Ku is associated with the telomere in mammals

Hsin-Ling Hsu^{*†}, David Gilley^{**}, Elizabeth H. Blackburn[‡], and David J. Chen^{§¶}

[†]Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545; [‡]Department of Microbiology and Immunology University of California, San Francisco, CA 94143-0414; and [§]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Edited by Joseph G. Gall, Carnegie Institution of Washington, Baltimore, MD, and approved August 31, 1999 (received for review June 14, 1999)

Telomeres are specialized DNA/protein complexes that comprise the ends of eukaryotic chromosomes. The highly expressed Ku heterodimer, composed of 70 and 80 K_d subunits (Ku70 and Ku80), is the high-affinity DNA binding component of the DNA-dependent protein kinase. Ku is critical for nonhomologous DNA double-stranded break repair and site-specific recombination of V(D)J gene segments. Ku also plays an important role in telomere maintenance in yeast. Herein, we report, using an *in vivo* crosslinking method, that human and hamster telomeric DNAs specifically coimmunoprecipitate with human Ku80 after crosslinking. Localization of Ku to the telomere does not depend on the DNA-dependent protein kinase catalytic component. These findings suggest a direct link between Ku and the telomere in mammalian cells.

Vertebrate telomeric DNA is composed of (T₂AG₃) tandem repeats, with the number of repeats varying between different species (1, 2). The GT-rich strand of telomeric DNA is synthesized by telomerase, a specialized ribonucleoprotein reverse transcriptase. A minimal telomeric DNA length and, in some situations, an active telomerase are required for chromosome stability and cellular viability (3, 4), with failure to maintain telomere length or function leading to a form of replicative senescence in *Tetrahymena*, yeasts, and mammalian cells (5–7). Activation of telomerase is characteristic of most established mammalian cell lines and tumors (8). Correspondingly, experimental activation of telomerase also allows certain virus-transformed human cell lines to bypass authentic cellular replicative senescence and crises and continue proliferation (4, 6, 9).

Several lines of evidence have recently converged supporting the conclusion that the Ku heterodimer is critical for telomere maintenance in yeast. *Saccharomyces cerevisiae* null for Ku70 or Ku80 have defects in telomere silencing, abnormally short telomeres, and a senescence-like phenotype at 37°C (10–13). Strikingly, despite the relatively low sequence conservation between yeast and human Ku subunits at the amino acid level, the exogenous expression of human Ku subunits rescues Ku null yeast from cell death at 37°C (14). Direct evidence for the physical localization of the Ku heterodimer to yeast telomeres came from an *in vivo* crosslinking experiment linking Ku to telomeric DNA (15). Recently, Martin *et al.* (16) demonstrated in yeast that Ku80 colocalizes with the telomere binding protein Rap1 at telomeric foci. These types of experiments have not been possible to perform in mammalian cells because of the large abundance and uniform distribution of Ku throughout the mammalian nucleus.

However, nothing has been reported concerning the Ku heterodimer's role at the mammalian telomere. Using an *in vivo* crosslinking method, we show that Ku is localized to mammalian telomeric repeats. We also determined that the DNA-dependent protein kinase catalytic component (DNA-PKcs) is not required *in vivo* for the association of Ku with telomeric repeats.

Materials and Methods

Cell Cultures. Human cervical carcinoma cell line HeLa and human malignant glioma cell lines MO59J and MO59K were cultured in RPMI medium 1640 (GIBCO/BRL) supplemented with 10% (vol/vol) FBS (HyClone). Human skin fibroblast (HSF-6) and Chinese hamster ovary (CHO) cell lines, XHB104 and xrs-6c, respectively, were cultured in MEM- α medium

(GIBCO/BRL) supplemented with 10% (vol/vol) FBS. These cells were passaged with trypsin-EDTA to maintain subconfluence.

