Functional Relationships between Rad18 and WRNIP1 in Vertebrate Cells

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The WRNIP1 protein interacts with WRN, the product of the causative gene for Werner syndrome. Mutation of the *Saccharomyces cerevisiae* gene *MGS1*, the yeast counterpart of *WRNIP1*, confers synthetic lethality with mutation of *RAD18*. To examine the functional relationship between WRNIP1 and Rad18 in higher eukaryotic cells, we generated $WRNIP1^{-/-/-}/RAD18^{-/-}$ lines from chicken DT40 cells and compared them with single mutant cell lines. Unlike the corresponding yeast mutant, $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells are viable but grow more slowly than single mutants and wild type cells, and they show an additive or synergistic elevation in the frequency of sister chromatid exchanges. As reported, $WRNIP1^{-/-/-}$ cells and $RAD18^{-/-}$ cells are moderately and severely sensitive to camptothecin (CPT), respectively. Unexpectedly, the severe CPT sensitivity of $RAD18^{-/-}$ cells is slightly suppressed by disruption of the *WRNIP1* gene.

Key words   Rad18; WRN interacting protein 1 (WRNIP1); DT40; maintenance of genome stability 1 (Mgs1); RecQ; proliferating cell nuclear antigen (PCNA)

Werner syndrome (WS) is a rare autosomal recessive disorder characterized by premature aging associated with an early onset of age-related diseases, including arteriosclerosis, malignant neoplasms, melituria, and cataracts.[1] The gene responsible for WS encodes WRN, a member of the RecQ family of DNA helicases.[2] The protein possesses DNA helicase and exonuclease activities.[3—6] Accumulating evidence suggests that WRN has roles relevant to telomere function since it efficiently prevents telomere degradation and consequent genomic instability.[7,8] Other functions of WRN are not well understood, although additional roles in many aspects of cellular metabolism may be inferred by its physical interaction with proteins involved in DNA replication, repair, and recombination, including DNA polymerase δ.[9]

To obtain further insights into the function of WRN, we searched for WRN-interacting proteins by a two-hybrid strategy and found a novel protein which we initially denoted WHIP (Werner helicase interacting protein)[10] but which is now called WRNIP1 according to the nomenclatural conventions of HUGO. Interaction between the two proteins was further confirmed by their co-immunoprecipitation from cell extracts.

The amino acid sequence of WRNIP1 is similar to that of replication factor C (RFC), and it also contains the Walker A and B motifs for ATP binding and/or ATPase activity.[10] A homologue of *WRNIP1*, *MGS1*, has been identified in budding yeast.[11] Previous studies have shown that overproduction of Mgs1 is lethal in mutants defective in proteins related to DNA replication, such as DNA polymerase δ, RFC, PCNA, and RPA.[12,13] Moreover, we showed that mutation of *mgs1* partially alleviates the growth defect of the *pol31* mutant, which bears a mutation in the second subunit of DNA polymerase δ.[13] Consequently, we proposed that Mgs1 (yWRNIP1) interacts with the DNA synthesis machinery to modulate the function of DNA polymerase δ during replication or replication-associated repair.[13] Indeed, we demonstrated that human WRNIP1 interacts with three of the four subunits of human DNA polymerase δ and stimulates its activity.[14]

To investigate the function of WRNIP1 in higher eukaryotic cells, we previously generated *WRNIP1* gene knockout cells from chicken DT40 cells.[15] Because the *WRNIP1* gene resides on chromosome 2, which is trisomic in DT40 cells, all three alleles were disrupted by gene targeting. $WRNIP1^{-/-/-}$ cells showed a slight elevation of sister chromatid exchange (SCE) and moderate sensitivity to the anticancer drug camptothecin (CPT),[15] an inhibitor of DNA topoisomerase I (Top1), which forms a complex with DNA.

