seen with NAADP; however, future work needs to be carried out to clarify this point.

Besides a two-pool/two-receptor model — i.e. TPC2 or TRP-ML1 on lysosomes as producers of the Ca^{2+} trigger upon NAADP binding and further amplification by RyRs or IP_3Rs at the SR/ER — Hohenegger *et al.* [2] introduced RyR1 as a Ca^{2+} channel that was directly activated by NAADP. These authors used a similar approach as Zhang and colleagues [3] and analyzed the open probability of RyR1 in lipid planar bilayers. In contrast to the work with TRP-ML1, Hohenegger and colleagues [2] used highly purified RyR1 preparations, as judged by protein staining and western blot. Single channel currents typical for RyR1 were obtained; the open probability was increased in a concentration-dependent manner in a similar NAADP range as reported for TRP-ML1 [3] or TPC2 [4]. However, a bell-shaped curve for NAADP was not seen under these conditions [2]. Nevertheless, the mechanism of action of NAADP was also determined: NAADP shifted RyR1 into a state with longer open times, while the single channel amplitude remained unchanged [2]. As specificity controls, 2′-phosphate and 3′-phosphate forms of nicotinamide adenine dinucleotide phosphate (NADP) were used and found not to have any effect. Moreover, the RyR antagonist ruthenium red inhibited NAADP-evoked activation of RyR1 [2]. In Hohenegger's study, no RNAi or knock-out approach was used to verify the data; however, gene silencing of RyR by an antisense construct in Jurkat T cells has since been reported to lead to a loss of both small local and global Ca^{2+} signals upon microinjection of NAADP [6,7], adding support to this story. Further evidence for RyR1 directly acting as an NAADP-sensitive Ca^{2+} channel, at least in some cell systems, comes from a recent study showing a significant enhancement of channel opening of highly purified RyR1 upon NAADP addition [8].

In conclusion, convincing evidence has been presented now that RyR1 and TPC2 are NAADP-sensitive Ca^{2+} channels. Given that the two-pool model includes RyR (or IP_3R) as amplifiers, one may argue that, in all studies favoring RyR1 as NAADP receptor, TPC2 was present as an impurity, giving rise to (undetectable) small Ca^{2+} signals that were then further amplified by RyR1. Although the studies with highly purified RyR1 [2,8] would argue against such an interpretation, it cannot be totally excluded. Now that the molecular identity and potential functional role of TPC2 has been released to the community, it can, however, be put to the test tube. Hopefully, the controversial issues around the NAADP receptor will be solved in the near future.

References

1. Guse, A.H., and Lee, H.C. (2008). NAADP: a universal Ca^{2+} trigger. Sci. Signal. *1*, re10.
2. Hohenegger, M., Suko, J., Gscheidlinger, R., Drobny, H., and Zidar, A. (2002). Nicotinic acid-adenine dinucleotide phosphate activates the skeletal muscle ryanodine receptor. Biochem. J. *367*, 423–431.
3. Zhang, F., and Li, P.-L. (2007). Reconstitution and characterization of a nicotinic acid adenine dinucleotide phosphate (NAADP)-sensitive Ca2+ release channel from liver lysosomes of rats. J. Biol. Chem. *282*, 25259–25269.
4. Calcraft, P.J., Ruas, M., Pan, Z., Cheng, X., Arredouani, A., Hao, X., Tang, J., Rietdorf, K., Teboul, L., Chuang, K.T., *et al*. (2009). NAADP mobilizes calcium from acidic organelles through two-pore channels. Nature *459*, 596–600.
5. Churchill, G.C., Okada, Y., Thomas, J.M., Genazzani, A.A., Patel, S., and Galione, A. (2002). NAADP mobilizes Ca^{2+} from reserve granules, lysosome-related organelles, in sea urchin eggs. Cell *111*, 703–708.
6. Dammermann, W., and Guse, A.H. (2005). Functional ryanodine receptor expression is required for NAADP-mediated local Ca^{2+} signaling in T-lymphocytes. J. Biol. Chem. *280*, 21394–21399.
7. Langhorst, M.F., Schwarzmann, N., and Guse, A.H. (2004). Ca^{2+} release via ryanodine receptors and Ca^{2+} entry: major mechanisms in NAADP-mediated Ca^{2+} signaling in T-lymphocytes. Cell. Signal. *16*, 1283–1289.
8. Dammermann, W., Zhang, B., Nebel, M., Cordiglieri, C., Odoardi, F., Kirchberger, T., Kawakami, N., Dowden, J., Schmid, F., Dornmair, K., *et al*. (2009). NAADP mediated Ca^{2+} signaling via type 1 ryanodine receptor in T cells revealed by a synthetic NAADP antagonist. Proc. Natl. Acad. Sci. USA, in press. DOI: 10.1073/pnas.0809997106.
9. Kiselyov, K., Chen, J., Rbaibi, Y., Oberdick, D., Tjon-Kon-Sang, S., Shcheynikov, N., Muallem, S., and Soyombo, A. (2005). TRP-ML1 is a lysosomal monovalent cation channel that undergoes proteolytic cleavage. J. Biol. Chem. *280*, 43218–43223.
10. Zissimopoulos, S., and Lai, F.A. (2007). Ryanodine receptor structure, function and pathophysiology. In Calcium: A Matter of Life or Death, J. Krebs and M. Michalak, eds. (Amsterdam: Elsevier), pp. 287–342.
11. Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N., Lai, F.A., Meissner, G., and MacLennan, D. (1990). Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. J. Biol. Chem. *265*, 2244–2256.

