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DNA Repair: Bacteria Join In Dispatch

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In higher organisms, a major pathway for repairing double stranded breaks in DNA is non-homologous end-joining. Now a similar pathway has been shown to operate in bacterial cells, indicating that this important repair mechanism has been conserved through evolution.

For the genetic integrity of chromosomes to be maintained, it is essential that double-stranded breaks in DNA are repaired efficiently. These DNA breaks may arise as a consequence of exposure to harmful DNA damaging agents, such as ionizing radiation, or through errors in cellular functions, such as replication. Furthermore, double-stranded DNA breaks also occur as normal intermediates during V(D)J recombination, the mechanism for assembling antibody genes from multiple gene segments. Because a single unrepaired DNA break may be lethal to a cell, organisms have developed specific repair pathways to deal with these potentially catastrophic lesions.

One important pathway for repairing double-stranded DNA breaks, homologous DNA repair, is used by both bacteria and higher organisms and takes advantage of the fact that, after replication, each DNA molecule has an identical copy present in the cell. Damage to one DNA molecule can therefore be repaired using its identical copy as a template without loss of any genetic information. Another important pathway is non-homologous end joining (NHEJ), in which broken DNA ends are directly re-joined regardless of sequence. But as a few bases are often lost at the repair site, non-homologous end-joining is a lower fidelity repair pathway than homologous DNA repair. Until recently, repair of DNA breaks by NHEJ had been described only in eukaryotic organisms. But now Weller and colleagues [1] report that bacteria also repair DNA breaks by NHEJ; their new results indicate that this important pathway has been conserved from bacteria to man.

Many of the genes involved in NHEJ in higher organisms have been identified and their protein products characterised (reviewed in [2]). The first clue that bacteria also repair double-stranded DNA breaks by NHEJ was the identification of a bacterial homologue of the mammalian repair protein, Ku [3,4]. Interestingly, whereas eukaryotic organisms encode two Ku proteins, Ku70 and Ku80, the bacterium *Bacillus subtilis* has just a single Ku-like protein, YkoU, with homology to both its mammalian counterparts. In eukaryotic organisms, Ku proteins function by facilitating the activity of the DNA end-joining enzyme, DNA ligase IV (Figure 1). Intriguingly, analysis of the *ykoU* locus revealed that it is part of an operon with another gene, *ykoV*, which encodes a bacterial DNA ligase. Furthermore, like DNA ligase IV, the YkoV ligase was found to act specifically in DNA repair, rather than more generally during replication.

Genetic experiments showed that YkoU and YkoV function in a pathway that repairs double-stranded DNA breaks caused by exposure to ionizing radiation. They do not, however, contribute to repair of other types of DNA damage, for example that caused by exposure to ultraviolet light or alkylating agents. Moreover, this repair pathway is distinct from homologous DNA repair, as strains carrying inactivating mutations in both *ykoU* and *recA* are more sensitive to ionizing radiation than either single mutant. Surprisingly, however, a strain defective for *RecA, YkoU* and *YkoV* is less sensitive to ionizing radiation than strains lacking either *RecA* and *YkoU*, or *RecA* and *YkoV*, suggesting that, in the absence of homologous DNA repair, loss of both *YkoU* and *YkoV* is less detrimental than loss of either gene alone.

Perhaps the simplest interpretation of these observations is that the bacterial homologues of Ku and ligase function together, and that in the absence of either YkoU or YkoV, the remaining enzyme acts on DNA breaks in a way that interferes with a third, minor repair pathway. Inactivation of both *YkoU* and *YkoV* curtails this aberrant activity, which then permits repair of breaks by the third pathway and consequently increases survival against the effects of ionizing radiation. One might speculate that the coordinate regulation of *ykoU* and *ykoV* as an operon may have evolved to minimize the chances of such interference occurring.

What, then, is the role of Ku in NHEJ? Studies in mammalian cells have shown that Ku physically interacts with both DNA ligase IV and DNA ends to facilitate end-joining [5] (Figure 1). By examining the biochemical properties of the Ku and ligase proteins purified from another bacterium, *Mycobacterium tuberculosis* — Mt-Ku and Mt-Lig — Weller *et al.* [1] demonstrated many functional similarities between the bacterial and mammalian homologues (Figure 1). While the mammalian Ku70 and Ku80 proteins associate to form a heterodimeric complex, Mt-Ku forms a stable homodimer. Moreover, like its mammalian counterpart and consistent with a role in end-joining, the Mt-Ku protein binds specifically to DNA ends but not to circular DNA. In addition, Mt-Ku diffuses along DNA away from the end allowing the binding and loading of additional Mt-Ku molecules at the break site.

