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Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.

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Genome Stability: A Self-Sufficient DNA Repair Machine

The repair of DNA double-strand breaks often requires the broken ends to be processed prior to religation. New results describe a bacterial enzyme with processing and rejoining activities encoded in a single polypeptide chain.

Stuart A. MacNeill

In all three kingdoms of life, DNA double-strand break repair is crucial for maintaining genome integrity. Repair can occur either by homologous recombination or by non-homologous end-joining [1]. In mammals, non-homologous end-joining (NHEJ) accounts for most double-strand break repair. NHEJ requires that broken DNA ends are recognized and processed before being rejoined by DNA ligase. The Ku protein heterodimer plays a vital role in these processes, by bridging the DNA ends and recruiting DNA ligase IV [1]. Unsurprisingly, Ku null mice display a heightened sensitivity to ionising radiation and, as NHEJ is required for V(D)J recombination, they also have immunological defects [2].

NHEJ was originally thought to be confined to eukaryotes, but sensitive database searching showed that certain bacterial genomes had the ability to encode primitive Ku-like proteins [3,4]. Strikingly, many of the bacterial Ku genes were found to occur adjacent to open reading frames encoding putative DNA ligases (Figure 1). This genomic co-localisation suggested that the Ku and ligase proteins were likely to interact functionally, if not physically, to form a novel bacterial DNA repair system [3].

This suggestion was quickly confirmed by biochemical analysis of the Ku and LigD proteins encoded by the *Mycobacterium tuberculosis* genome [5]. LigD is an ATP-dependent DNA ligase, one of three encoded by the organism [6]. Like its eukaryotic counterpart [1], bacterial Ku forms a stable dimer in solution and preferentially binds double-stranded DNA ends. In addition, Ku stimulates LigD activity and is capable of recruiting LigD to DNA [5]. Again, this parallels the situation seen in eukaryotic cells, where the Ku heterodimer acts to recruit DNA ligase IV to sites of DNA damage [1].

These *in vitro* results identified the Ku and LigD proteins as potential components of an NHEJ pathway. To confirm this, the functions of the two proteins were analyzed genetically in both *M. tuberculosis* and *Bacillus subtilis* [5,6]. Deletion of the *ligD* gene from *M. tuberculosis* significantly reduced the ability of cells to repair a linearised double-stranded plasmid when compared to wild type [6], while *B. subtilis* cells lacking either Ku or LigD were shown to be hypersensitive to ionising radiation [5]. Taken together, these results point to Ku and LigD playing important roles in bacterial double-strand break repair. Now, details of the mechanism of bacterial NHEJ have been revealed by two new reports [7,8].

Classical bacterial DNA ligase enzymes are members of the highly conserved NAD^+-dependent DNA ligase family [9]. All bacteria possess at least one such ligase and studies on a number of organisms have shown that these enzymes are essential for microbial cell viability [9]. Certain species additionally contain a varying number of ATP-dependent DNA ligases. Enzymes of this family are characteristic of archaeal and eukaryotic cells (indicating that the bacterial enzymes were most likely acquired by lateral gene transfer) and have a well-defined catalytic domain.

As mentioned above, LigD is one such ATP-dependent DNA ligase, but there is more to this enzyme than just the catalytic domain (Figure 1). In the case of *B. subtilis*, the ligase domain is fused at its carboxyl terminus to a domain that is related to a family of enzymes whose members display either DNA polymerase or primase activities [3,10]. A similar domain is found in *M. tuberculosis* LigD, but this time the domain is located amino-terminal to the ligase domain, and is separated from the latter by a short domain with similarity to the exonuclease III/AP endonuclease family [3,10]. In other species, the same three

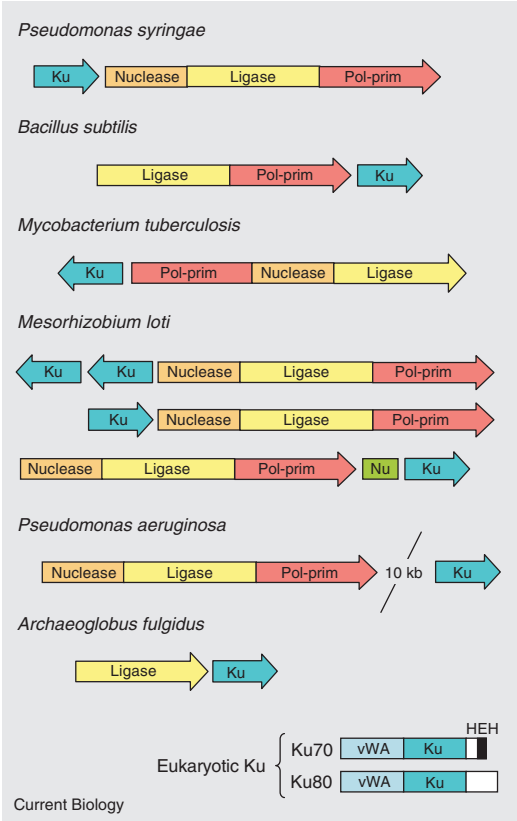

Figure 1. Gene organization and domain structure of NHEJ components in bacteria.

