BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **246,** 137–141 (1998)
ARTICLE NO. RC988589

Non-histone Chromosomal Proteins HMG1 and 2 Enhance Ligation Reaction of DNA Double-Strand Breaks

Sumiko Nagaki,*,† Mayumi Yamamoto,* Yoshiko Yumoto,*,† Hitoshi Shirakawa,† Michiteru Yoshida,† and Hirobumi Teraoka*,[1]

Department of Pathological Biochemistry, Medical Research Institute, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan; and †Department of Biological Science and Technology, Science University of Tokyo, Noda, Chiba 278-8510, Japan

Received April 3, 1998

DNA ligase IV in a complex with XRCC4 is responsible for DNA end-joining in repair of DNA double-strand breaks (DSB) and V(D)J recombination. We found that non-histone chromosomal high mobility group (HMG) proteins 1 and 2 enhanced the ligation of linearized pUC119 DNA with DNA ligase IV from rat liver nuclear extract. Intra-molecular and inter-molecular ligations of cohesive-ended and blunt-ended DNA were markedly stimulated by HMG1 and 2. Recombinant HMG2-domain A, B, and (A + B) polypeptides were similarly, but non-identically, effective for the stimulation of DSB ligation reaction. Ligation of single-strand breaks (nicks) was only slightly activated by the HMG proteins. The DNA end-binding Ku protein singly or in combination with the catalytic component of DNA-dependent protein kinase (DNA-PK) as the DNA-PK holoenzyme was ineffective for the ligation of linearized pUC119 DNA. Although the stimulatory effect of HMG1 and 2 on ligation of DSB *in vitro* was not specific to DNA ligase IV, these results suggest that HMG1 and 2 are involved in the final ligation step in DNA end-joining processes of DSB repair and V(D)J recombination.

Among rodent ionization-radiation repair complementation groups (IR), IR4, 5, 6, and 7 are deficient both in repair of DNA double-strand breaks (DSB) and in recombination of antigen-receptor gene segments, which are complemented by corresponding human *XRCC*4, 5, 6, and 7 genes (1). Recently *XRCC4* gene product has been shown to be tightly bound to DNA ligase IV as its activator protein, indicating that DNA ligase IV in conjunction with XRCC4 functions on non-homologous DNA end-joining in the processes of DSB repair and V(D)J recombination (2,3). This is confirmed by the observation that the homologue of DNA ligase IV in yeast has been demonstrated to mediate non-homologous DNA end-joining (4,5). The*XRCC*5 and *XRCC*7 genes have been proved to encode p80 subunit of heterodimeric Ku protein (p70/p80) and the catalytic component of DNA-dependent protein kinase (DNA-PK), respectively, and IR6 complemented by the*XRCC*6 gene is devoid of Ku protein (6). Although DNA-PK composed of the p470 catalytic component (DNA-PKcs) and the DNA-end binding regulatory component Ku protein is considered to function in the process of DNA end-joining in DSB repair and V(D)J recombination, the molecular mechanism of DNA end-joining remains to be clarified (1). DNA-PK is a nuclear serine/threonine kinase catalyzing *in vitro* phosphorylation of a variety of proteins, including simian virus 40 large T antigen, Sp1, Fos, Jun, c-Myc, p53, RPA (p34), TBP, TFIIB, and the largest subunit of RNA polymerase II (7-19).

We previously reported that DNA-PK activity in highly purified preparations from Raji nuclear extracts is stimulated by non-histone chromosomal high mobility group (HMG) proteins 1 and 2 (16), and recently found that HMG1 and 2 function as DNA-binding regulatory components for DNA-PK like Ku protein *in vitro* (Y. Yumoto, *et al.;* manuscript in submission). Ubiquitous HMG1 and 2 with molecular masses of about 25 kDa are composed of two similar repeats of DNA binding domains, termed HMG-domains A and B, and an acidic C-terminal region (20). HMG1 and 2 bind to DNA sequence-non-specifically and bend DNA (21). Interestingly HMG1 and 2 enhance RAG1 and RAG2-mediated cleavage at a V(D)J recombination signal *in vitro* (22,23).

In this paper, we show that HMG1 and 2 stimulated

[1] Corresponding author. Fax: 81-3-5280-8075. E-mail: hteraoka.pbc@mri.tmd.ac.jp.

