32. M. B. Kirkley, J. J. Gurney, A. A. Levinson, *Gems Gemol.* **27**, 2 (1991).
33. Diamonds with no or low (<20 ppm) N are termed Type II. Diamonds with spectroscopically detectable N (termed Type Ia, Table 1) show a N distribution that progresses from C-centers (single N) through A-centers (paired N) to B-centers (clusters of four N and a vacancy) in proportion with geological time (hundreds of Ma), temperature, and nitrogen content. Of these three variables, N aggregation will be sensitive chiefly to temperature. See (*49*) for details.
34. R. V. Danchin, in *The Mantle Sample: Inclusions in Kimberlites and Other Volcanics*, F. R. Boyd, H. O. A. Meyer, Eds. (American Geophysical Union, Washington, DC, 1979), vol. 2, pp. 104–126.
35. M. Q. W. Jones, *J. Geophys. Res.* **93**, 3243 (1988).
36. T. H. Jordan, in *The Mantle Sample; Inclusions in Kimberlites and Other Volcanics*, F. R. Boyd, H. O. A. Meyer, Eds. (American Geophysical Union, Washington, DC, 1979), vol. 2, pp. 1–14.
37. R. W. Carlson *et al.*, in *The J. B. Dawson Volume*, J. J. Gurney, J. L. Gurney, M. D. Pascoe, S. H. Richardson, Eds. (Red Roof Design, Cape Town, 1999), pp. 99–108.
38. G. J. Irvine, D. G. Pearson, R. W. Carlson, *Geophys. Res. Lett.* **28**, 2505 (2001).
39. R. J. Walker, R. W. Carlson, S. B. Shirey, F. R. Boyd, *Geochim. Cosmochim. Acta* **53**, 1583 (1989).
40. D. Canil, K. Wei, *Contrib. Mineral. Petrol.* **109**, 421 (1992).
41. T. Stachel, K. S. Viljoen, G. Brey, J. W. Harris, *Earth Planet. Sci. Lett.* **159**, 1 (1998).
42. M. J. Walter, *J. Petrol.* **39**, 29 (1998).
43. S. W. Parman, J. C. Dann, T. L. Grove, *Geophys. Res. Lett.* **28**, 2513 (2001).
44. S. E. Haggerty, *Nature* **320**, 34 (1986).
45. N. T. Arndt, A. C. Kerr, J. Tarney, *Earth Planet. Sci. Lett.* **146**, 289 (1997).
46. M. D. Schmitz, thesis, Massachusetts Institute of Technology (2002).
47. J. W. Valley, P. D. Kinny, D. J. Schulze, M. J. Spicuzza, *Contrib. Mineral. Petrol.* **133**, 1 (1998).
48. P. Cartigny, J. W. Harris, M. Javoy, *Earth Planet. Sci. Lett.* **185**, 85 (2001).
49. O. Navon, in *The P.H. Nixon Volume*, J. J. Gurney, J. L. Gurney, M. D. Pascoe, S. H. Richardson, Eds. (Red Roof Design, Cape Town, 1999), pp. 584–604.
50. P. E. Janney, R. W. Carlson, S. B. Shirey, D. R. Bell, A. P. le Roex, paper presented at the Ninth Annual V. M. Goldschmidt Conference, Cambridge, MA, 22 to 26 August 1999.
51. J. W. Harris, unpublished data.
52. P. Deines, J. W. Harris, D. N. Robinson, J. J. Gurney, S. R. Shee, *Geochim. Cosmochim. Acta* **55**, 515 (1991).
53. H. J. Milledge *et al.*, *Nature* **303**, 791 (1983).
54. P. Deines, F. Viljoen, J. W. Harris, *Geochim. Cosmochim. Acta* **65**, 813 (2001).
55. C. B. Smith *et al.*, *Geochim. Cosmochim. Acta* **55**, 2579 (1991).
56. S. H. Richardson, unpublished data.
57. S. Shirey *et al.*, unpublished data.

58. Discussions with D. Bell, R. Carlson, I. Chinn, D. G. Pearson, and K. Westerlund are greatly appreciated, as is the assistance of T. Mock and M. Horan in the DTM mass spectrometry and chemistry labs, respectively. We appreciate V. Anderson, E. van Blerk, R. Ferraris, R. Hamman, W. Moore, A. Ntidisang, G. Parker, and others at Harry Oppenheimer House, Kimberley, for their skill in selecting specimens, and we thank the De Beers Diamond Trading Company for making them available. The manuscript was improved by the reviews of F. Boyd, K. Burke, R. Carlson, E. Hauri, T. Stachel, and E. Vicenzi. Support was chiefly from NSF EAR Continental Dynamics Grant 9526840.

Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5586/1683/DC1

Fig. S1

29 March 2002; accepted 29 July 2002

REPORTS

Identification of a DNA Nonhomologous End-Joining Complex in Bacteria

Geoffrey R. Weller,[1]* Boris Kysela,[2]* Rajat Roy,[3]* Louise M. Tonkin,[1] Elizabeth Scanlan,[4] Marina Della,[1] Susanne Krogh Devine,[4] Jonathan P. Day,[5] Adam Wilkinson,[5] Fabrizio d'Adda di Fagagna,[3] Kevin M. Devine,[4] Richard P. Bowater,[5] Penny A. Jeggo,[2] Stephen P. Jackson,[3] Aidan J. Doherty[1]†

In eukaryotic cells, double-strand breaks (DSBs) in DNA are generally repaired by the pathway of homologous recombination or by DNA nonhomologous end joining (NHEJ). Both pathways have been highly conserved throughout eukaryotic evolution, but no equivalent NHEJ system has been identified in prokaryotes. The NHEJ pathway requires a DNA end-binding component called Ku. We have identified bacterial Ku homologs and show that these proteins retain the biochemical characteristics of the eukaryotic Ku heterodimer. Furthermore, we show that bacterial Ku specifically recruits DNA ligase to DNA ends and stimulates DNA ligation. Loss of these proteins leads to hypersensitivity to ionizing radiation in *Bacillus subtilis*. These data provide evidence that many bacteria possess a DNA DSB repair apparatus that shares many features with the NHEJ system of eukarya and suggest that this DNA repair pathway arose before the prokaryotic and eukaryotic lineages diverged.

Double-strand breaks (DSBs) in DNA arise during exposure to ionizing radiation (IR) and as intermediates during site-specific rearrangement events such as mating-type switching in *Saccharomyces cerevisiae* and V(D)J recombination in vertebrates (*1*, *2*). In eukaryotic cells, the primary DNA end-binding component of NHEJ, Ku, is a heterodimer of two sequence-related subunits [Ku70 (69 kD) and Ku80 (83 kD)] (*3*–*5*) that forms an open ringlike structure through which a variety of DNA end structures can be threaded (*6*, *7*). DNA-bound Ku helps to recruit the ligase IV/XRCC4 complex, thereby enhancing its ligation activity (*8*–*10*). In vertebrates, Ku also recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), thereby activating its kinase activity, which is required for DSB rejoining (*11*–*13*). Mammalian cells deficient in these NHEJ proteins are defective in DSB rejoining and are hypersensitive to IR (*1*, *4*, *13*).

In contrast to the conservation between these components in higher and lower eukaryotes, NHEJ has not been reported in prokaryotes. However, genes with significant homology to Ku70 and Ku80 have been identified in some bacterial genomes (*14*, *15*), which raises the possibility that prokaryotes might have a NHEJ apparatus that is fundamentally homologous to that of eukaryotic cells. Significantly, the Ku-like gene exists in some bacterial species in an operon that includes a gene predicted to encode an adenosine triphosphate (ATP)–dependent DNA ligase (*14*–*16*). Operons frequently co-regulate functionally interacting proteins (*17*); perhaps then, these putative ligases interact with the Ku-like proteins.

We exploited the genetically amenable bacterium *Bacillus subtilis* to generate strains bearing inactivating mutations in *YkoV* (Ku-like gene; *ykoV*) and *YkoU* (ligase-like gene; *ykoU*) and double mutants defective in *YkoU* and *YkoV* (ykoU ykoV) (*18*). None of the strains had any observable growth defect at temperatures ranging from 10° to 37°C (*19*), which indicates that neither *YkoU* nor *YkoV* is essential. These findings are consistent with the notion that the *B. subtilis* nicotinamide adenine dinucleotide (NAD^+)–dependent ligase *YerG* functions during DNA replication (*20*). To investigate the role of *YkoU* and *YkoV* in DNA repair, we examined the sensitivity of the mutant strains to DNA-damaging agents (*18*). No sensitivity to ultraviolet light or to methyl methanesulfonate (MMS) was observed, which suggests that nucleotide excision repair functions normally and alkylation damage induced by MMS is repaired efficiently (*19*). In contrast,