In Vivo Crosslinking. Exponentially growing cells were harvested and washed twice with PBS. Harvested cells were resuspended in growth medium (1×10^7 cells per ml) and fixed in a formaldehyde solution containing 1% formaldehyde (Sigma), 1% methanol stock solution (Merck), 10 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 5 mM Tris-HCl (pH 8.0) at 4°C for 60 min (17). Fixation was stopped by washing twice in PBS. The cells were resuspended in SDS buffer (1% SDS/10 mM EDTA/50 mM Tris-HCl, pH 8.0) or buffer A [1% Triton X-100/150 mM NaCl/2.5 mM KCl/5 mM Na₂HPO₄/1.5 mM KH₂PO₄/10 mM EDTA/25 mM Tris-HCl, pH 7.4/1 mM DTT/10% (vol/vol) glycerol/1 mM PMSF] at a concentration of 2.5×10^7 cells per ml. The cell suspension was incubated on ice for 30 min, followed by sonication in five bursts of 1-min intervals. DNA was sheared to an average size of 2–5 kilobases. The suspension was centrifuged at $10,000 \times g$ for 15 min to pellet insoluble materials.

Immunoprecipitation. Buffer A extracts were used directly, and SDS extracts were diluted 5-fold with buffer A before immunoprecipitation. An aliquot of extract (2.5×10^7 cells) was immunoprecipitated with Ku80 monoclonal antibody-coupled protein A/G beads (Amersham Pharmacia; Roche Molecular Biochemicals) for 12 h at 4°C in the presence or absence of 3 μ g of λ -DNA. Protein A/G beads were washed six times with NET buffer (0.5% Nonidet P-40/1 mM EDTA/1 mM EGTA/150 mM NaCl/5 mM MgCl₂/50 mM Tris-HCl, pH 8.0). To isolate coimmunoprecipitated DNA, immunocomplexes were extracted with 100 μ l of 1% SDS/0.1 M NaHCO₃ and then washed five times with 100 μ l of TE (10 mM Tris-HCl, pH 7.5/1 mM EDTA) buffer. The pooled eluates were heated at 65°C for 8 h to reverse the formaldehyde crosslinks (18, 19). The decrosslinked samples were extracted twice with phenol/chloroform/isoamylalcohol (25:24:1, vol/vol) and ethanol precipitated. The immunoprecipitated DNA was resuspended in 15 μ l of TE and treated with 50 μ g/ml DNase-free RNase A for 30 min at 37°C.

Hybridization Analysis by Dot Blot. To characterize coimmunoprecipitated DNA, DNA probes [(C₃TA₂)₁₆, λ -DNA, genomic DNA, actin DNA, and human repetitive Alu DNA] were labeled by oligonucleotide random-primed DNA synthesis (Promega) with [α -³²P]dCTP. An equivalent amount of immunoprecipitated DNA (from 6.7×10^6 cells) was denatured with 1 M NaOH and placed onto a Hybond-N⁺ filter with a dot blot apparatus (Bio-Rad). Filters were prehybridized in buffer [10% (vol/vol)

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: DNA-PKcs, DNA-dependent protein kinase catalytic component; HSF, human skin fibroblast; CHO, Chinese hamster ovary.

^{*}Present address: Life Sciences Division, Lawrence Berkeley National Laboratory, Mail stop 74, 1 Cyclotron Road, Berkeley, CA 94720.

[¶]To whom reprint requests should be addressed. E-mail: djchen@lbl.gov.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Fig. 1. Human telomeric DNA coimmunoprecipitated with Ku80 monoclonal antibody on *in vivo* crosslinking. DNA isolated from immunoprecipitates (IP) was placed onto a membrane by using a dot-blotting apparatus and then analyzed by hybridization with ^{32}P -labeled DNA probes. Cell lysates were immunoprecipitated with either p21 monoclonal antibody (α -p21) or Ku80 monoclonal antibody (α -Ku80). The following probes were used: $(C_3TA_2)_{16}$ (C_3TA_2), λ -DNA (Lambda), total human genomic DNA from HeLa cells (Total DNA), actin DNA (Actin), and Alu sequence repeats (Alu); formaldehyde treatment for 60 min (F60); no formaldehyde treatment (F0). (A) *In vivo* crosslinking of HeLa cells. Dot blots were exposed for the following times: C_3TA_2 , 48 h; Lambda, 3 h; Total DNA, 3 h; Actin, 18 h; Alu, 30 min. (B) *In vivo* crosslinking of HSF cells. Exposure times: C_3TA_2 , 7 days; Total DNA, 8 h. (C) Differential telomeric DNA levels coimmunoprecipitated with and without formaldehyde crosslinking. The hybridized signals of telomeric DNA repeats c-immunoprecipitated with Ku monoclonal antibody before (F0) or after (F60) formaldehyde treatment were analyzed by PhosphorImager and quantitated by IMAGEQUANT software. Differential amounts of telomeric DNA associated with Ku were normalized to the levels of total DNA that was coimmunoprecipitated with Ku80 antibody. The background level of F0 telomeric DNA signal was set at 1 unit.