Abbreviations used: HMG, high mobility group; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, catalytic subunit of DNA-PK; XRCC, X-ray repair cross-complementing; DSB, DNA double-strand breaks.

0006-291X/98 $25.00

cohesive-end and blunt-end ligations with DNA ligase IV highly purified from rat liver nuclear extracts. In addition, DNA-PKcs and Ku protein, singly or in combination as DNA-PK, were ineffective for the dsDNA ligation reaction.

MATERIALS AND METHODS

Materials. [α-^{32}P]ATP and [γ-^{32}P]ATP were purchased from NEN (Boston, MA, USA). Other chemicals of reagent grade were described previously (10,15,16,19).

DNA-PKcs and Ku protein. DNA-PK was partially purified from Raji nuclear extracts as described (15), and then subjected to glycerol gradient (15-30%) centrifugation to separate DNA-PKcs and Ku protein from each other. DNA-PKcs and Ku protein were purified to near-homogeneity by successive column chromatography. These purification procedures will be published in detail (Y. Yumoto *et al.*, manuscript in submission).

HMG1 and 2. HMG1 and 2 proteins were purified to homogeneity from pig thymus as described (24). Recombinant HMG2-domain A (residues 1-76), -domain B (residues 88-164), and -domains (A + B) (residues 1-164) proteins were purified from lysates of *Escherichia coli* BL21 cells harboring the pGEM plasmids with the corresponding cDNA sequences downstream the T7 promoter (K. Yoshioka *et al.*, manuscript in preparation; see Ref. 25).

Purification of DNA ligase IV. Purification procedures were performed at 0-4°C. Nuclei were purified from rat liver (70 g) as described previously (26). To the nuclear pellet, 50 ml of Buffer A (50 mM NaPi, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA, and 1 μg/ml each of aprotinin, leupeptin and pepstatin A) containing 0.4 M NaCl was added with subsequent stirring for 15 min. The suspension was subjected to centrifugation at 100,000 *g* for 30 min to obtain nuclear extract. The extract was passed through DEAE-cellulofine equilibrated with Buffer A containing 0.4 M NaCl. The pass-through fraction was dialyzed against Buffer A containing 0.05 M NaCl, and then applied to a DEAE-cellulofine column (2.7 $\times$ 10 cm) equilibrated with Buffer A/0.05 M NaCl. After washing the column with the above buffer, proteins were eluted with 100 ml of a 0.05 to 1 M NaCl linear gradient in Buffer A. The active fractions were combined and dialyzed against Buffer A containing 0.05 M NaCl. The dialysate was applied to a column (2.7 $\times$ 8 cm) of phosphocellulose equilibrated with Buffer A/0.05 M NaCl, and proteins were eluted with 80 ml of a 0.05-0.8 M NaCl gradient in Buffer A. The active fractions were combined and dialyzed against Buffer B (50 mM Tris-HCl, pH 7.5, 0.5 mM dithiothreitol, 1 mM EDTA, 5% glycerol, and 1 μg/ml each of aprotinin, leupeptin and pepstatin A) containing 25 mM NaCl. The dialysate was applied to an FPLC Mono S HR5/5 column (Pharmacia Biotech) equilibrated with the above buffer. Proteins were eluted with 24 ml of a 25-400 mM NaCl gradient in Buffer B. DNA ligase activity was determined by cohesive-end ligation and adenylylation reaction (see Fig. 1). The active fractions were frozen at -80°C until use. Protein was determined by the Bio-Rad dye-binding assay with BSA as a standard.

Formation of DNA-ligase-[^{32}*P*]*AMP.* DNA ligase was adenylylated in a reaction mixture (20 μl) containing 75 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 2 μg BSA, 5 mM dithiothreitol, 0.1 μM PPi and 0.5 μM ATP (3 μCi [α-^{32}P]ATP) at 30°C for 30 min (27,28). After addition of 3 $\times$ SDS sample buffer, the products were analyzed by electrophoresis on a 0.1% SDS/8% polyacrylamide gel with subsequent autoradiography. Rainbow colored protein molecular weight markers (Amersham) were used.