[1]Cambridge Institute for Medical Research & Department of Haematology, University of Cambridge, Hills Road, Cambridge CB2 2XY, UK. [2]Genome Damage and Stability Centre, University of Sussex, Brighton BN1 9RQ, UK. [3]Wellcome Trust/Cancer Research UK, Institute of Cancer and Developmental Biology and Department of Zoology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UK. [4]Department of Genetics, Smurfit Institute, Trinity College Dublin, Dublin 2, Republic of Ireland. [5]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: AJD42@cam.ac.uk.

the mutant strains were sensitive to IR relative to a wild-type control (WT168) (Fig. 1A). The *ykoU* and *ykoUV* strains showed similar sensitivity to IR, more marked than that shown by the *ykoV* mutant strain (Fig. 1A), which indicates that *ykoV* and *ykoU* are epistatic but that defects in *ykoU* have the greatest impact. The major cytotoxic lesions induced by IR are DNA DSBs, which suggests a role for *YkoV* and *YkoU* in DSB repair.

In *B. subtilis*, *RecA* plays a crucial role in homologous recombination. Therefore, we generated *YkoV* and *YkoU* mutations (*recA ykoU* and *recA ykoV*) in a *recA* mutant background and examined the response of the multiple mutant strains to IR. As expected, the *recA* mutant strain was very sensitive to IR, as were the *recA ykoU* and *recA ykoV* double-mutant strains (Fig. 1B). However, we noted that the triple-mutant strain (*recA ykoU ykoV*) was reproducibly slightly more resistant to lower doses of IR than the *recA* single mutant or the double-mutant strains. We interpret these survival experiments as indicating that there may be an alternative, inefficient DSB repair system that can operate in cells lacking *RecA*, *YkoV*, and *YkoU*. In *recA* mutant strains that still possess either *YkoV* or *YkoU*, however, it may be that the remaining NHEJ factor interacts with IR-induced DNA damage in a nonproductive manner so that it not only precludes NHEJ but also interferes with the alternative DSB repair pathway.

The genes for *YkoU* and *YkoV* and their homologs were cloned from *B. subtilis* and additional bacteria, and the proteins were overexpressed in *Escherichia coli* (*18*). Many of the proteins, including *B. subtilis YkoU* and *YkoV*, expressed poorly or were insoluble. By contrast, recombinant histidine-tagged versions of *Mycobacterium tuberculosis* Ku-like protein [open reading frame (ORF) Rv0937c] and the genetically linked putative ATP-dependent ligase (ORF Rv0938) were readily overexpressed in soluble form. We analyzed these proteins, henceforth called Mt-Ku and Mt-Lig, further after we purified them by nickel-agarose affinity chromatography (fig. S1).

Eukaryotic Ku70 and Ku80 form a stable heterodimeric complex, and this 1:1 stoichiometry is essential for interaction with DNA ends (*6*, *7*). Analysis of recombinant Mt-Ku by gel-filtration chromatography indicated that Mt-Ku exists as a homodimer in solution (fig. S2). This species was very stable, even at high salt concentrations, which suggests a strong homodimeric interaction (see supplemental text; fig. S3). Eukaryotic Ku binds to double-stranded (ds) DNA ends with high affinity (*21*). Electrophoretic mobility-shift assays (EMSAs), with a 33–base-pair (bp) dsDNA oligonucleotide with either 5′ or 3′ overhangs, demonstrated that Mt-Ku, like eukaryotic Ku, forms a specific complex with either type of DNA end (fig. S4). Excess nonlabeled linear dsDNA but not closed circular plasmid DNA or single-stranded DNA competed for binding (Fig. 2A), which demonstrates that Mt-Ku binds preferentially to dsDNA ends.

Multiple molecules of eukaryotic Ku can bind to the same DNA molecule to give multimeric Ku-DNA complexes; because of this, the maximum number of Ku molecules that can bind to a DNA molecule is directly proportional to DNA length (*21*). Titration of Mt-Ku against a fixed concentration of labeled 33-nucleotide oligomer resulted in a single retarded band (Fig. 2B), presumably representing a 1:1 Ku-DNA