It has been reported that the *mgs1* (*wrnip1*) *rad18* double mutation confers synthetic lethality in the budding yeast *Saccharomyces cerevisiae*.[12] The yeast Rad18 protein is known to function in post-replication repair pathways, including an error-free damage bypass pathway involving Rad30 (Polη) and an error-prone damage bypass pathway involving Rev3/7 (Polζ).[16—20] The *rad18* mutants of lower eukaryotic cells show hypersensitivity to DNA damaging agents such as ultraviolet (UV), methyl methanesulfonate (MMS), and mytomycin C (MMC).[21—23] In the case of higher eukaryotic cells, $RAD18^{-/-}$ mouse embryonic stem cells and $RAD18^{-/-}$ DT40 cells also show hypersensitivity to various DNA damaging agents such as UV and MMS. Moreover, both spontaneous and DNA damage-induced sister chromatid exchange (SCE) are elevated in *RAD18* knockout vertebrate cells.[24,25] Interestingly, $RAD18^{-/-}$ DT40 cells were found to be hypersensitive to CPT, while $RAD30^{-/-}$ and $REV3^{-/-}$ cells, which are defective in Polη and Polζ, respectively, are as sensitive to CPT as wild type cells.[26] These data suggest a previously unanticipated role for vertebrate Rad18 in the processing of replication forks that encounter lesions induced by CPT. These circumstances prompted us to examine whether *WRNIP1*/*RAD18* double gene knockout cells exhibit synthetic lethality, as observed for budding yeast, or if they are viable, whether they show a synergistically higher sensitivity to CPT.

Since both DT40 $RAD18^{-/-}$ and $WRNIP1^{-/-/-}$ cell lines have been generated,[15,25] we constructed $WRNIP1^{-/-/-}/$

$RAD18^{-/-}$ DT40 cells by transfecting two $RAD18$ targeting vectors sequentially into $WRNIP^{-/-/-}$ cells. We characterized growth, frequency of SCE, and sensitivity to DNA damaging agents including CPT in $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells and obtained unexpected results, as compared to data previously reported for budding yeast. We discuss functional relationships between WRNIP1 and Rad18 in higher eukaryotic cells.

MATERIALS AND METHODS

Cell Culture and DNA Transfection DT40 cells were cultured in RPMI 1640 supplemented with $100\,\mu g/ml$ kanamycin, 10% fetal bovine serum, and 1% chicken serum at $39.5\,°C$. For gene targeting, 10^7 cells were electroporated with $30\,\mu g$ of linearized $RAD18$ gene targeting constructs[25] using a Gene Pulser apparatus (BioRad, Hercules, CA, U.S.A.) at 550 V and $25\,\mu F$. Drug-resistant colonies were selected in 96-well plates with medium containing $0.5\,\mu g/ml$ Puromycin or $20\,\mu g/ml$ Mycophenolic acid. Gene disruption was confirmed by Southern and Northern blot analyses and RT-PCR.[15,25]

Growth Curves Cells (2×10^4) were inoculated and cultured at $39.5\,°C$ for the specified periods. To maintain exponential cell growth, 3 ml cultures were started in 30 mm diameter dishes, from which all cells were successively transferred into 60 and 100 mm dishes. The cells were counted and growth rates were estimated.

Measurements of MMS, CDDP, and CPT Sensitivity Cells (3×10^2) were plated into dishes containing various concentrations of methyl methanesulfonate (MMS), cisplatin (CDDP) or CPT in D-MEM/F-12 medium supplemented with 1.5% (w/v) methylcellulose, 1.5% chicken serum, and 15% fetal bovine serum. Visible colonies were counted after 7 to 10 d, and sensitivity to the treated reagent was represented as the percentage of cells forming colonies relative to that of untreated cells.