DNA ligase assay. Linearized pUC119 DNA was prepared by treatment with *Bam* HI or *Hinc* II with subsequent phenol-chloroform treatment. Ligation of linearized pUC119 DNA was performed in a reaction mixture (10 μl) containing 75 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 0.2 mM ATP, 2.5 mM dithiothreitol, 1 μg BSA, 0.5 μg linearized pUC119 DNA, and enzyme (27,28). The reaction was performed at 30°C for 20 min unless otherwise specified, and stopped by addition of 0.5 μl of 0.5 M EDTA (pH 8) and 0.5 μl of proteinase K (10 mg/ml) with subsequent incubation at 37°C for 20 min. The samples were subjected to electrophresis on a slab gel composed of 0.8% agarose, 90 mM Tris, 90 mM boric acid, 2 mM EDTA, and 0.5 μg/ml ethidium bromide. The nick-ligation reaction was performed as described previously (27,28) in the above reaction mixture (10 μl) containing [5′-^{32}P]dT_{20} hybridized with dA_n or [5′-^{32}P]$rA_{12\text{-}18}$ hybridized with dT_n instead of linearized pUC119 DNA.

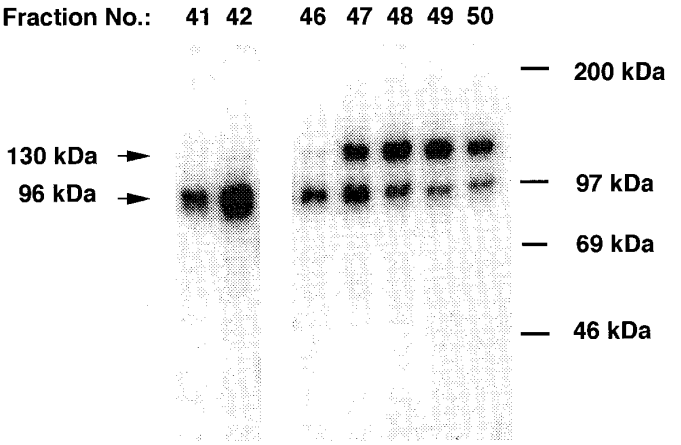

FIG. 1. Detection of DNA ligase-[^{32}P]AMP for DNA ligases purified from rat liver nuclear extract. To detect DNA ligase-[^{32}P]AMP complex, 2 μl each of Mono S fractions (fraction numbers 41, 42, and 46-50) was incubated with [α-^{32}P]ATP as described in Materials and Methods. Molecular weight markers are indicated in the right. DNA ligase IV with molecular mass of 96 kDa was observed in Fractions 41 and 42, and DNA ligase I with an apparent molecular mass of 130 kDa and DNA ligase III (100 kDa) were in fractions 47-50.

RESULTS

Purification of DNA ligase IV from rat liver nuclear extract. In the final Mono S column chromatography, DNA ligase activity was eluted as two peaks at about 0.22 and 0.28 M NaCl as described in Ref. 29. As shown in Fig. 1, the faster eluting ligase corresponded to DNA ligase IV with a molecular mass of 96 kDa (29,30). This is confirmed by the observation that DNA ligase IV was not able to react with oligo(rA) hybridized with poly(dT) (see Ref. 29). In the later eluting fractions two DNA ligase bands of DNA-ligase-[^{32}P]AMP were observed, which are considered to be DNA ligase I with an apparent molecular mass of 130 kDa and probably DNA ligase III with 100 kDa (Fig. 1) (31).

Stimulation of cohesive-end ligation by HMG1 and 2. In general, purified DNA ligases are capable of catalyzing ligation reaction of single-strand breaks on dsDNA and DSB with cohesive-ends extremely faster than that of DSB with blunt-ends. As shown in Fig. 2, cohesive-ended pUC119 DNA was ligated by DNA ligase IV, resulting in circular DNA by intra-molecular