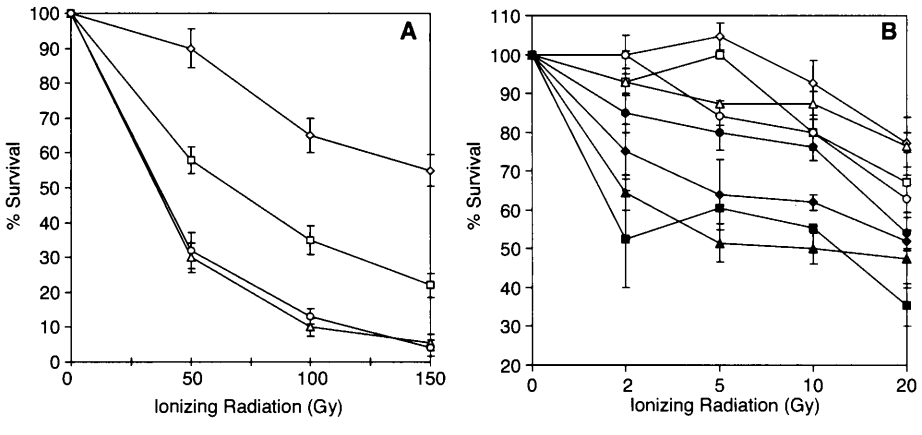

Fig. 1. IR sensitivity of *B. subtilis* Ku (*ykoV*) and ligase (*ykoU*) knockout strains. *B. subtilis* wild type (WT168), *ykoV*, *ykoU*, or *ykoV ykoU* double-mutant and *B. subtilis recA* mutant cells were treated with x-rays. The percentage survival for each strain over the indicated range of doses was determined, and the mean from three independent experiments was plotted. Error bars denote standard deviation from the mean for each point. (**A**) Deletion of *YkoV* and *YkoU* genes (strains *ykoU* and *ykoV*) resulted in significant IR hypersensitivity of these strains compared with the wild type, and the *ykoV ykoU* double-knockout strain did not show any higher sensitivity than the *ykoU* single mutant, which suggests that these genes act in the same pathway. (**B**) *B. subtilis recA* mutant strains were extremely sensitive to irradiation. However, at lower IR dosage, deletion of *ykoV* and *ykoU* together (*recA ykoU ykoV*) could partially rescue the hypersensitivity of *recA* cells.

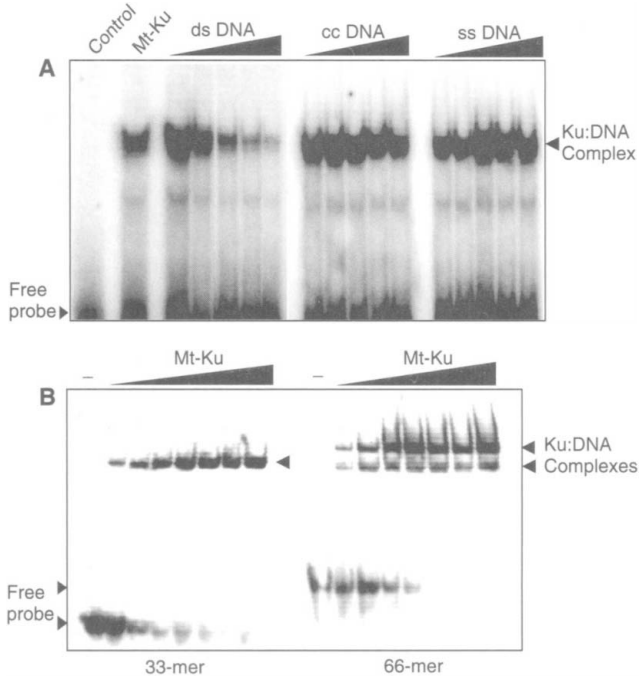

Fig. 2. DNA binding properties of Mt-Ku on dsDNA substrates. (**A**) Mt-Ku interacts specifically with dsDNA ends. Reaction mixtures contained 90 fmol of labeled 33-mer dsDNA and 2.0 pmol of Mt-Ku, plus the following where indicated. Control, no competition, increasing unlabeled ds 33-mer (9, 90, 180, 450, and 900 fmol), closed-circular plasmid (ccDNA; 9, 90, 180, 450, and 900 fmol), and unlabeled single-stranded 33-mer DNA (ssDNA; 9, 90, 180, 450, and 900 fmol). For each reaction Mt-Ku was added last. (**B**) Effect of DNA length on binding of Mt-Ku to dsDNA. Multiple Mt-Ku molecules load onto dsDNA in a length-dependent manner, about one Ku per 30 bp. Reaction mixtures contained 90 fmol of labeled 33-mer or 66-mer dsDNA and increasing concentrations of Mt-Ku (2.5, 5, 10, 15, 20, 25, and 50 pmol).