Measurements of Spontaneous and CPT-Induced SCE Frequencies Cells (5×10^5) were treated with 5 nM CPT for the last 8 h of incubation with $10\,\mu M$ BrdU for 16 h. The cells were also treated with $0.1\,\mu g/ml$ colcemid for the final 3 h of incubation to increase the proportion of mitotic cells. The cells were harvested and treated with 75 mM KCl for 20 min at room temperature and then fixed with methanol–acetic acid (3 : 1) for 30 min. The cell suspension was dropped onto ice-cold wet glass slides and air-dried. The cells on the slides were incubated with $10\,\mu g/ml$ Hoechst 33258 in phosphate buffer (pH 6.8) for 20 min and rinsed with MacIlvaine solution (164 mM Na_2HPO_4, 16 mM citric acid, pH 7.0). The cells were exposed to a black light (352 nm) at a distance of 1 cm for 30 min, incubated in $2\times SSC$ (0.3 M NaCl, 0.03 M sodium citrate) at $62\,°C$ for 30 min and stained with 3% Giemsa for 10 min.

RESULTS AND DISCUSSION

Generation of $WRNIP1^{-/-/-}/RAD18^{-/-}$ DT40 Cells The observation that the $wrnip1$ ($mgs1$) $rad18$ double mutant of budding yeast exhibits synthetic lethality[12] prompted us to examine whether mutation of the $WRNIP1$ gene affects higher eukaryotic cells with the $RAD18^{-/-}$ genotype.
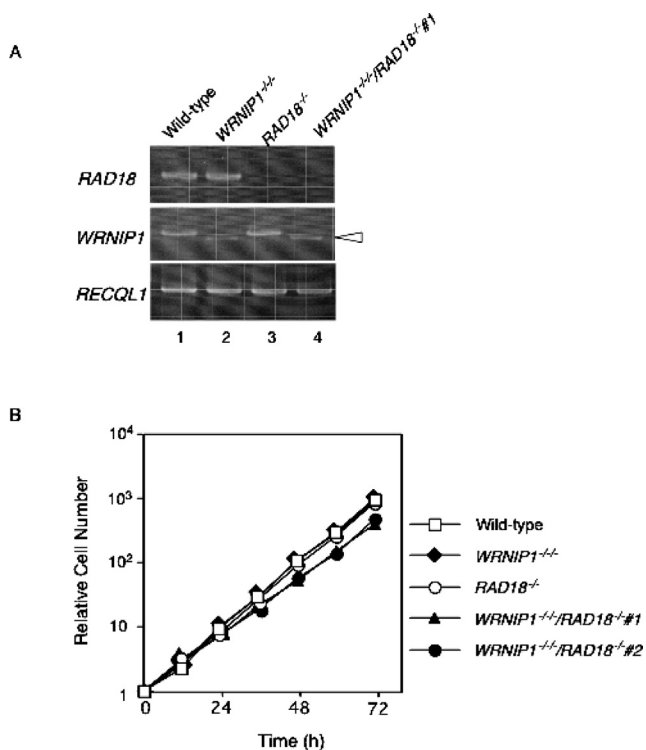

Fig. 1. Generation of $WRNIP1^{-/-/-}/RAD18^{-/-}$ DT40 Cells

(A) RT-PCR analysis of total RNA from wild-type (lane 1), $WRNIP1^{-/-/-}$ (lane 2), $RAD18^{-/-}$ (lane 3) and $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells (lane 4). Arrowhead shows truncated form of WRNIP1 expressed in $WRNIP1^{-/-/-}$ cells.[15] (B) Growth curves of wild-type, $RAD18^{-/-}$, $WRNIP1^{-/-/-}$, and $WRNIP1^{-/-/-}/RAD18^{-/-}$ DT40 cells. Cells were inoculated into 30 mm dishes and counted after the indicated periods. Cells that were negative for trypan blue staining were counted as viable cells.