Conserved protein domains are indicated as follows: ligase, ATP-dependent DNA ligase, yellow; pol–prim, polymerase-primase homology domain, red; nuclease, exonuclease III/AP endonuclease homology domain, orange; Ku, Ku core domain, green. With the exception of *Pseudomonas aeroginosa*, all the genes shown are adjacent to one another on the chromosome, suggesting that they are likely to interact functionally. The open-reading frame labeled Nu encodes a protein of unknown function with similarity to the Slx1–YhbQ nuclease family [3,11]. The structures of eukaryotic Ku70 and Ku80 are shown for comparison: vWA, von Willebrand factor A domain; HEH, helix–extended-region–helix domain [3].

domains are present but their order is different (Figure 1).

The identification of these three functional domains in a single polypeptide chain led to the suggestion that LigD might be capable of processing DNA ends prior to ligating them, but direct evidence for this was lacking. The two new reports describe *in vitro* studies of the *M. tuberculosis* [7] and *Pseudomonas aeruginosa* LigD enzymes [8] and provide striking evidence that Ku and LigD form a self-sufficient NHEJ machine, capable of efficiently recognizing, processing and ligating double-strand breaks.

To begin with, Doherty and coworkers [7] demonstrated that recombinant *M. tuberculosis* LigD protein can fill a non-ligatable single nucleotide gap in a synthetic NHEJ-type substrate *in vitro*. Gap filling was abolished by mutation of presumptive active site residues within the LigD polymerase-primase domain. Next, they tested LigD's ability to deal with unannealed flap structures, finding that the enzyme has intrinsic 3′-to-5′ single-stranded nuclease activity capable of digesting 3′ flaps (Figure 2). Once again, mutation of presumptive active site residues, this time within the nuclease domain, led to loss of activity. Finally, by using templates that required both 3′ flap removal and gap filling prior to ligation, the authors showed that the single LigD polypeptide has all three activities [7].

In addition to performing template-directed DNA synthesis of the type shown for *M. tuberculosis* LigD, biochemical analysis showed that *P. aeruginosa* LigD is also capable of performing non-templated DNA synthesis [8]. This activity was seen when the enzyme was challenged with a blunt-ended DNA substrate: the 3′ termini were typically extended by addition of a single nucleotide. This suggests that blunt-ended DNA breaks may be repaired *in vivo* by a mechanism that involves non-templated single nucleotide addition followed by end ligation. Consistent with this, Zhu and Shuman [8] refer to unpublished work showing that blunt-ended double-strand breaks in *Mycobacterium smegmatis* are repaired by a LigD-dependent NHEJ pathway that results in a high frequency of single-nucleotide insertions at the repair junction.

Non-templated synthesis is also seen when the *P. aeruginosa* LigD enzyme is supplied with a template with a recessed 3′ end [8]. In this situation, the enzyme first performs template-directed DNA synthesis to form a blunt-end, at which point the blunt-end is modified by non-templated addition of a single nucleotide to the 3′ end. Recessed 3′ ends processed in this way can presumably then be ligated in the same way as described above for modified blunt-ends, resulting in an additional nucleotide being inserted at the repair junction. *In vivo*, this type of repair is likely to be highly mutagenic. Interestingly, when faced with a 3′ flap structure, Ku appears able to prevent the LigD nuclease from digesting beyond the end of the flap into the duplex DNA [7]. In this way, Ku acts to minimise the loss of DNA sequence information at the breakpoint, minimising the potential for mutagenesis.