The Calcium Signalling Group, Institute of Biochemistry and Molecular Biology I, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany.
E-mail: guse@uke.de

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DNA Repair: Common Approaches to Fixing Double-Strand Breaks

In bacteria, the RecF pathway plays an important role in the repair of DNA breaks and gaps. Reconstitution of this reaction *in vitro* has revealed similarities with double-strand break repair in eukaryotes.

Kevin Hiom

Single-strand or double-strand breaks are potentially lethal forms of DNA damage found in all organisms [1]. To counteract this threat, organisms have evolved several mechanisms to repair these lesions. One strategy, used widely in eukaryotes, archaea and some bacteria, is simply to rejoin broken DNA ends, regardless of the DNA sequence at the break, a process referred to as non-homologous end-joining. However, because one or more nucleotides are often lost at the site of the break, this mechanism is potentially mutagenic. A second strategy, which enables more accurate repair of DNA breaks, is homologous recombination. This makes use of a second undamaged copy of the chromosome, generated during DNA replication, as a template to faithfully restore the genetic information at the repaired break.

In bacteria such as *Escherichia coli*, homologous recombination is the

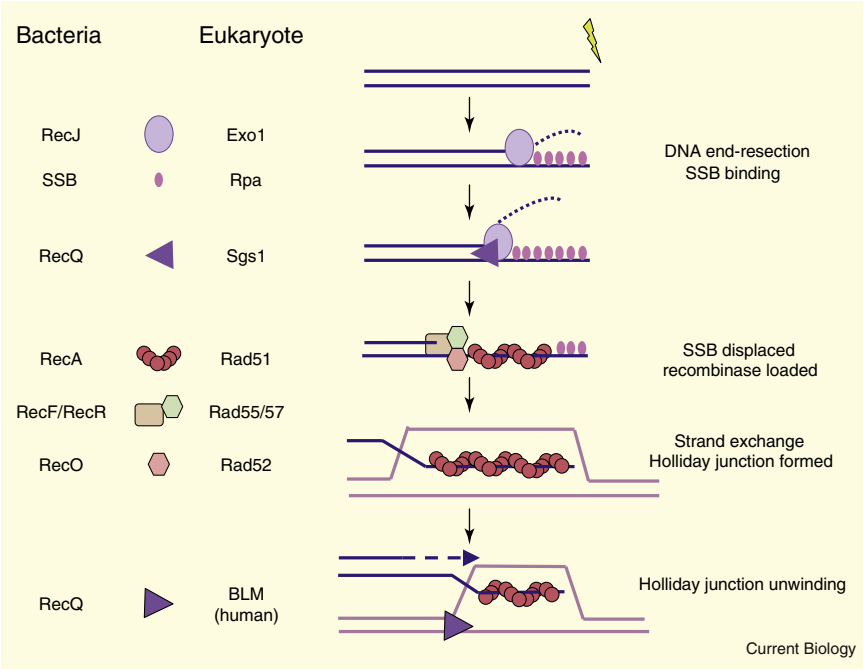

Figure 1. A model for the repair of DNA double-strand breaks by the RecF pathway.

The components shown by Handa *et al.* [3] to be involved in each step of the RecF pathway in bacteria are indicated. Also indicated are the proteins that perform an equivalent function in eukaryotes. Repair of a double-strand break is initiated by the degradation of one strand of the DNA duplex in a 5′–3′ direction by the RecJ endonuclease. This process is stimulated in the presence of the RecQ helicase. The single-stranded DNA generated by DNA end-resection is rapidly bound by SSB. RecFOR binds the junction between single-stranded DNA and double-stranded DNA where it displaces SSB and recruits the RecA recombinase. RecA binds cooperatively to form a nucleoprotein filament along single-stranded DNA, which promotes the search for homology and exchange of homologous DNA strands. After DNA synthesis to restore the genetic information at the break site the Holliday junction intermediate may be dissolved by the action of a RecQ helicase to give a gene conversion type recombination product. The functional similarities with proteins in eukaryotes from yeast to man suggest that the RecF pathway may provide the blueprint for double-strand break repair in humans. (Adapted with permission from [3].)