SDS/7% (vol/vol) polyethylene glycol/200 μ g/ml heat-denatured salmon sperm DNA] for 2 h at 62°C. Heat-denatured DNA probes were added directly to prehybridization solution and incubated at 62°C overnight. Filters were washed twice in $0.5\times$ SSC (0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.1% SDS at 55°C for 30 min for $(C_3TA_2)_{16}$ probe. Filters were washed twice in $0.1\times$ SSC/0.1% SDS for 30 min for λ -DNA, total DNA, actin DNA, and Alu DNA probes.

Immunoblotting. Before (from 5×10^4 cells) and after (from 5×10^5 cells) immunoprecipitation, samples were added to reducing Laemmli sample buffer and boiled for 10 min. Protein samples were fractionated by SDS/4–12% PAGE and transferred to nitrocellulose filters. The filters were immunoblotted with antibodies [α -Ku70 (Santa Cruz Biotechnology), α -Ku80, α -DNA-PKcs (Neomarker, Lab Vision, Fremont, CA), or α -Abl (Santa Cruz Biotechnology)] and reacted with peroxidase-conjugated goat α -mouse IgG (Bio-Rad) or rabbit α -goat IgG (Santa Cruz

Biotechnology). The antibody-reactive proteins were visualized by chemiluminescence (Amersham Pharmacia).

Results and Discussion

Association of the Ku Heterodimer with Telomeric DNA in Human Cells.

We performed *in vivo* crosslinking experiments to determine whether the Ku heterodimer is present at the mammalian telomere. When whole cells were treated with the crosslinking reagent formaldehyde, we observed enrichment of telomeric DNA coimmunoprecipitated with Ku80 monoclonal antibody (Fig. 1). Dot blot hybridization membranes were first probed with a telomeric sequence-specific probe C_3TA_2 (see *Materials and Methods*) to determine relative levels of coimmunoprecipitated telomeric DNA from formaldehyde-treated and untreated control samples (Fig. 1A). This same membrane was stripped of the C_3TA_2 telomeric probe and probed with total genomic DNA and actin DNA probes to allow normalization of nontelomeric DNAs coimmunoprecipitated by Ku80 antibody (Fig. 1A). These

nontelomeric probes indicated that nearly equal levels of non-telomeric DNAs were coimmunoprecipitated by Ku80 antibody regardless of formaldehyde treatment (Fig. 1A). Quantitative analysis after normalizing for amounts of total DNA on the membrane revealed that telomeric DNA was enriched by ≈ 20 -fold in crosslinked samples compared with noncrosslinked controls from HeLa cells (Fig. 1C). Crosslinked and noncrosslinked extracts were also incubated with p21 antibody to determine whether the enrichment of telomeric DNA was specific to Ku80 antibody (Fig. 1A). The p21 antibody coimmunoprecipitated lower amounts of total DNA compared with Ku80 antibody, and, as with the immunoprecipitation with Ku80 antibody, nearly equal amounts of total DNA were coimmunoprecipitated regardless of formaldehyde treatment (Fig. 1A). Samples were also incubated with IgG from normal mouse serum, with the results being essentially identical to those seen with p21 antibody (data not shown). These results suggested that enrichment of telomeric DNA in the Ku80 coimmunoprecipitate was specific to Ku80 antibody and was not caused by aggregation of protein–DNA complexes during formaldehyde treatment.

In additional controls, λ -DNA was added during cell lysis to determine whether Ku bound to exogenously added DNA under these conditions. The Ku80 monoclonal antibody coimmunoprecipitated higher amounts of λ -DNA than did the p21 monoclonal antibody (Fig. 1A), suggesting that Ku also bound to exogenously added λ -DNA in these extracts. Importantly, prior treatment of cells with formaldehyde caused no enrichment of λ -DNA, with nearly equal amounts of λ -DNA being coimmunoprecipitated in both treated and untreated samples (Fig. 1A). The same membrane was also probed with a repetitive Alu element probe to determine whether another repetitive element (20) besides telomeric repeats was enriched on formaldehyde treatment. As shown in Fig. 1A, nearly equal amounts of Alu repeat sequences were immunoprecipitated by formaldehyde treatment.