Taken together, these observations suggest that the three domain LigD enzymes have the capacity to process all three types of DNA end that can be generated by double-strand breakage — 5′-recessed, 3′-recessed and blunt — with polymerase and ligase activities being required for all three types of end-processing reaction and the 3′-to-5′ nuclease activity playing a role in processing recessed 5′ ends only. Ku and LigD can therefore be thought of as a self-sufficient NHEJ repair machine. Consistent with this, co-expression of Ku and LigD proteins in NHEJ-defective yeast cells was found to restore NHEJ to around 50% of wild-type levels [7]. Given the good effect to which NHEJ has been put by certain bacteria, it is perhaps surprising that this system is not more widespread in this domain of life. For many bacteria, however, the presence of multiple copies of the genome during phases of rapid growth presumably facilitates highly efficient double-strand

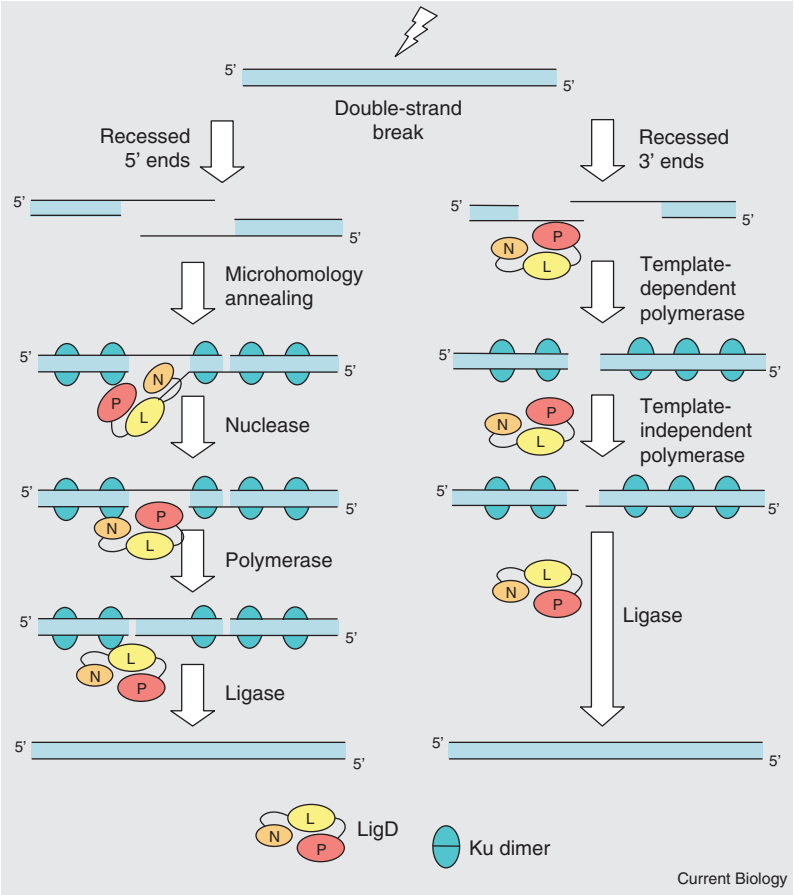

Figure 2. A model for non-homologous end joining in bacteria.

The model is based on biochemical analysis of *M. tuberculosis* LigD protein (left, processing 5′ recessed ends [5]) and *P. aeruginosa* LigD (right, processing 3′ recessed ends [8]). The repair of blunt ends appears to be accomplished by template-independent single-nucleotide addition, followed by ligation (shown as lower parts of 3′ recessed end processing reactions).

break repair by homologous recombination. As noted by Weller *et al*. [5], NHEJ may offer a particular advantage to bacterial species that spend at least part of their life cycle either as spores, like *B. subtilis*, or in stationary phase, like *M. tuberculosis*, where homologous recombination may not be possible.

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Wellcome Trust Centre for Cell Biology, University of Edinburgh, Michael Swann Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK.
E-mail: s.a.macneill@ed.ac.uk

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Nuclear Envelope: Nuclear Pore Complexity

A new study shows that the filamentous fungus, Aspergillus nidulans, which has a closed mitosis, does not maintain a continuous permeability barrier during mitosis. This work challenges current views of the differences between closed and open mitosis and has implications for understanding mitotic specific changes in the nuclear pore complex and Ran GTPase system in lower eukaryotes.

Shelley Sazer

The presence of a lipid bilayer called the nuclear envelope that keeps the genetic material separate from the rest of the cell distinguishes eukaryotes from prokaryotes. Nuclear pore complexes (NPCs) traverse the nuclear envelope and form channels that allow the diffusion of small molecules and the selective transport of larger molecules between the nucleus and the cytoplasm (reviewed in [1]). The asymmetric distribution of the GTP-bound form of the Ran GTPase across the nuclear envelope is essential for regulation of nucleocytoplasmic