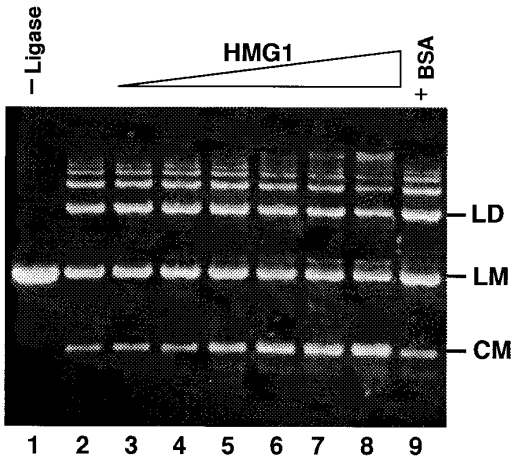

FIG. 2. HMG1 stimulates cohesive-end ligation with rat liver DNA ligase IV. DNA ligase IV (lanes 2-9) was incubated at 30°C for 20 min in a reaction mixture containing *Bam* H1-digested pUC119 DNA. Lane 1, no enzyme. Increasing amounts of HMG1 were added: 0 μg for lane 2; 0.05 μg for lane 3; 0.1 μg for lane 4; 0.2 μg for lane 5; 0.5 μg for lane 6; 1 μg for lane 7; 2 μg for lane 8. Lane 9 contained additional 2 μg BSA. CM, circular monomer; LM, linear monomer; LD, linear dimer.

ligation and linear oligomeric products by inter-molecular ligation. HMG1 stimulated both the inter-molecular and intra-molecular ligations in a dose-dependent manner (Fig. 2). Although blunt-end ligation was hardly detectable with DNA ligase IV as seen in other species of mammalian DNA ligase, a weak activity and the stimulatory effect of HMG1 were observed in a prolonged incubation (Fig. 3). HMG2 similarly enhanced cohesive-end ligation with DNA ligase IV (Fig. 4b).

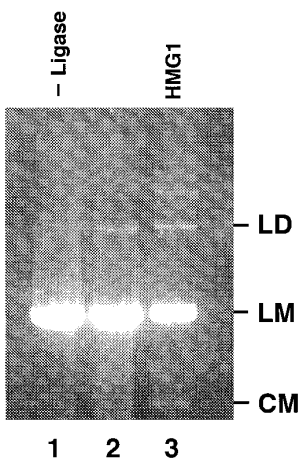

FIG. 3. Stimulatory effect of HMG1 on blunt-end ligation with DNA ligase IV. DNA ligase IV (lanes 2 and 3) was incubated at 30°C for 90 min in a reaction mixture (20 μl) containing *Hinc* II-digested pUC119 DNA in the absence (lane 2) and presence of 2 μg HMG1 (lane 3). Lane 1, no enzyme. CM, circular monomer; LM, linear monomer; LD, linear dimer.