predominant pathway for repairing DNA breaks. Moreover, there are two overlapping, but distinct, homologous recombination pathways through which this occurs. Most commonly, double-strand breaks are processed and repaired through a pathway involving the multifunctional helicase/nuclease complex RecBCD (the RecBCD pathway). This pathway has been well-characterised biochemically, and the recently solved crystal structure of RecBCD has provided great insight into the molecular mechanism underlying this pathway [2].

Double-strand breaks in *E.coli* can also be repaired through a second pathway, called the RecF pathway. While this pathway is primarily involved in the homology-directed repair of single-stranded DNA gaps formed during replication of a damaged DNA template, it can also substitute for RecBCD in the repair of double-strand breaks. Compared with the RecBCD pathway, however, the RecF pathway is much less well understood.

Now, Handa *et al.* [3] have reconstituted the early steps in RecF-mediated double-strand break repair *in vitro*, revealling a ‘new’ activity for an ‘old’ protein, which explains how broken DNA ends are processed to initiate homologous recombination through this pathway. Moreover, the biochemical similarities between the components of the RecF pathway and several eukaryotic proteins involved in homologous recombination suggest that this pathway may provide the blueprint for homologous recombination-mediated double-strand break repair in higher organisms.

Handa *et al.* [3] used purified components of the RecF pathway to examine the formation of recombination intermediates, called joint molecules, from linear double-stranded DNA and homologous supercoiled DNA. This reaction resembles the early steps of homologous recombination-mediated double-strand break repair involving DNA end-processing and homologous pairing. They found that, *in vitro*, joint molecules can be formed in a minimal reaction comprising only seven proteins: RecF, RecO, RecR, RecA, RecJ, RecQ and the single-strand-binding protein SSB. They further found that, whereas RecO, RecR, RecA and RecJ are essential for joint molecule formation, RecQ, RecF and SSB are stimulatory.

A key step shared by all homologous recombination pathways is the recruitment of a recombinase, which in bacteria is RecA, where it binds co-operatively to single-stranded DNA to form a nucleoprotein filament [4]. It is this filament which drives the pairing and exchange of homologous DNA molecules. First, however, the broken DNA ends must be processed to generate single-stranded DNA tails onto which RecA can load.

Previous studies identified the RecJ endonuclease as an important component in the resection of DNA ends. Until now it was thought that this nuclease worked only on single-stranded DNA and, therefore, first required the action of a DNA helicase, such as RecQ, to unwind the DNA duplex. However, Handa *et al.* [3] found that RecJ can act alone to degrade one strand of a DNA duplex, generating the 3′-single-stranded DNA tails that are perfect for the recruitment of RecA and formation of joint molecules. Although RecQ is not required for DNA end-resection, joint molecules are formed more efficiently in its presence, suggesting that it may increase the processivity of RecJ on DNA.

In cells, single-stranded DNA is rapidly coated with SSB, which must be displaced in order to load RecA. This function is performed by RecF, RecO and RecR [5]. More specifically, a complex of RecO and RecR binds to SSB-covered DNA, displaces the SSB from DNA and nucleates binding of RecA [6]. Once bound, RecA cooperatively assembles a nucleoprotein filament along single-stranded DNA with a defined 5′–3′ polarity. For efficient homologous recombination it is important that the entire 3′-single-stranded DNA tail

is covered by RecA. Handa *et al.* [3] show that this is achieved through the action of RecF, which binds the single-stranded DNA tail 5′ at the junction with double-stranded DNA, where it tethers RecOR to load RecA.

What then is the role of RecQ? Genetic analyses suggest that RecQ may have roles at both early and late steps in recombination [7]. This appears to be true for the biochemical system too. As outlined above, RecQ acts early in recombination to promote the formation of joint molecules by stimulating RecJ mediated DNA-end resection. Paradoxically, at high concentrations, RecQ also disrupts formation of joint molecules. However, this may not be as perverse as it seems. For example, Holliday junctions, formed to provide a template for DNA repair via synthesis in synthesis-dependent strand annealing, are subsequently unwound to generate non-crossover, gene-conversion-type repair products. Similarly, topologically linked recombination intermediates are resolved through the concerted Holliday junction unwinding and decatenation by RecQ and topoisomeraseIII [8].

The study by Handa *et al.* [3] shows that, together, the components of the RecF pathway act in a concerted manner to promote double-strand break repair through homologous recombination (Figure 1). But can this tell us anything about the repair of double-strand breaks in more complex organisms?

