is proposed to occur by a copy-choice process now called template switching. In this model, first proposed in 1976 independently by Bernard Strauss and coworkers (Higgins et al., 1976) and by Fujiwara and Tatsumi (Fujiwara and Tatsumi, 1976), the stalled leading strand is extended using the correctly replicated nascent lagging sister strand through a combined action of strand displacement and branch migration (see Figure 1) that reverses the replication fork, allows repair synthesis, and then reassociates the newly replicated strands with the parental template strands, thus reversing the replication fork again and allowing replication to continue downstream of the site of the lesion and leading strand stalling. Experimental evidence for such a model has come from density shift experiments that identified paired newly replicated sister strands after DNA damage and from electron microscopy that visualized reversed forks, the so-called "chicken-foot" structure and four-way junction molecules (Higgins et al., 1976; Fujiwara and Tatsumi, 1976; Cotto-Ramusino et al., 2005).

Genetic studies in *S. cerevisiae* have identified three genes, *MMS2*, *UBC13*, and *RAD5*, which are essential for this mode of damage tolerance and

are separate from the translesion DNA polymerases (Xiao et al., 2000). *MMS2*, *UBC13*, and *RAD5* form a separate sub-branch of the *RAD6*/*RAD18* pathway; Ubc13 is a ubiquitin-conjugating enzyme that forms a heterodimer with Mms2, whereas Rad5, a member of the SWI/SNF family, has ATPase and E3 ubiquitin ligase activities. These activities are required for DNA damage tolerance and act on monoubiquitinated PCNA to form lysine 63-linked polyubiquitin chains. Thus strong genetic evidence linked Rad5 to template switching, but no biochemical activity for a direct action on stalled replication forks was known because Rad5 exhibits no DNA helicase activity in vitro, using a standard DNA helicase reaction that involves displacement of a short paired strand from a single-stranded molecule (Johnson et al., 1994). Now, Blastyák et al. (2007) show that Rad5 can reverse a modeled stalled replication fork in a helicase-like reaction that does not expose any single-stranded DNA.

The first hint that Rad5 could act on nonlinear DNA structures came from the finding that Rad5 ATPase activity was stimulated by branched DNAs formed from oligonucleotide reannealing to yield three-way and four-way junctions. Further studies showed that Rad5 binds to and unwinds three-way junctions that resembled replication forks, but only if the arms are homologous. This finding implied that reannealing of the nascent strands, which could form a four-way junction or chicken foot (see Figure 1), is an important part of the Rad5 activity. Lastly, the authors show that Rad5 can reverse an asymmetric fork, similar to one that would be formed after uncoupling of leading and lagging strand synthesis. These experiments provide biochemical support for the action of Rad5 in replication fork reversal. While accumulation of a chicken-foot structure in the absence of a functional replication checkpoint has been termed pathological, transient fork reversal in the context of a stable replication fork might not be so. An alternative structure might involve pairing of the nascent sister strands promoted by a hemicatenane linkage (see Figure 1).

Replication fork reversal has been proposed as a way to restart stalled replication forks through several modes: through template switching and repair synthesis on the paired nascent sister strands, through repair of damage through an excision-repair process on the annealed parental strands, or through cleavage of the four-way junction of the chicken foot and subsequent homologous recombination into the sister chromatid for a recombination-based reinitiation of replication event. *RAD5* also acts in a double-strand break repair pathway that is independent of its ubiquitin ligase activity (Chen et al., 2005), and sister-strand recombination dependent on Rad5 activity has been proposed as a mechanism for DNA damage tolerance (Zhang and Lawrence, 2005). It is possible that the Rad5 E3 ligase activity promotes the synthesis part of the template switching reaction while the ATPase-based helicase-annealing activity is required for any DNA configuration need for template switching or recombination-based replication restart.

A new player in postreplication repair might be the *EXO1* endonuclease, which acts on reversed replication forks that accumulate when the DNA polymerase is destabilized at stalled replication forks (Cotto-Ramusino et al., 2005). Here, Exo1 might limit the extent of replication fork reversal by processing the paired nascent sister strands. This might be important because the reversed forks have to be regressed after repair synthesis. It is not known whether Rad5 also promotes this reaction and, if so, whether there is a limit to the length of paired structure that could be regressed. Exo1 could promote regression in concert with Rad5. Regardless of the mechanisms for replication reversal and regression, the identification of the Rad5 helicase/branch migration activity identified by Blastyák et al. opens the door for more exciting biochemistry on this reaction.

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