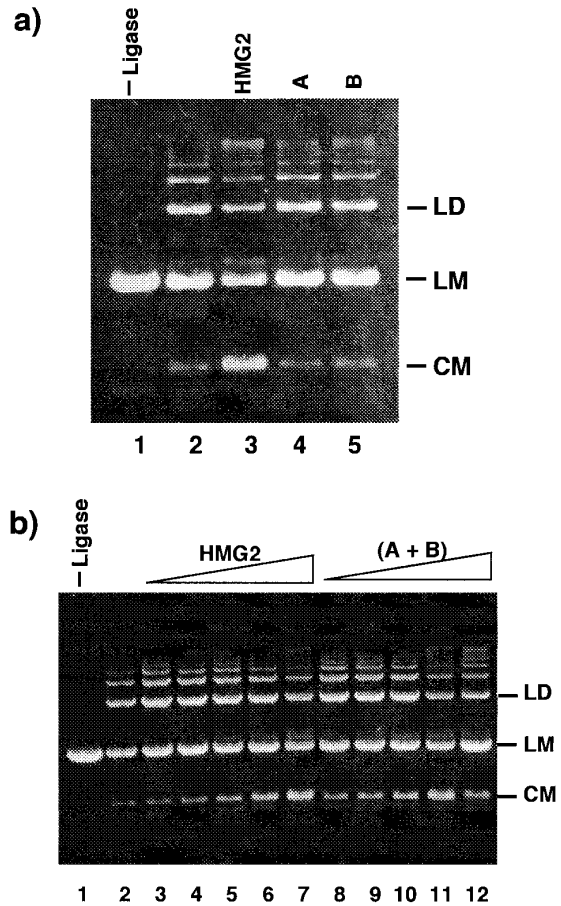

FIG. 4. Effect of recombinant HMG2 polypeptides containing HMG-domain A, B, and (A + B). (a) DNA ligase IV (lanes 2-5) was incubated at 30°C for 20 min in a reaction mixture (20 μl) containing *Bam* H1-digested pUC119 DNA. Lane 1, no enzyme. Lanes 3-5 contained 2 μg HMG2, 1 μg HMG2-domain A, and 1 μg HMG2-domain B, respectively. (b) DNA ligase IV (lanes 2-12) was incubated at 30°C for 20 min in a reaction mixture containing *Bam* H1-digested pUC119 DNA. Lane 1, no enzyme. Increasing amounts of HMG2 were added: 0 μg for lane 2; 0.125 μg for lane 3; 0.25 μg for lane 4; 0.5 μg for lane 5; 1 μg for lane 6; 2 μg for lane 7. Increasing amounts of HMG2-domains (A + B) were added: 0.125 μg for lane 8; 0.25 μg for lane 9; 0.5 μg for lane 10; 1 μg for lane 11; 2 μg for lane 12. CM, circular monomer; LM, linear monomer; LD, linear dimer.

Effect of recombinant HMG-domains A and B on ligation reaction. HMG1 and 2 are composed of HMG-domains A and B, and the acidic C-terminal region (20). In order to verify which domains are required for stimulation of DNA ligation reaction, we examined recombinant polypeptides containing HMG2-domain A, B, and (A + B). Compared with full-length HMG2, the stimulatory effect of HMG2-domain A and B polypeptides on DNA ligation reaction was weak (Fig. 4a), but (A + B) polypeptide was effective at lower amounts (Fig. 4b). The effectiveness for the stimulation of DNA end-ligation seems to be parallel to the affinity for dsDNA, because dissociation constants for linear dsDNA of full-length HMG2 and HMG2-domains (A +

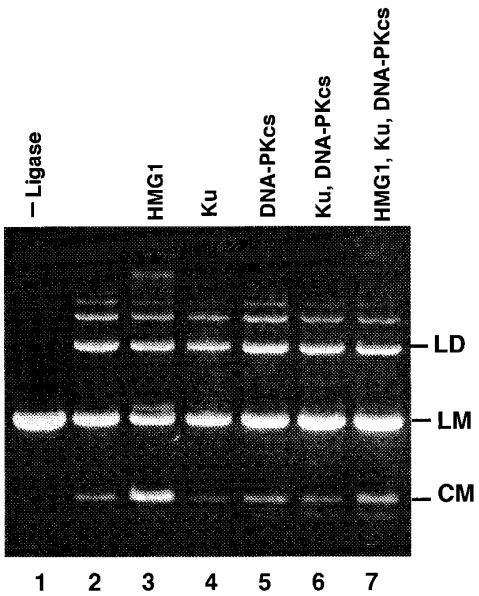

FIG. 5. Effect of Ku and DNA-PKcs on cohesive-end ligation. DNA ligase IV (lane 2-7) was incubated at 30°C for 20 min in a reaction mixture (20 μl) containing *Bam* H1-digested pUC119 DNA. Lane 1, no enzyme. Lanes 3-7 contained 2 μg HMG1, 0.24 μg Ku protein , 0.08 μg DNA-PKcs, Ku + DNA-PKcs and HMG + Ku + DNA-PKcs, respectively. CM, circular monomer; LM, linear monomer; LD, linear dimer.

B) polypeptide were estimated to be 3.0 and 1.1 $\times$ 10^{-6} M^{-1}, respectively (25).

Influence of HMG proteins on nick sealing reaction. To examine whether HMG1 and 2 influence on ligation reaction of single-strand breaks on DNA, [5′-^{32}P]dT_{20} hybridized with dA_n was used as a substrate. Addition of HMG1 and 2 slightly activated the nick ligation reaction (1.1 to 1.3-fold). These results indicate that HMG1 and 2 markedly stimulate ligation reaction of DSB, but do not significantly ligation reaction of single-strand breaks.

DNA-end binding Ku protein does not stimulate DNA double-strand break ligation. Ku protein is known to be a typical DNA end-binding protein, which is the regulatory component of DNA-dependent protein kinase involved in DNA end-joining process in DSB repair and V(D)J recombination (1). It is attractive to know whether Ku protein singly or in combination with DNA-PKcs as the holoenzyme affects the final ligation step in DNA end-joining process. Ku protein singly and in combination with DNA-PKcs and HMG1 did not enhance cohesive-end ligation, but it was rather inhibitory (Fig. 5). For blunt-end ligation, Ku protein, DNA-PKcs, and the mixture of the both were also ineffective (data not shown).