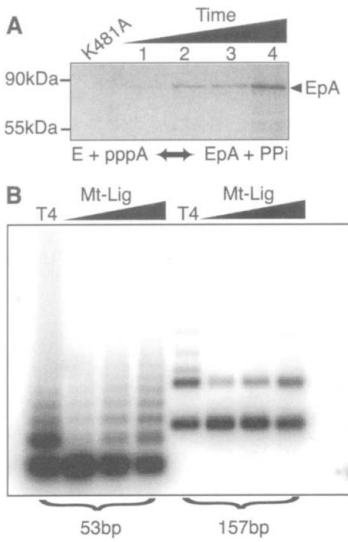

Fig. 3. Mt-Lig is an ATP-dependent dsDNA ligase. (**A**) Mt-Lig forms a covalent adenylate adduct in the presence of ATP. One microgram of Mt-Lig or K481A mutant was incubated with 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 5 mM dithiothreitol, [α-^{32}P]ATP for 1, 30, 60, and 120 min (lanes 1–4, respectively) at 25°C. K481A was incubated for 120 min. The reactions were stopped by boiling in SDS-PAGE loading buffer and analyzed by electrophoresis on a 12% SDS–polyacrylamide gel. (**B**) Mt-Lig efficiently ligates DSBs in vitro. Equimolar concentrations of the DNA ends (70 fmol) were used in ligase reactions and the efficiency of ligation was compared with that mediated by T4 DNA ligase (330 ng). Labeled DNA fragments (53 bp to 2.6 kbp) were incubated with increasing amounts of Mt-Lig (0.5, 1.5, and 2.5 μg). Efficiency of ligation was monitored by converting the substrates into higher order concatamers.