Finally, Weller *et al.* [1] showed that Mt-Ku also interacts directly with Mt-Lig. But while the biochemical functions of Mt-Ku and Mt-lig are conserved through evolution, the proteins themselves have diverged. Although Mt-Ku clearly promotes end-joining by Mt-Lig *in vitro*, it does not facilitate ligation of DNA ends by mammalian DNA ligase IV. Similarly, mammalian Ku did not enhance end-joining by Mt-Lig.

Previous studies showed that the ability of Ku protein to facilitate end-joining by ligase IV *in vitro*

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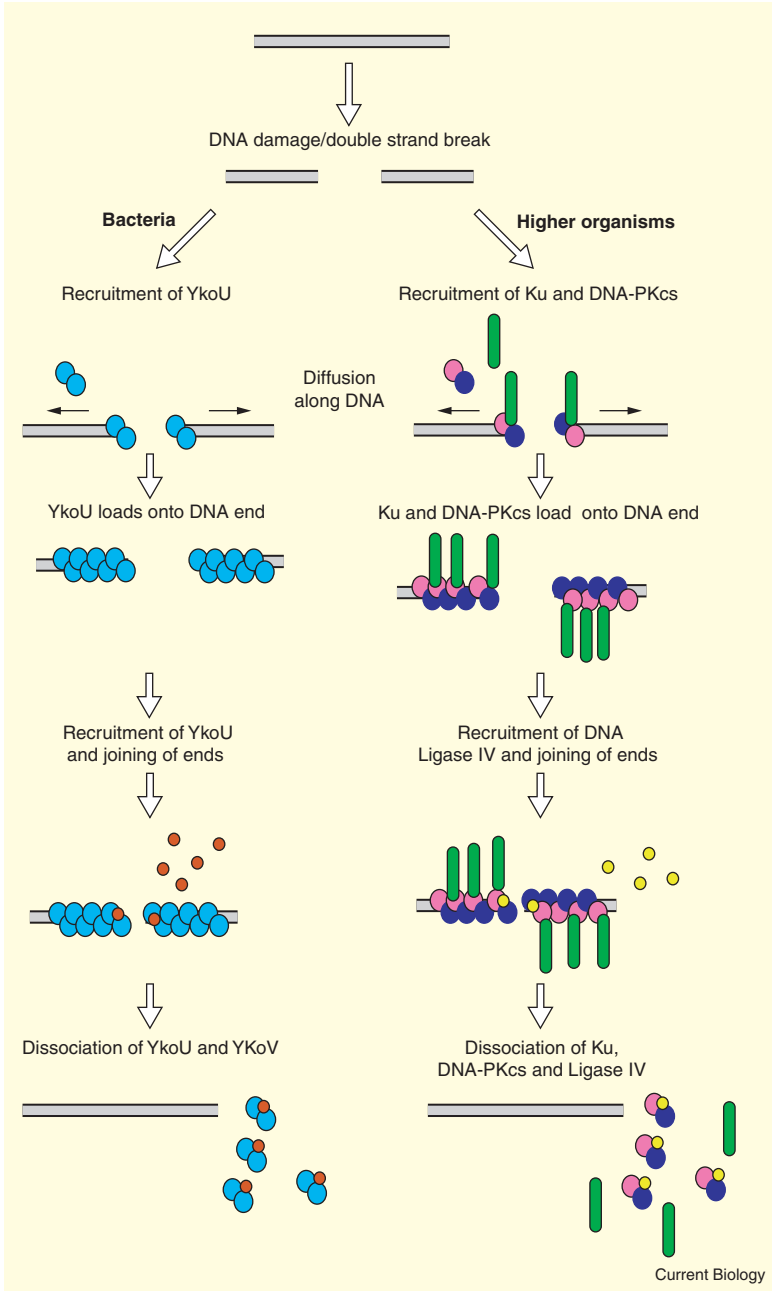

Figure 1. A model describing the non-homologous end-joining in bacteria and higher organisms.

DNA damage by ionizing radiation or errors in cellular processes generates a double strand break. In higher organisms, broken DNA ends are bound by a heterodimeric complex of Ku70 and Ku80, which also recruit the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). In bacteria the Ku-like protein YkoU binds as a homodimer to the DNA end. In both higher organisms and bacteria the Ku-like proteins diffuse along DNA, away from the break, enabling the loading of further Ku-like complexes. Next the DNA ends associate, most likely through interactions between Ku-like molecules bound to different DNA ends. The DNA end-joining enzymes, DNA ligase IV in higher organisms and YkoV in bacteria, are then recruited by Ku and YkoU and the broken ends are re-joined. The proteins of the non-homologous end-joining pathway probably then dissociate from the repaired break. In this diagram, YkoU is represented by light blue circles, YkoV by red circles, Ku70 and Ku80 by dark blue and pink circles, DNA ligase IV by yellow circles and DNA-dependent protein kinase by the green shapes.

depended on the structure of the broken DNA end [5]. While Ku greatly facilitates joining of blunt DNA ends, and ends with a one or two base complimentary overhang, its effect is less marked on ends with complimentary four base overhangs. This suggests that Ku functions by stabilizing unstable DNA ends in order for them to be joined. Indeed, in yeast strains that are defective for Ku, the joining of blunt ends is markedly reduced and instead these cells use a pathway in which the repair of DNA breaks is mediated through a search for small regions of microhomology [6]. Although Weller *et al.* [1] did not examine the repair of double-stranded DNA breaks in the absence of bacterial Ku, it would be interesting to know if prokaryotic organisms can also repair DNA breaks through a microhomology-mediated repair pathway.