DISCUSSION

Our present results demonstrate that both intra-molecular and inter-molecular ligations of the linearized pUC119 DNA (3.16 kbp) with DNA ligase IV were enhanced by HMG1 and 2. The intra-molecular ligation resulting in the circular monomer was stimulated maximally 5 to 6-fold by addition of HMG1 (Fig. 1) and 2 (Fig. 4). For the inter-molecular ligation of linearized pUC119 DNA, linear oligomers were observed on agarose gels, and interestingly longer products were obtained in the presence of HMG proteins (see Figs. 2-4). Although it was hard to determine precisely activation ratio by HMG1 and 2 in inter-molecular ligation, we roughly estimated about 3-fold stimulation of inter-molecular ligation by HMG1 and 2. HMG1 has been reported to be capable of inducing DNA bend as revealed by the formation of circular oligomers of short dsDNA fragments (87-123 bp) with T4 DNA ligase (21). In the absence of HMG1, linear oligomers were exclusively formed by T4 ligase, implying that HMG1 stimulates intra-molecular ligation of short dsDNA.

Since similar stimulation of the ligation reaction by HMG1 and 2 was observed with other DNA ligases tested, such as human DNA ligase I and T_4 DNA ligase, HMG1 and 2 seem to interact directly with DNA substrates but not DNA ligase itself. It remains to be clarified what kind of DNA conformation favorable for end ligation is induced by HMG 1 and 2. HMG1 and 2 binds dsDNA, especially supercoiled and cruciform DNA, and shows low affinity for linear dsDNA (Kd = about 10^{-6} M^{-1}) (25). Physiologically HMG1 and 2 proteins seems to be involved at least in transcription and cell proliferation, respectively (32,33).

Mutant cells devoid of either XRCC4 as the activator for DNA ligase IV, Ku protein, or DNA-PKcs are deficient in DNA end-joining for DSB repair and V(D)J recombination (1-5). DNA ligase IV is not necessarily preferable for ligation of blunt-ended DNA, but rather DNA ligase I can catalyze blunt-end ligation (2,5,31). This suggests that alignment factor(s) will be required for ligation reaction of linearized dsDNA, especially for blunt-end ligation, with DNA ligase IV. Despite of the lack of ligase-species specificity, HMG1 and 2 are considered to be the candidates *in vivo* for blunt-end ligation with DNA ligase IV.

Our data indicate that DNA end-binding Ku protein singly or in combination with DNA-PKcs had no significant effect on the ligation of linearized pUC119 DNA. Ku protein tightly, preferentially binds to dsDNA ends with a Kd of about 10^{-9} M^{-1} (34), and can translocate on DNA strand without ATP (35). In addition, Ku protein as the regulatory component of DNA-PK is essential for DNA end-joining process in DSB repair and V(D)J recombination. Most recently Ramsden and Gellert (36) reported that Ku protein extremely enhance inter-molecular ligation of 60-bp DNA fragments by mammalian DNA ligases under appropriate reaction conditions. This means a direct role of Ku protein in the absence of collaboration with DNA-PKcs in DSB joining. Discrepancy between their and our observa-

tions may be due to drastic difference in length of DNA substrate employed.