Identical *in vivo* crosslinking experiments were performed with a primary HSF cell line. Again, we found that telomeric DNA coimmunoprecipitation with Ku80 antibody was enriched greatly on treatment of whole cells with formaldehyde (Fig. 1B and C). Formaldehyde treatment did not result in enrichment of telomeric DNA in the p21 antibody immunoprecipitate, and the level of total genomic DNA coimmunoprecipitating with Ku80 antibody was unchanged by formaldehyde treatment (Fig. 1B). Quantitative analysis revealed that telomeric DNA in the Ku80 antibody coimmunoprecipitate was ≈ 16 -fold enriched by formaldehyde treatment (Fig. 1C). These results, which were similar for both HeLa and HSF cells, strongly suggest that the Ku heterodimer is in close proximity to the telomere in human cells.

Association of Human Ku with Telomeric DNA in a CHO Cell Line. The CHO cell line xrs-6 was shown previously to be repair-deficient because of the absence of Ku80. The repair-proficient x-ray hybrid cell line XHB104, derived from the CHO cell line xrs-6, contains a small fragment of human chromosome 2, which includes the human Ku80 gene (21, 22). We took advantage of these cell lines to test whether, as the results above predicted, enrichment of telomeric DNA by crosslinking requires the presence of human Ku80. Such a finding would also indicate that human Ku protein can localize to CHO telomeric DNA. Fig. 2 shows the results of using these CHO lines in the same type of crosslinking experiments described above for HeLa and HSF cells. Significantly, regardless of crosslinking, the repair-deficient line xrs-6 failed to coimmunoprecipitate telomeric DNA and total DNA above background levels (Fig. 2B). In contrast, crosslinking significantly enriched the level of coimmunoprecipitated telomeric DNA in the XHB104 line (Fig. 2B and C). XHB104 cells contained similar levels of Ku80 before or after immunoprecipitation (Fig. 2A). Equal numbers of cells

Fig. 2. Telomeric DNA is crosslinked to human Ku80 in a CHO hybrid cell line. (A) Western analysis before and after immunoprecipitation. Western analysis after immunoprecipitation (post-IP) probed with Ku80 antibody with a dash marking Ku80 and Ig heavy chain (IgH). Western analysis before immunoprecipitation (pre-IP) probed with both Ku80 and c-Abl antibodies with a dash marking Ku80 and c-Abl, respectively. (B) Dot blot analysis of DNA coimmunoprecipitated with Ku80 antibody. Lysates from xrs-6 and XHB104 were immunoprecipitated with Ku80 antibody. Coimmunoprecipitated DNA was then placed on a membrane and probed with either $(C_3TA_2)_{16}$ (C_3TA_2) or total genomic DNA from CHO cells (Total DNA). Exposure times: C_3TA_2 , 72 h; Total DNA, 4 h. (C) Differential telomeric DNA levels coimmunoprecipitated with and without formaldehyde crosslinking. The hybridized signals of telomeric DNA repeats coimmunoprecipitated with Ku80 monoclonal antibody before (F0) or after (F60) formaldehyde treatment were analyzed as described in the legend to Fig. 1C.

were used in each assay, as shown by the nearly equal levels of c-Abl protein in each sample (Fig. 2A). The absence of coimmunoprecipitated telomeric DNA in the xrs-6 samples is consistent with a requirement for Ku80 to coimmunoprecipitate telomeric DNA (Fig. 2B). These experiments render it unlikely that the Ku80 antibody nonspecifically crossreacts with other proteins that have become crosslinked to telomeric DNA by formaldehyde treatment.