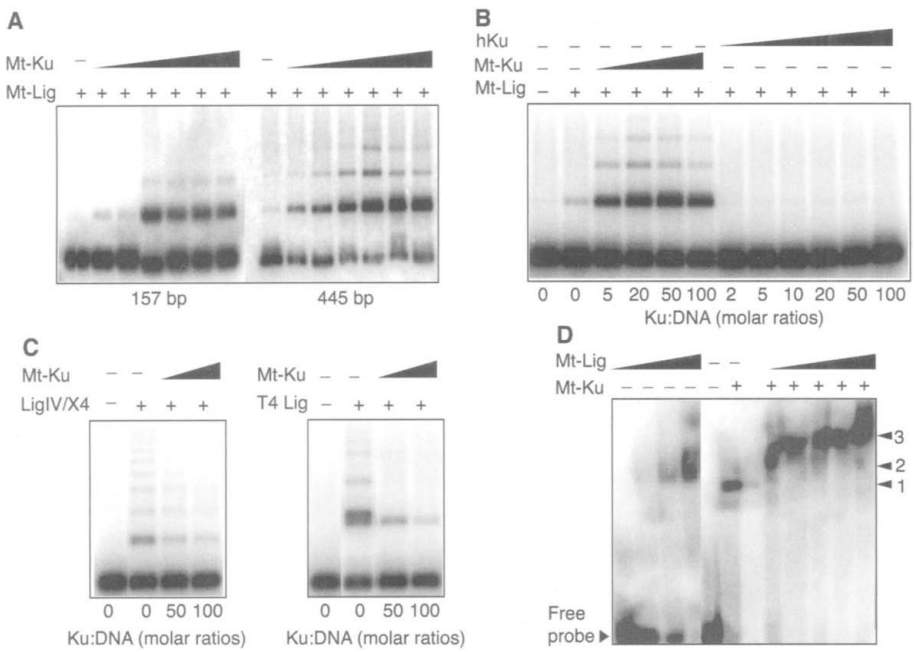

Fig. 4. (**A**) Mt-Lig end-joining activity is greatly stimulated by the Mt-Ku complex. dsDNA fragments (70 fmol) (157 or 445 bp) were preincubated with increasing amounts of Mt-Ku (0, 20, 40 80, 200, 400, and 800 ng). The ligase reaction was started by addition of 1 μg of Mt-Lig. Optimum stimulation was achieved between 80 and 200 ng of Mt-Ku per reaction. (**B**) Stimulation of Mt-Lig end-joining activity is specific to the bacterial Ku complex. DNA fragments (445 bp) were preincubated with increasing amounts of Mt-Ku (20, 80, 200, and 400 ng) or human recombinant Ku70/80 complex (hKu; 20, 60, 120, 240, 500, and 1100 ng). Ligase reactions were started by adding 1 μg of Mt-Lig. No stimulation but significant inhibition has been observed in reaction mixtures preincubated with hKu. (**C**) Mt-Ku significantly inhibits end-joining ligation catalyzed by human recombinant LigaseIV/XRCC4 complex (LigIV/X4) or T4 DNA ligase. Ligase reaction mixtures were preincubated with Mt-Ku and ligation was started by adding 150 ng of LigIV/X4 complex or T4 Lig. In both cases, the presence of increasing amounts of Mt-Ku (200 and 400 ng, respectively) significantly inhibited end-joining reactions, which suggests that stimulation of ligation is critically dependent on interaction of the correct physiological partners, Mt-Ku and Mt-Lig. (**D**) Mt-Ku recruits Mt-Lig to DNA. Three distinct DNA-protein complexes formed by Mt-Ku (arrowhead 1), Mt-Lig (arrowhead 2), and Mt-Ku and Mt-Lig together (arrowhead 3) in EMSA. Reaction mixtures contained 90 fmol of 33-mer dsDNA and 2.5 pmol of Mt-Ku, where indicated (+). In reactions with ligase (left), DNA was incubated with increasing amounts of Mt-Lig (0, 0.5, 1.0, and 2.0 pmol). For the supershift reactions (right), DNA was preincubated with Mt-Ku (2.5 pmol) followed by the addition of increasing amounts of Mt-Lig (0.5, 1, 2, 3, and 4 pmol).

complex (see supplementary text). When we doubled the length of the DNA (66-nucleotide oligomer), we observed two progressively retarded bands (Fig. 2B). Multiple Ku-DNA complexes were formed on all dsDNA linear substrates of >60-mer tested, and the number of retarded species was directly proportional to the length of the DNA (*19*), which suggests that upon binding to the end the Mt-Ku can freely move along the DNA.

The putative bacterial ATP-dependent DNA ligases (*14–16*) share a common core domain with other ATP-dependent ligases, and this encompasses the signature catalytic motif KXDG-R (motif I) (*22*) found at the active site of DNA ligases (*23*) (fig. S5). The first step of the ligation reaction involves the active site lysine attacking the α-phosphorus of ATP or NAD^+, yielding a covalent intermediate (ligase-adenylate) in which adenosine monophosphate (AMP) is linked via a phosphoamide bond to the ε-amino group of the lysine (*23*). To test whether Mt-Lig uses ATP or NAD^+, we incubated Mt-Lig with either [α-^{32}P]ATP or NAD^+ and magnesium. In the presence of ATP, but not NAD^+, a radiolabeled covalent ligase-adenylate adduct was formed that comigrated with the Mt-Lig polypeptide during SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 3A). This demonstrates that Mt-Lig is active in covalent nucleotidyl transfer with a specific preference for ATP as the AMP donor. Substitution of the motif I residue Lys^{481} by alanine (K481A) abolished ligase-AMP formation (Fig. 3A), as has been observed with analogous mutations in other ATP-dependent DNA ligases (*24*, *25*).

To examine whether Mt-Lig is a dsDNA ligase, we used dsDNA substrates of various sizes (53 to 2560 bp) in ligation reactions and compared the efficiency of ligation to that mediated by T4 DNA ligase. Mt-Lig catalyzed the joining of the various dsDNA fragments of different lengths to equivalent extents (Fig. 3B). We conclude that *M. tuberculosis* Mt-Lig, and by inference *B. subtilis* YkoU, are functional DNA ligases capable of catalyzing DSB rejoining in an ATP-dependent manner.

Eukaryotic Ku can stimulate dsDNA ligation catalyzed by DNA ligase IV/XRCC4 (*8*, *9*, *26–29*). Notably, the DNA ligation activity of Mt-Lig was stimulated >30-fold by the addition of Mt-Ku (Fig. 4, A and B). Stimulation was abolished by heat denaturation of Mt-Ku (*19*). By contrast, Mt-Lig was not stimulated by the human Ku heterodimer (Fig. 4B) and, conversely, human ligase IV/XRCC4 and T4 ligase were not stimulated by Mt-Ku (Fig. 4C). Indeed, amounts of Mt-Ku that stimulated Mt-Lig inhibited both ligase IV and T4 ligase activity (Fig. 4C). Consistent with these observations, Mt-Ku stimulated the activity of Mt-Lig by >20-fold but not T4 ligase in an in vitro plasmid repair assay (fig. S6). Thus, stimulation of ligation by Mt-Ku is highly specific for Mt-Lig

and raises the possibility that these proteins might physically interact.