Since we had $RAD18^{-/-}$ and $WRNIP1^{-/-/-}$ cells derived from chicken DT40 cells in hand, we generated $WRNIP1^{-/-/-}/RAD18^{-/-}$ DT40 cells lines by sequentially transfecting two $RAD18$ targeting vectors into $WRNIP1^{-/-/-}$ cells. Disruption of both $RAD18$ alleles was confirmed by Southern blotting (data not shown) and by RT-PCR (Fig. 1A). A truncated $WRNIP1$ mRNA was expressed in $WRNIP1^{-/-/-}$ and $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells, and the putative truncated WRNIP1 protein appeared to have no biological activity *in vivo*, based on our previous characterization.[15] Although the $wrnip1$ ($mgs1$) $rad18$ double mutation in budding yeast confers synthetic lethality, $WRNIP1^{-/-/-}/RAD18^{-/-}$ DT40 cells were viable and grew slightly more slowly than either single mutant (Fig. 1B). We did not detect apparent differences among $WRNIP1^{-/-/-}/RAD18^{-/-}$, $RAD18^{-/-}$, or $WRNIP1^{-/-/-}$ cells with respect to cell cycle distribution and spontaneous cell death, as monitored by flow cytometry (data not shown).

Additive Increase of Spontaneous SCE in $WRNIP1^{-/-/-}/RAD18^{-/-}$ Cells Increases in mitotic recombination frequency are suggested to be the signal of replication defects. Sister chromatid exchange (SCE) (Fig. 2A) in vertebrate cells is thought to be an excellent indicator of such defects.[27] The frequency of SCE in $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells was increased compared to that of either single mutant (Fig. 2B). As reported previously,[25] $RAD18^{-/-}$ cells exhibited an elevation in SCE (Fig. 2B, panel b). Since SCE arise mainly *via* homologous recombination, the elevation of spontaneous SCE in $RAD18^{-/-}$
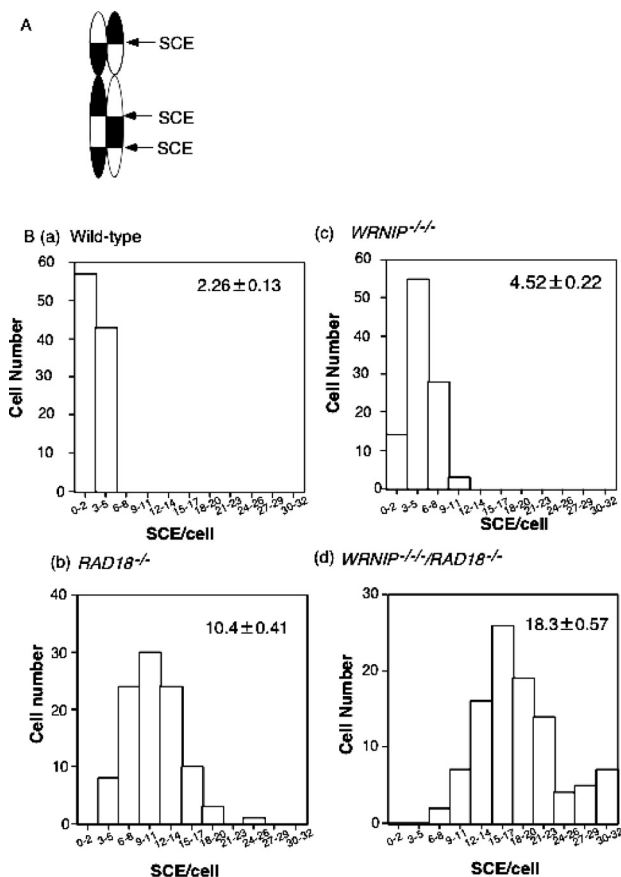
Fig. 2. Frequency of Spontaneous SCE in Various Cells

(A) Schematic presentation of SCE. (B) Histograms of the incidence of SCE in wild-type (a), $RAD18^{-/-}$ (b), $WRNIP1^{-/-/-}$ (c), and $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells (d). The number of SCE in the macro-chromosomes of 100 metaphase cells were counted. Histograms show the number of cells having the indicated number of SCE. The mean and standard error are shown in the upper right corner of each histogram.