Interestingly, although most double-strand break repair in bacteria occurs through the RecBCD pathway, no homologue of this complex has been identified in eukaryotes. By contrast many of the components of the RecF pathway do have structural or functional equivalents in archaea and also in eukaryotes (Figure 1). Most obvious is the close structural and functional similarity of the RecA and RAD51 recombinases of bacteria and eukaryotes, respectively, which supports the notion that homologous pairing and strand exchange are mechanistically similar in all organisms [9].

No obvious eukaryote homologue of RecJ has been found; instead, DNA end-resection in eukaryotes is performed by the nuclease Exo1. Nevertheless, Handa *et al.* [3] note that, like RecJ in bacteria, Exo1 in yeast is assisted in DNA resection by a RecQ-like helicase, Sgs1 [10,11]. Similarly, in the thermophilic archaea *P. furiosus*, end resection is achieved through the cooperative action of a helicase and nuclease, in this case HerA and NurA [12].

In yeast, the role of bacterial RecFOR in the displacement of single-strand binding protein (RPA in eukaryotes) and loading of the recombinase is performed by mediator proteins. While Rad52 takes the role of RecO, the functional equivalents of RecF and RecR are likely Rad55/57 in yeast or one of the various RAD51 paralogs (RAD51B, C and D) in humans.

In complex eukaryotes, there are several RecQ-like helicases, including WRN and BLM proteins, which are associated with the inherited human disorders Werner's syndrome and Blooms syndrome, respectively [13]. Whereas in yeast the RecQ-like protein Sgs1 acts early in homologous recombination with Exo1, other RecQ homologues may, like their bacterial counterpart, also have a later role. BLM, for example, can be seen as a direct functional equivalent of bacterial RecQ in the resolution of catenanes with topoisomerase III [14].

The functional similarities between components of the RecF pathway and homologous recombination proteins in archaea and eukaryotes are clearly evident (Figure 1). Moreover, it appears that, in evolution, double-strand break repair by homologous recombination in complex organisms more closely resembles the RecF 'back-up pathway' in bacteria than it does the 'primary' RecBCD pathway. And, once again, we find that the mechanism through which humans protect their genome from harmful DNA damage is foreshadowed by our bacterial relatives.

References

1. Kuzminov, A. (1999). Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. Microbiol. Mol. Biol. Rev. *63*, 751–813.
2. Singleton, M.R., Dillingham, M.S., Gaudier, M., Kowalczykowski, S.C., and Wigley, D.B. (2004). Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. Nature *432*, 187–193.
3. Handa, N., Morimatsu, K., Lovett, S.T., and Kowalczykowski, S.C. (2009). Reconstitution of initial steps of dsDNA break repair by the RecF pathway of E. coli. Genes Dev. *23*, 1234–1245.
4. Stasiak, A., DiCapua, E., and Koller, T. (1983). Unwinding of duplex DNA in complexes with recA protein. Cold Spring Harb. Symp. Quant. Biol. *2*, 811–820.
5. Morimatsu, K., and Kowalczykowski, S.C. (2003). RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. Mol. Cell *11*, 1337–1347.
6. Umezu, K., Chi, N.W., and Kolodner, R.D. (1993). Biochemical interaction of the Escherichia coli RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. Proc. Natl. Acad. Sci. USA *90*, 3875–3879.
7. Lovett, S.T., and Sutera, V.A., Jr. (1995). Suppression of recJ exonuclease mutants of Escherichia coli by alterations in DNA helicases II (uvrD) and IV (helD). Genetics *140*, 27–45.
8. Harmon, F.G., Brockman, J.P., and Kowalczykowski, S.C. (2003). RecQ helicase stimulates both DNA catenation and changes in DNA topology by topoisomerase III. J. Biol. Chem. *278*, 42668–42678.
9. Benson, F.E., Stasiak, A., and West, S.C. (1994). Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. EMBO J. *13*, 5764–5771.
10. Gravel, S., Chapman, J.R., Magill, C., and Jackson, S.P. (2008). DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes Dev. *22*, 2767–2772.
11. Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell *134*, 981–994.
12. Hopkins, B.B., and Paull, T.T. (2008). The P. furiosus mre11/rad50 complex promotes 5′ strand resection at a DNA double-strand break. Cell *135*, 250–260.
13. Wu, L., and Hickson, I.D. (2006). DNA helicases required for homologous recombination and repair of damaged replication forks. Annu. Rev. Genet. *40*, 279–306.
14. Wu, L., and Hickson, I.D. (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature *426*, 870–874.

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK.
E-mail: hiom@mrc-lmb.cam.ac.uk

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