In primary Chinese hamster cells, about 50% of telomeric repeats in chromosomal DNA are interstitially localized, nearly

exclusively at the centromeres (P. Slijepcevic, personal communication). In CHO cells, approximately 97% of the telomeric tracts are interstitial because of the reduction of telomeric length from an average of 38 kilobases in primary Chinese hamster cells to 1 kilobase in CHO cells (P. Slijepcevic, personal communication). Therefore, although the crosslinking results obtained with the CHO cells indicated that Ku is localized to telomeric tracts, these could have been interstitially located in the chromosomes. In addition, the large proportion of interstitial telomeric tracts in CHO cells might account for the high background of telomeric sequences coimmunoprecipitated with Ku80 antibody without crosslinking (Fig. 2 *B* and *C*; F0 samples). We propose that Ku binds DNA ends containing interstitial telomeric tracts once CHO cells are lysed and chromosomal DNA is fragmented by sonication (≈ 2 –5 kilobases). This process may be analogous to the binding of Ku to exogenously added λ -DNA in HeLa extracts (Fig. 1*A*). Alternatively, the terminal telomeric tracts in CHO cells are greatly reduced compared with HeLa and HSF cells. Therefore, less telomeric DNA is available to coimmunoprecipitate with Ku. Given that human chromosomes contain very few interstitial telomeric tracts and that telomeric DNA in anti-Ku immunoprecipitates is greatly enriched by crosslinking, together, these results provide strong evidence that Ku is localized to the telomeres in mammals.

DNA-PKcs Subunit Is Not Essential *In Vivo* for Ku Association with the Telomere. To determine whether the DNA-PKcs is essential for the association of Ku80, presumably as the Ku heterodimer, with the mammalian telomere, we used human glioma cell lines, which lack (cell line MO59J) or express (cell line MO59K) DNA-PKcs (23). As previously shown and repeated here, MO59J is deficient in the expression of DNA-PKcs, and MO59K, though from the same tumor (24), expresses DNA-PKcs (Fig. 3*A*). However, despite lacking DNA-PKcs, telomeric DNA in MO59J cells was still greatly enriched by coimmunoprecipitation with Ku80 antibody with formaldehyde treatment (Fig. 3 *B* and *C*). MO59K cells showed similar results (Fig. 3 *B* and *C*).

In this report, we have presented evidence suggesting an association of the Ku heterodimer with the telomere in mammalian cells. Association of Ku with the telomere must be functionally distinct from its role in double-stranded break repair to prevent telomeric end-to-end fusions (25, 26). Several possibilities could account for the proximity of Ku to telomeric DNA. Ku might bind initially to telomeric DNA ends and/or single-stranded/double-stranded junctions in T loops (27) and then translocate to internal telomeric DNA sites, or Ku might bind to internal telomeric DNA sites directly in a sequence-dependent manner, analogous to the way that Ku binds to specific enhancer regions (28, 29). Alternatively, Ku might not bind telomeric DNA directly but be held in close proximity to telomeric DNA by telomere binding proteins. The fact that specific crosslinking can be detected in CHO cells despite the preponderance of interstitial telomeric tracts suggests that a specialized DNA-protein complex is required to localize Ku to the terminal, as opposed to interstitial, telomeric tracts. In fact, the chromatin structure of interstitial and terminal telomeric tracts in CHO cells seems different, as indicated by their differing sensitivities to *in situ* nuclease digestion (30). Perhaps a combination of interactions involving both telomeric DNA and telomere binding proteins underlies the association of Ku with the telomeres in mammalian cells.

In the human glioma cell line MO59J, Ku is localized to telomeric DNA despite the absence of DNA-PKcs (23). Previously, it was shown that DNA-PKcs is not required for *in vitro* Ku binding to DNA ends (31). It is possible that other cellular kinases might function to compensate for the absence of DNA-PKcs. Alternatively, phosphorylation of Ku by the DNA-PKcs might not be required for telomere localization in cells (32). Scid

Fig. 3. DNA-PKcs is not required for the Ku heterodimer's association with the telomere. (*A*) Western blot probed with Ku70 antibody after immunoprecipitation (post-IP). Extracts were treated with 1% SDS before immunoprecipitation by Ku80 antibody to disrupt noncovalent interactions between heterodimer components Ku70 and Ku80. Extracts were diluted and immunoprecipitations were performed. IgH, Ig heavy chain. Western blot before immunoprecipitation (pre-IP). Membranes were probed with Ku70, Ku80, and DNA-PKcs antibodies. Dashes mark Ku70, Ku80, IgH and c-Abl proteins. (*B*) Dot blot analysis of DNA coimmunoprecipitated with Ku80 antibody. Lysates from MO59J and MO59K with 60 min of (F60) or without (F0) crosslinking were immunoprecipitated with Ku80 antibody. Coimmunoprecipitated DNA was probed with either (C₃TA₂)₁₆ (C₃TA₂) or total human genomic DNA from HeLa cells (Total DNA). Exposure times: C₃TA₂, 72 h; Total DNA, 4 h. (*C*) Differential telomeric DNA levels coimmunoprecipitated with and without formaldehyde crosslinking. The hybridized signals of telomeric DNA repeats coimmunoprecipitated with Ku80 monoclonal antibody before (F0) or after (F60) formaldehyde treatment were analyzed as described in the legend to Fig. 1*C*.