Fig. 3. Sensitivity of $RAD18^{-/-}$, $WRNIP1^{-/-/-}$ and $WRNIP1^{-/-/-}/$ $RAD18^{-/-}$ DT40 Cells to MMS, CDDP and CPT

Cells were treated with the indicated concentrations of MMS (A), CDDP (B), or CPT (C) as described in Materials and Methods. Survival is expressed as a percentage of the number of colonies that grow in the absence of the indicated genotoxin. The bars represent standard deviations based on data from three independent experiments.

cells can be explained by the proposal that lesions that remain on replicating DNA in $RAD18^{-/-}$ cells are channeled to a homologous recombination repair pathway that uses a sister chromatid as a template.[25] The frequency of spontaneous SCE in $WRNIP1^{-/-/-}$ cells was slightly increased compared with that of wild-type cells (Fig. 2B, panel c) as previously described.[15] It is therefore likely that the additive or synergistic increase of spontaneous SCE in $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells (Fig. 2B, panel d) indicates a further impairment of DNA replication in cells simultaneously lacking WRNIP1 and Rad18 functions, producing lesions that are processed by homologous recombination. Interestingly, the synthetic lethality of the *wrnip1* (*mgs1*) *rad18* double mutant of budding yeast is suppressed by enhancing homologous recombination.[12] One possible explanation for the viability of $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells is that a homologous recombination pathway is constitutively active in higher eukaryotic cells for responding to replication mishaps that occur in the absence of Rad18 and WRNIP1. Indeed, DT40 $RAD18^{-/-}$ cells are reportedly inviable in the absence of Rad54, which is an essential component of homologous recombination,[25] suggesting that the Rad18 functional defect is mainly compensated for by the Rad54-dependent pathway but not by the WRNIP1 pathway in higher eukaryotic cells.

CPT Sensitivity of $WRNIP1^{-/-/-}/RAD18^{-/-}$ Cells $WRNIP1^{-/-/-}$ cells are not more sensitive to cisplatin
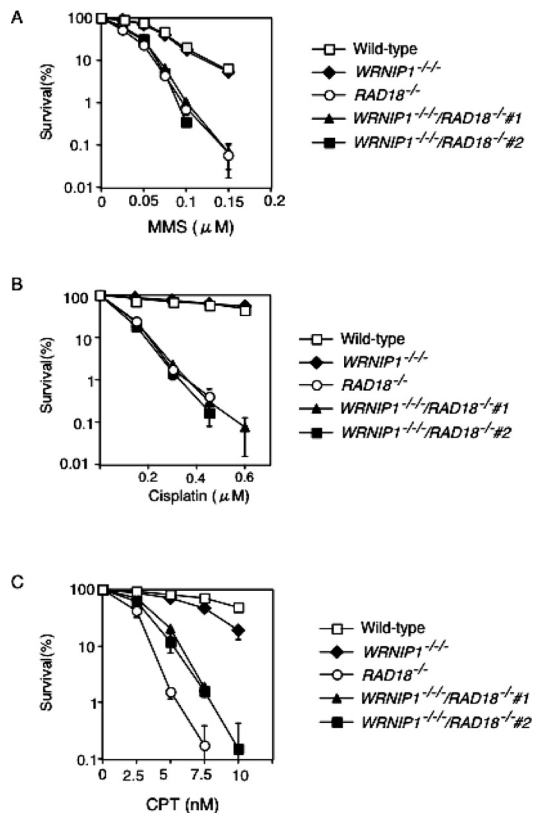
(CDDP) or MMS than wild type cells and they show moderate sensitivity to CPT.[15] In contrast, $RAD18^{-/-}$ cells are severely sensitive to all of these drugs, as previously reported (Fig. 3).[15,25,26] Here we examined the sensitivity of $WRNIP1^{-/-/-}/RAD18^{-/-}$ cells to MMS, CDDP, and CPT. These cells were as sensitive to MMS and CDDP as $RAD18^{-/-}$ cells, suggesting that WRNIP1 has no role in repairing or tolerating lesions generated by MMS or CDDP, even in the absence of Rad18 (Figs. 3A, B). Surprisingly, the severe CPT sensitivity of $RAD18^{-/-}$ cells was slightly suppressed by disruption of the $WRNIP1$ gene (Fig. 3C).