Although there are many functional similarities between the Ku proteins from higher and lower organisms, there are some clear differences (Figure 1). Indeed, the earliest identification of Ku in mammalian cells was as the DNA-binding subunit of a complex known as DNA-dependent protein kinase (DNA-PK) [7]. This large complex consists of a Ku70–Ku80 heterodimer and a large 450 kDa catalytic subunit (DNA-PKcs) and plays an important role in the repair, not only of double-stranded DNA breaks arising from DNA damage, but also those generated during V(D)J recombination. Mice lacking Ku are thus sensitive to radiation-induced DNA damage and have a defective

immune system [8,9]. As bacteria do not encode an obvious DNA-PKcs homologue, it seems probable that there are aspects of end-joining in mammalian cells which have developed later in evolution. Most likely, Ku plays a dual role in DNA repair. Firstly, in bacteria and higher organisms, Ku has a direct role in NHEJ through its interaction with a specific repair ligase; and secondly, it plays an additional role in the targeting of DNA-PKcs to the DNA break for a function that is specifically required in higher organisms.

How important is the NHEJ pathway for repair of double-stranded DNA breaks in bacteria? Bacteria that are defective in homologous DNA repair through a mutation in *recA* are more sensitive to killing by ionizing radiation than bacteria with inactivating mutations in either *ykoU* or *ykoV*. Hence it appears that homologous DNA repair plays a more important role than NHEJ in repair of double-stranded DNA breaks. This is at least true for actively growing bacteria, which are rapidly replicating their DNA. It makes some sense, as bacterial genomes have very little non-coding DNA so it is important that repair occurs primarily through a high fidelity mechanism. In the wild, however, many bacterial populations live in environments with limiting nutrients, so that they are not rapidly growing and not replicating. In this situation, homologous recombination is not possible and therefore NHEJ is likely to play a critical role in the repair of DNA breaks.

The evidence that a bacterium, like *B. subtilis*, repairs DNA breaks through non-homologous end-joining is overwhelming. But is it surprising? Probably not. It seems inconceivable that prokaryotes would not use a simple re-joining mechanism to repair broken DNA ends, in addition to the more complex, but higher fidelity, homologous DNA repair pathway. What is undoubtedly more interesting, however, is that the end-joining machinery in these bacteria appears to be a direct ancestor of the NHEJ pathway used by higher organisms, including man. Therefore once again, even though identification of NHEJ in prokaryotes has been late arriving, it is now clear that, not only do bacteria repair double-stranded DNA breaks through non-homologous end-joining, but that they did it first.

References

1. Weller, G.R., Kysela, B., Roy, R., Tonkin, L.M., Scanlan, E., Della, M., Devine, S.K., Day, J.P., Wilkinson, A., di Fagagna, F. *et al*. (2002). Identification of a DNA nonhomologous end-joining complex in bacteria. Science *297*, 1686–1689.
2. Critchlow, S.E. and Jackson, S.P. (1998). DNA end-joining: from yeast to man. Trends Biochem. Sci. *23*, 394–398.
3. Doherty, A.J., Jackson, S.P. and Weller, G.R. (2001). Identification of bacterial homologues of the Ku DNA repair proteins. FEBS Lett. *500*, 186–188.
4. Aravind, L. and Koonin, E.V. (2001). Prokaryotic homologs of the eukaryotic DNA-end-binding protein Ku, novel domains in the Ku protein and prediction of a prokaryotic double-strand break repair system. Genome Res. *11*, 1365–1374.
5. Ramsden, D.A. and Gellert, M. (1998). Ku protein stimulates DNA end joining by mammalian DNA ligases: a direct role for Ku in repair of DNA double-strand breaks. EMBO J. *17*, 609–614.
6. Boulton, S.J. and Jackson, S.P. (1996). Saccharomyces cerevisiae Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. EMBO J. *15*, 5093–5103.
7. Gottlieb, T.M. and Jackson, S.P. (1993). The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*, 131–142.
8. Zhu, C., Bogue, M.A., Lim, D.S., Hasty, P. and Roth, D.B. (1996). Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. Cell *86*, 379–389.
9. Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., Nussenzweig, M.C. and Li, G.C. (1996). Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. Nature *382*, 551–555.