Eukaryotic Ku recruits DNA ligase IV/XRCC4 to sites of DNA damage (*8–10*). Therefore, we looked for potential interactions between Mt-Ku and Mt-Lig by EMSAs with a radiolabeled dsDNA probe (33 bp). As shown in Fig. 4D, including Mt-Lig and Ku together led to the generation of a DNA-protein complex with a mobility distinct from that of the complexes formed by either protein alone. However, the addition of increasing amounts of Mt-Ku did not abolish the appearance of the novel DNA-protein complex, which strongly suggests that it does not inhibit the binding of Mt-Lig to DNA. Formation of the new complex did not occur if Mt-Lig had been heat denatured (*19*), which indicates that it reflects the binding of Mt-Lig and is not mediated by a buffer component (Fig. 4D). Biacore studies with a biotinylated dsDNA (33-mer) bound to a streptavidin-coated chip and isothermal titration calorimetry studies also confirmed that Mt-Ku specifically recruits Mt-Lig to DNA (*19*).

Despite the importance of XRCC4 in the eukaryotic NHEJ system, we have so far been unable to detect any XRCC4 homologs in bacteria (*19*). It has been reported that many of the Ku-associated DNA ligases possess additional domains with significant homology to eukaryotic DNA primases and nucleases (*15*, *16*). Therefore, it is possible that these domains enhance ligase activity in the absence of XRCC4 and may also be directly involved in the DSB repair process (*16*). The similarity between the eukaryotic and bacterial Ku proteins suggests that they have evolved from a common ancestor and shows that NHEJ is a much more ancient process than was previously believed. The eukaryotic Ku proteins may have acquired additional domains—such as the VWA, SAP, and DNA-PKcs interaction domains—to enhance the repair activities of the complex and to provide additional functions, such as roles in telomere maintenance and V(D)J recombination. It is notable that only one characterized archaeal species, *Archaeglobus fulgidus*, contains the Ku ligase system, which suggests that this was acquired by lateral transfer. The Mu phage protein, Gam, and related bacterial orthologs may also represent a distinct family of functional homologs of eukaryotic Ku (*30*). It is also interesting to note that many of the bacteria that contain the Ku ligase system are capable of sporulation (*B. subtilis*, *S. coelicolor*) and/or spend long periods of their life cycle in the stationary phase (*M. tuberculosis*, *Mesorhizobium loti*, *Sinorhizobium loti*). It is tempting to speculate that a Ku-based NHEJ system is particularly important for the repair of DSBs that arise during these states of relative inactivity, where the better characterized homologous recombination-based repair pathways may be less effective.