CPT is an inhibitor of DNA topoisomerase I (Top1), which forms a complex with DNA. A Top1-mediated transient DNA single-strand break is formed by reversible transfer of a phosphodiester bond to a tyrosine residue in the catalytic site of the enzyme to form a covalently linked Top1-DNA cleavage complex.[28—32] CPT associates with Top1 and stabilizes cleavage complexes, which may result in DNA double-strand breaks (DSBs) followed by the ATR/ATM-dependent phosphorylation of H2AX when DNA replication forks encounter these lesions.[33,34] It has been shown that DSBs induced by CPT are mainly repaired by homologous recombination in DT40 cells.[35]

Defect in the Induction of SCE by CPT in $RAD18^{-/-}$ and $WRNIP1^{-/-/-}/RAD18^{-/-}$ Cells How does impairment of the WRNIP1 function slightly suppress the severe CPT sensitivity of $RAD18^{-/-}$ cells? Since overproduction of a protein involved in homologous recombination rescues the
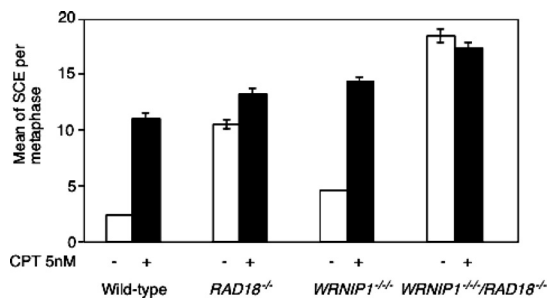
Fig. 4.   Induction of SCE by CPT in Various Cells

The numbers of SCEs in the macro-chromosomes of 100 metaphase cells were counted. Cells were treated with 5 nM CPT for 8 h (filled column) or not treated (open column). Columns and bars represent means and standard errors.

lethality of *wrnip1* (*mgs1*) *rad18* cells in budding yeast,[12] we examined the induction of SCE in various cells upon exposure to CPT.

In the presence of CPT, SCE frequencis in wild-type cells and *WRNIP1*$^{-/-/-}$ cells were higher than those of spontaneous SCE by about 5-fold and 3-fold, respectively, while only 1.3-fold increase in SCE frequency was observed in *RAD18*$^{-/-}$ cells. In the case of *WRNIP1*$^{-/-/-}$/*RAD18*$^{-/-}$ cells, no induction of SCE by CPT occurred although the frequencies of spontaneous and CPT-induced SCE in the cells were higher than those of either *RAD18*$^{-/-}$ or *WRNIP1*$^{-/-/-}$ cells (Fig. 4). These data indicate that the induction of SCE by CPT is as proficient in *WRNIP1*$^{-/-/-}$ cells like as in wild type cells but that it is defective in both *RAD18*$^{-/-}$ and *WRNIP1*$^{-/-/-}$/*RAD18*$^{-/-}$ cells. Thus, it is unlikely that the partial suppression of the CPT sensitivity of *RAD18*$^{-/-}$ cells by disruption of the *WRNIP1* gene is due to the induction of recombination.