mice, which are deficient in DNA-PKcs, have abnormally long telomeres (33). Therefore, the DNA-PKcs influences telomere maintenance, possibly by modification of telomeric factors, and it is likely that the entire DNA-PK complex (Ku and DNA-PKcs) as a unit plays an important role in telomere maintenance.

We propose that, as has been found in yeast, Ku plays a critical

role in mammalian telomere maintenance. Several lines of evidence show that telomere maintenance is a critical event controlling the ability of cells to continue proliferating (4, 6, 9). Therefore, factors that control telomere maintenance are potential candidates for components that block proliferation and lead to cellular senescence. Such factors could include Ku, given that Ku-deficient mouse embryonic fibroblasts are reduced in their replicative capacity possibly because of loss of telomere length regulation (34, 35). In addition, there are several proteins that have been shown to play roles in telomere maintenance, possibly through signal transduction pathways including DNA-PKcs, ATM, Tankyrase, and poly(ADP-ribose) polymerase (33, 36–38).

We are only beginning to discover the protein components of the telomere, and much needs to be learned about how the telomere is actually regulated. In mammals, different tissues

have different modes of telomere regulation (39). Activation or deactivation of telomerase is only one aspect of telomere regulation. For example, in human white blood cells and TERT-transformed fibroblasts, telomerase activity is present, but telomeres still shorten (4, 40, 41). Understanding the factors involved in telomere regulation should lead to a better understanding of the control of cellular proliferation and cellular senescence.

We thank Joan Tuner for providing MO59J and MO59K cells lines; Ning-Hsin Yeh for generously supplying anti-Ku80 hybridoma; and Sandeep Burma, Judith Campisi, and Priscilla Cooper for critical reading of the manuscript and for helpful suggestions. This work was supported by the U.S. Department of Energy and by National Institutes of Health Grants CA50519 (to D.J.C.) and GM26259 (to EHB).