References and Notes

1. S. E. Critchlow, S. P. Jackson, *Trends Biochem. Sci.* **23**, 394 (1998).
2. A. Shinohara, T. Ogawa, *Trends Biochem. Sci.* **20**, 387 (1995).
3. W. S. Dynan, S. Yoo, *Nucleic Acids Res.* **26**, 1551 (1998).
4. C. Featherstone, S. P. Jackson, *Mutat. Res.* **434**, 3 (1999).
5. D. Gell, S. P. Jackson, *Nucleic Acids Res.* **17**, 3494 (1999).
6. J. R. Walker, R. A. Corpina, J. Goldberg, *Nature* **412**, 607 (2001).
7. A. J. Doherty, S. P. Jackson, *Curr. Biol.* **11**, R920 (2001).
8. S. A. Nick McElhinny, C. M. Snowden, J. McCarville, D. A. Ramsden, *Mol. Cell. Biol.* **20**, 2996 (2000).
9. L. Chen, K. Trujillo, P. Sung, A. E. Tomkinson, *J. Biol. Chem.* **275**, 26196 (2000).
10. S. H. Teo, S. P. Jackson, *Curr. Biol.* **10**, 165 (2000).
11. A. Dvir, S. R. Peterson, M. W. Knuth, H. Lu, W. S. Dynan, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11920 (1992).
12. T. M. Gottlieb, S. P. Jackson, *Cell* **72**, 131 (1993).
13. G. C. Smith, S. P. Jackson, *Genes Dev.* **13**, 916 (1999).
14. A. J. Doherty, S. P. Jackson, G. R. Weller, *FEBS Lett.* **500**, 186 (2001).
15. L. Aravind, E. V. Koonin, *Genome Res.* **11**, 1365 (2001).
16. G. R. Weller, A. J. Doherty, *FEBS Lett.* **505**, 340 (2001).
17. T. Dandekar, B. Snel, M. Huynen, P. Bork, *Trends Biochem Sci.* **23**, 324 (1998).
18. Materials and methods are available as supporting material on *Science* Online.
19. G. R. Weller *et al.*, unpublished data.
20. M. A. Petit, S. D. Ehrlich, *Nucleic Acids Res.* **28**, 4642 (2000).
21. T. M. Bliss, D. P. Lane, *J. Biol. Chem.* **272**, 5765 (1997).
22. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
23. A. J. Doherty, S. W. Suh, *Nucleic Acids Res.* **28**, 4051 (2000).
24. K. Kodama, D. E. Barnes, T. Lindahl, *Nucleic Acids Res.* **19**, 6093 (1991).
25. V. Sriskanda, S. Shuman, *Nucleic Acids Res.* **26**, 4618 (1998).
26. P. Pfeiffer, S. Thode, J. Hancke, W. Vielmetter, *Mol. Cell. Biol.* **14**, 888 (1994).
27. D. Pang, S. Yoo, W. S. Dynan, M. Jung, A. Dritschilo, *Cancer Res.* **57**, 1412 (1997).
28. R. B. Cary *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4267 (1997).
29. D. A. Ramsden, M. Gellert, *EMBO J.* **17**, 609 (1998).
30. F. d'Adda di Fagagna, G. R. Weller, A. J. Doherty, S. P. Jackson, unpublished data.

31. We thank J. Bradbury for comments on the manuscript, L. Serpell for assistance with figures, T. Kieser for providing *M. tuberculosis* DNA, and P. J. Piggot for supplying *B. subtilis* strains. G.R.W. was supported by a UK Medical Research Council (MRC) studentship. A.J.D. is a Royal Society University Research Fellow, and work in the A.J.D. laboratory is supported by grants from the UK Biotechnology and Biological Sciences Research Council, Cancer Research UK Leukaemia Research Fund, the Association for International Cancer Research, and the Royal Society. Research in the S.P.J. laboratory is supported by grants from Cancer Research UK. B.K. is funded by an MRC grant to P.A.J. R.R. is recipient of a Cambridge Commonwealth Trust Scholarship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5587/1686/DC1
Materials and Methods
SOM Text
Figs. S1 to S6

31 May 2002; accepted 19 July 2002

Mast Cells: A Cellular Link Between Autoantibodies and Inflammatory Arthritis

David M. Lee,[1,3] Daniel S. Friend,[2] Michael F. Gurish,[1,3] Christophe Benoist,[1,4] Diane Mathis,[1,4] Michael B. Brenner[1,3]*

Previous studies have revealed that autoantibodies, complement components, and Fc receptors each participate in the pathogenesis of erosive arthritis in K/BxN mice. However, it is not known which cellular populations are responsive to these inflammatory signals. We find that two strains of mice deficient in mast cells, W/W^v and Sl/Sl^d, were resistant to development of joint inflammation and that susceptibility was restored in the W/W^v strain by mast cell engraftment. Thus, mast cells may function as a cellular link between autoantibodies, soluble mediators, and other effector populations in inflammatory arthritis.

The pathogenic mechanisms at play in inflammatory arthritis, such as rheumatoid arthritis, remain poorly understood both systemically and in the microenvironment of the diarthrodial joint. A large number of soluble inflammatory mediators and cellular effector populations have been implicated in arthritis; however, the early clinical events remain elusive. Recent studies using the serum of an engineered mouse model, K/BxN, have revealed that autoantibodies directed against a ubiquitously expressed antigen can selectively provoke inflammatory, hyperplastic, and erosive synovitis (*1–3*). It is known that members of the complement network (the alternative pathway and C5a), Fc receptors (FcγRIII), and cytokines [interleukin

[1]Department of Medicine and [2]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [3]Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital; Harvard Medical School, Boston, MA 02115, USA. [4]Section on Immunology and Immunogenetics, Joslin Diabetes Center, Harvard Medical School, One Joslin Place, Boston, MA 02215, USA.

*To whom correspondence should be addressed. E-mail: mbrenner@rics.bwh.harvard.edu