In conclusion, WRNIP1 and Rad18 in higher eukaryotic cells deal with DNA lesions encountered by replication forks in different pathways, and the lesions that persist in the absence of these proteins are processed by homologous recombination. Thus, both WRNIP1 and Rad18 suppress spontaneously occurring SCE. However, when replication forks encounter lesions induced by CPT, Rad18 processes lesions in a pathway that results in homologous recombination. The behavior of Rad18 is not surprising because Sgs1, the yeast homolog of RecQ, suppresses spontaneous homologous recombination, and it is required for homologous recombination induced by DNA damaging agents such as MMS.[36] In addition, a pathway involving both WRNIP1 and Rad18, in which WRNIP1 functions upstream of Rad18, operates in the presence of CPT. In the absence of WRNIP1, the lesions processed by this pathway are channeled to other pathways, thereby partially suppressing the CPT sensitivity of *WRNIP1*$^{-/-/-}$/*RAD18*$^{-/-}$ cells as compared with *RAD18*$^{-/-}$ cells. The elucidation of this pathway is one of our future goals. Recently, it has been reported that yeast Mgs1 (yWRNIP1) interacts with PCNA, which is essential for polymerase δ to replicate DNA processively.[37] Interestingly, both human WRNIP1 and yeast Mgs1 interact with DNA polymerase δ.[14,38] Taking into account that Rad18 is required for the modification of PCNA with ubiquitin, some interplays among Rad18, WRNIP1, PCNA, and DNA polymease δ, seem to operate to deal with CPT-induced DNA lesions. Thus, simultaneous deletion of WRNIP1 and Rad18

affects such interplays and then activates other pathway to deal with CPT lesions. Finally, the functional interaction of Rad18 and WRNIP1 is dynamic, and it changes depending on the type of lesion and situation of cells.

REFERENCES

1) Epstein C. J., Martin G. M., Schultz A. L., Motulsky A. G., *Medicine* (Baltimore), **45**, 177—221 (1966).
2) Yu C. E., Oshima J., Fu Y. H., Wijsman E. M., Hisama F., Alisch R., Matthews S., Nakura J., Miki T., Martin G. M., Mulligan J., Schellenberg G. D., *Science*, **272**, 258—262 (1996).
3) Suzuki N., Shimamoto A., Imamura O., Kuromitsu J., Kitao S., Goto M., Furuichi Y., *Nucleic Acids Res.*, **25**, 2973—2978 (1997).
4) Gray M. D., Shen J. C., Kamath-Loeb A. S., Blank A., Sopher B. L., Martin G. M., Oshima J., Loeb L. A., *Nat. Genet.*, **17**, 100—103 (1997).
5) Huang S., Li B., Gray M. D., Oshima J., Mian I. S., Campisi J., *Nat. Genet.*, **20**, 114—116 (1998).
6) Suzuki N., Shiratori M., Goto M., Furuichi Y., *Nucleic Acids Res.*, **27**, 2361—2368 (1999).
7) Crabbe L., Verdun R. E., Haggblom C. I., Karlseder J., *Science*, **306**, 1951—1953 (2004).
8) Du X., Shen J., Kugan N., Furth E. E., Lombard D. B., Cheung C., Pak S., Luo G., Pignolo R. J., DePinho R. A., Guarente L., Johnson F. B., *Mol. Cell Biol.*, **24**, 8437—8446 (2004).
9) Kamath-Loeb A. S., Johansson E., Burgers P. M., Loeb L. A., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 4603—4608 (2000).
10) Kawabe Y., Branzei D., Hayashi T., Suzuki H., Masuko T., Onoda F., Heo S. J., Ikeda H., Shimamoto A., Furuichi Y., Seki M., Enomoto T., *J. Biol. Chem.*, **276**, 20364—20369 (2001).
11) Hishida T., Iwasaki H., Ohno T., Morishita T., Shinagawa H., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 8283—8289 (2001).
12) Hishida T., Ohno T., Iwasaki H., Shinagawa H., *EMBO J.*, **21**, 2019—2029 (2002).
13) Branzei D., Seki M., Onoda F., Enomoto T., *Mol. Genet. Genomics*, **268**, 371—386 (2002).
14) Tsurimoto T., Shinozaki A., Yano M., Seki M., Enomoto T., *Genes Cells*, **10**, 13—22 (2005).
15) Kawabe Y., Seki M., Yoshimura A., Nishino K., Hayashi T., Takeuchi T., Iguchi S., Kusa Y., Ohtsuki M., Tsuyama T., Imamura O., Matsumoto T., Furuichi Y., Tada S., Enomoto T., *DNA Repair*, **5**, 816—828 (2006).
16) Ulrich H. D., Jentsch S., *EMBO J.*, **19**, 3388—3399 (2000).
17) Hoege C., Pfander B., Moldovan G. L., Pyrovolakis G., Jentsch S., *Nature* (London), **419**, 135—141 (2002).
18) Haracska L., Torres-Ramos C. A., Johnson R. E., Parakash S., Prakash L., *Mol. Cell Biol.*, **24**, 4267—4274 (2004).
19) Broomfield S., Hryciw T., Xiao W., *Mutat. Res.*, **486**, 167—184 (2001).
20) Haracska L., Kondratick C. M., Unk I., Parakash S., Prakash L., *Mol. Cell*, **8**, 407—415 (2001).
21) Lawrence C. W., Chrintensen R., *Genetics*, **82**, 207—232 (1976).
22) Prakash L., *Mol. Gen. Genet.*, **184**, 471—478 (1981).
23) Tomita H., Soshi T., Inoue H., *Mol. Gen. Genet.*, **238**, 225—233 (1993).
24) Tateishi S., Niwa H., Miyazaki J., Fujimoto S., Inoue H., Yamaizumi M., *Mol. Cell Biol.*, **23**, 474—481 (2003).
25) Yamashita Y. M., Okada T., Matsusaka T., Sonoda E., Zhao G. Y., Araki K., Tateishi S., Yamaizumi M., Takeda S., *EMBO J.*, **20**, 5558—5566 (2002).
26) Yoshimura A., Nishino K., Takezawa J., Tada S., Kobayashi T., Sonoda E., Kawamoto T., Takeda S., Ishii Y., Yamada K., Enomoto T., Seki M., *DNA* Repair in press.
27) Sonoda E., Sasaki M. S., Morrison C., Yamaguchi-Iwai Y., Takata M., Takeda S., *Mol. Cell Biol.*, **19**, 5166—5169 (1999).