- Blasco, M., Lee, H., Hande, M., Samper, E., Lansdorp, P., DePinho, R. & Greider, C. (1997) *Cell* **91**, 25–34.
- de Lange, T. (1998) *Cancer J. Sci. Am.* **4**, S22–S25.
- Prescott, J. & Blackburn, E. H. (1997) *Genes Dev.* **11**, 528–540.
- Zhu, J., Wang, H., Bishop, J. M. & Blackburn, E. H. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 3723–3728.
- Lundblad, V. & Szostak, J. W. (1989) *Cell* **57**, 633–643.
- Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S. & Wright, W. E. (1998) *Science* **279**, 349–352.
- Yu, G. L., Bradley, J. D., Attardi, L. D. & Blackburn, E. H. (1990) *Nature (London)* **344**, 126–132.
- Shay, J. W. (1998) *Cancer J. Sci. Am.* **4**, S26–S34.
- Counter, C. M., Hahn, W. C., Wei, W., Caddle, S. D., Beijersbergen, R. L., Lansdorp, P. M., Sedivy, J. M. & Weinberg, R. A. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 14723–14728.
- Boulton, S. J. & Jackson, S. P. (1996) *Nucleic Acids Res.* **24**, 4639–4648.
- Boulton, S. J. & Jackson, S. P. (1996) *EMBO J.* **15**, 5093–5103.
- Porter, S. E., Greenwell, P. W., Ritchie, K. B. & Petes, T. D. (1996) *Nucleic Acids Res.* **24**, 582–585.
- Boulton, S. J. & Jackson, S. P. (1998) *EMBO J.* **17**, 1819–1828.
- Barnes, G. & Rio, D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 867–872.
- Gravel, S., Larrivee, M., Labrecque, P. & Wellinger, R. J. (1998) *Science* **280**, 741–744.
- Martin, S. G., Laroche, T., Suka, N., Grunstein, M. & Gasser, S. M. (1999) *Cell* **97**, 621–633.
- Orlando, V. & Paro, R. (1993) *Cell* **75**, 1187–1198.
- Solomon, M., Larsen, P. & Varshavsky, A. (1988) *Cell* **53**, 937–947.
- Braunstein, M., Rose, A. B., Holmes, S. G., Allis, C. D. & Broach, J. R. (1993) *Genes Dev.* **7**, 592–604.
- Szmulewicz, M. N., Novick, G. E. & Herrera, R. J. (1998) *Electrophoresis* **19**, 1260–1264.
- Chen, D. J., Marrone, B. L., Nguyen, T., Stackhouse, M., Zhao, Y. & Siciliano, M. J. (1994) *Genomics* **21**, 423–427.
- Chen, F., Peterson, S. R., Story, M. D. & Chen, D. J. (1996) *Mutat. Res.* **362**, 9–19.
- Lees-Miller, S. P., Godbout, R., Chan, D. W., Weinfeld, M., Day, R. S., III, Barron, G. M. & Allalunis-Turner, J. (1995) *Science* **267**, 1183–1185.
- Allalunis-Turner, M. J., Barron, G. M., Day, R. S., III, Dobler, K. D. & Mirzayans, R. (1993) *Radiat. Res.* **134**, 349–354.
- Bertuch, A. & Lundblad, V. (1998) *Trends Cell Biol.* **8**, 339–342.
- Shore, D. (1998) *Science* **281**, 1818–1819.
- Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H. & de Lange, T. (1999) *Cell* **97**, 503–514.
- Giffin, W., Torrance, H., Rodda, D. J., Prefontaine, G. G., Pope, L. & Hache, R. J. (1996) *Nature (London)* **380**, 265–268.
- Giffin, W., Kwast-Welfeld, J., Rodda, D. J., Prefontaine, G. G., Traykova-Andonova, M., Zhang, Y., Weigel, N. L., Lefebvre, Y. A. & Hache, R. J. (1997) *J. Biol. Chem.* **272**, 5647–5658.
- Fernandez, J. L., Goyanes, V. J., Ramiro-Diaz, J. & Gosalvez, J. (1998) *Cytogenet. Cell Genet.* **82**, 195–198.
- Cary, R. B., Peterson, S. R., Wang, J., Bear, D. G., Bradbury, E. M. & Chen, D. J. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 4267–4272.
- Lees-Miller, S. P., Chen, Y. R. & Anderson, C. W. (1990) *Mol. Cell. Biol.* **10**, 6472–6481.
- Hande, P., Slijepcevic, P., Silver, A., Bouffler, S., van Buul, P., Bryant, P. & Lansdorp, P. (1999) *Genomics* **56**, 221–223.
- Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., Nussenzweig, M. C. & Li, G. C. (1996) *Nature (London)* **382**, 551–555.
- Gu, Y., Seidl, K. J., Rathbun, G. A., Zhu, C., Manis, J. P., van der Stoep, N., Davidson, L., Cheng, H. L., Sekiguchi, J. M., Frank, K., *et al.* (1997) *Immunity* **7**, 653–665.
- Vaziri, H., West, M. D., Allsopp, R. C., Davison, T. S., Wu, Y. S., Arrowsmith, C. H., Poirier, G. G. & Benchimol, S. (1997) *EMBO J.* **16**, 6018–6033.
- Smith, S., Giriat, I., Schmitt, A. & de Lange, T. (1998) *Science* **282**, 1484–1487.
- d'Adda di Fagnana, F., Hande, M. P., Tong, W. M., Lansdorp, P. M., Wang, Z.-Q. & Jackson, S. P. (1999) *Nat. Genet.* **23**, 76–80.
- Blackburn, E. H. (1997) *Biochemistry* **62**, 1196–1201.
- Broccoli, D., Young, J. W. & de Lange, T. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 9082–9086.
- Counter, C. M., Gupta, J., Harley, C. B., Leber, B. & Bacchetti, S. (1995) *Blood* **85**, 2315–2320.