ACKNOWLEDGMENT

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

1. Jackson, S. P., and Jeggo, P. A. (1995) *Trend. Biochem. Soc.* **20,** 412–415.
2. Grawunder, U., Wilm, M., Wu, X., Kulesza, P., Wilson, T. E., Mann, M., and Lieber, M. R. (1997) *Nature* **388,** 492–495.
3. Critchlow, S. E., Bowater, R. P., and Jackson, S. P. (1997) *Curr. Biol.* **7,** 588–598.
4. Wilson, T. E., Grawunder, U., and Lieber, M. R. (1997) *Nature* **388,** 495–498.
5. Schar, P., Herrmann, G., Daly, G., and Lindahl, T. (1997) *Genes Dev.* **11,** 1912–1924.
6. Lee, S. E., He, D. M., and Hendrickson, E. A. (1996) *in* Current Topics in Microbiology and Immunology, (Jessberger, R., and Lieber, M. R., Eds.), Vol. 217 pp. 133–142, Springer–Verlag, Berlin/Heidelberg.
7. Carter, T., Vancurova, I., Sun, I., Lou, W., and DeLeon, S. (1990) *Mol. Cell. Biol* **10,** 6460–6471.
8. Lees-Miller, S. P., Chen, Y.-R., and Anderson, C. W. (1990) *Mol. Cell. Biol.* **10,** 6472–6481.
9. Dvir, A., Peterson, S. R., Knuth, M. W., Lu, H., and Dynan, W. S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11920–11924.
10. Iijima, S., Teraoka, H., Date, T., and Tsukada, K. (1992) *Eur. J. Biochem.* **206,** 595–603.
11. Lees-Miller, S. P., Sakaguchi, K., Ullrich, S. J., Appella, E., and Anderson, C. W. (1992) *Mol. Cell. Biol.* **12,** 5041–5049.
12. Dvir, A., Stein, L. Y., Calore, B. L., and Dynan, W. S. (1993) *J. Biol. Chem.* **268,** 10440–10447.
13. Anderson, C. W. (1993) *Trends Biochem. Soc.* **18,** 433–437.
14. Gottlieb, T. M., and Jackson, S. P. (1993) *Cell* **72,** 131–142.
15. Watanabe, F., Teraoka, H., Iijima, S., Mimori, T., and Tsukada, K. (1994) *Biochim. Biophys. Acta* **1223,** 255–260.
16. Watanabe, F., Shirakawa, H., Yoshida, M., Tsukada, K., and Teraoka, H. (1994) *Biochem. Biophys. Res. Comm.* **202,** 736–742.
17. Pan, Z.-Q., Amin, A. A., Gibbs, E., Niu, H., and Hurwitz, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8343–8347.
18. Brush, G. S., Anderson, C. W., and Kelly, T. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12520–12524.
19. Chibazakura, T., Watanabe, F., Kitajima, S., Tsukada, K., Yasukochi, Y., and Teraoka, H. (1997) *Eur. J. Biochem.* **247,** 1166–1173.
20. Shirakawa, H., Tsuda, K., and Yoshida, M. (1990) *Biochemistry* **29,** 419–4423.
21. Pil, P. M., Chow, C. S., and Lippard, S. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9465–9469.
22. Agrawal, A., and Schatz, G. (1997) *Cell* **89,** 43–53.
23. Sawchuk, D. J., Weis-Garcia, F., Malik, S., Besmer, E., Bustin, M., Nussenzweig, M. C., and Cortes, P. (1997) *J. Exp. Med.* **185,** 2025–2032.
24. Adachi, Y., Mizuno, S., and Yoshida, M. (1990) *J. Chromatogr.* **530,** 39–46.
25. Yamamoto, A., Ando, Y., Yoshioka, K., Saito, K., Tanabe, T., Shirakawa, H., and Yoshida, M. (1997) *J. Biochem. (Tokyo)* **122,** 586–594.
26. Ohmura, Y., Teraoka, H., and Tsukada, K. (1986) *FEBS Lett.* **208,** 451–454.
27. Teraoka, H., Minami, H., Iijima, S., Tsukada, K., Koiwai, O., and Date, T. (1993) *J. Biol. Chem.* **268,** 24156–24162.
28. Matsuda, S., Sakaguchi, K., Tsukada, K., and Teraoka, H. (1996) *Eur. J. Biochem.* **237,** 691–697.
29. Robins, P., and Lindahl, T. (1996) *J. Biol. Chem.* **271,** 24257–24261.
30. Wei, Y.-F., Robins, P., Carter, K., *et al.* (1995) *Mol. Cell. Biol.* **15,** 3206–3216.
31. Lindahl, T., and Barnes, D. E. (1992) *Ann. Rev. Biochem.* **61,** 251–281.
32. Ogawa, Y., Aizawa, S., Shirakawa, H., and Yoshida, M. (1995) *J. Biol. Chem.* **270,** 9272–9280.
33. Yamazaki, F., Nagatsuka, Y., Shirakawa, H., and Yoshida, M. (1995) *Biochem. Biophys. Res. Commun.* **210,** 1045–1051.
34. Blier, P. R., Griffith, A. J., Craft, J., and Hardin, J. A. (1993) *J. Biol. Chem.* **268,** 7594–7601.
35. de Vries, E., van Driel, W., Bergsma, W. G., Amberg, A. C., and van der Vliet, P. C. (1989) *J. Mol. Biol.* **208,** 65–78.
36. Ramsden, D. A., and Gellert, M. (1998) *EMBO J.* **17,** 609–619.