28) Liu L. F., *Annu. Rev. Biochem.*, **58**, 351—375 (1989).
29) Wang J. C., *Annu. Rev. Biochem.*, **65**, 635—692 (1996).
30) Champoux J. J., *Ann. N.Y. Acad. Sci.*, **922**, 56—64 (2000).
31) Hsiang Y. H., Hertzberg R., Hecht S., Liu L. F., *J. Biol. Chem.*, **260**, 14873—14878 (1985).
32) Hsiang Y. H., Lihou M. G., Liu L. F., *Cancer Res.*, **49**, 5077—5082 (1989).
33) Huang X., Traganos F., Darzynkiewicz Z., *Cell Cycle*, **2**, 614—619 (2003).
34) Furuta T., Takemura H., Liao Z. Y., Aune G. J., Redon C., Sedelnikova O. A., Pilch D. R., Rogakou E. P., Celeste A., Chen H. T., Nussenzweig A., Aladjem M. I., Bonner W. M., Pommier Y., *J. Biol. Chem.*, **278**, 20303—20312 (2003).
35) Adachi N., So S., Koyama H., *J. Biol. Chem.*, **279**, 37343—37348 (2004).
36) Onoda F., Seki M., Miyajima A., Enomoto T., *Mol. Gen. Genet.*, **264**, 702—708 (2001).
37) Hishida T., Ohya T., Kubota Y., Kamada Y., Shinagawa H., *Mol. Cell Biol.*, **26**, 5509—5517 (2006).
38) Davoodi Vijeh Motlagh N., Seki M., Branzei D., Enomoto T., *DNA Repair*, in press.