Structure and Enzymatic Properties of a Stable Complex of the Human REV1 and REV7 Proteins*

Received for publication, November 19, 2002, and in revised form, January 14, 2003
Published, JBC Papers in Press, January 15, 2003, DOI 10.1074/jbc.M211765200

Yuji Masuda, Mika Ohmae, Kenji Masuda, and Kenji Kamiya‡
From the Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8533, Japan

With yeast Saccharomyces cerevisiae, results from a variety of genetic and biochemical investigations have demonstrated that the REV genes play a major role in induction of mutations through replication processes that directly copy the damaged DNA template during DNA replication. However, in higher eucaryotes functions of homologues are poorly understood and appear somewhat different from the yeast case. It has been suggested that human REV1 interacts with human REV7, this being specific to higher eucaryotes. Here we show that purified human REV1 and REV7 proteins form a heterodimer in solution, which is stable through intensive purification steps. Results from biochemical analysis of the transreactions of the REV1-REV7 complex demonstrated, in contrast to the case of yeast Rev3 whose polymerase activity is stimulated by assembly with yeast Rev7, that human REV7 did not influence the stability, substrate specificity, or kinetic parameters of the transreactions of REV1 protein. The possible role of human REV7 is discussed.

In yeast Saccharomyces cerevisiae, rev mutants, rev1, rev3, and rev7 were initially isolated on the basis of reduced mutations after UV treatment (1, 2) and classified into a subbranch of the RAD6 epistasis group, with alteration in the error-prone postreplication repair pathway (2–4). Yeast strains carrying rev mutations exhibit a reduced frequency of mutations following treatments with a variety of DNA damaging agents (5). The majority of mutations are induced through replication processes, which directly copy the damaged DNA template during DNA replication, a function of REV genes. Lesion bypass DNA replication is one cellular system activated in response to DNA damage that functions to prevent cell death caused by replication arrest (5).

Rev1 protein, belonging to a family of translesion DNA polymerases (6, 7), contains a BRCA1 C terminus (BRCT) domain (8) and possesses deoxycytidyl transferase activity in template-directed reactions (9). It is capable of extending a primer terminus by insertion of dCMP opposite a variety of damaged bases and apurinic/apyrimidinic (AP) sites (9–11). The in vivo role of the REV1 gene in translesion replication of AP sites has been well studied using a yeast system (12, 13), and dCMP residues are known to be incorporated opposite AP sites in the majority of bypass events in the wild type but not the revΔ strain (13). Deoxycytidyl transferase activity of the Rev1 protein, first observed for the yeast enzyme (9), has been conserved throughout eucaryotic evolution (14–18), implying a contribution to survival (5). However, the other role of the REV1 gene in the mutagenesis pathway is not due to its action as a deoxycytidyl transferase. Although the Rev1 protein does not allow bypass of T-T (6–4) photoproducts in vitro, the gene is required for translesion DNA synthesis in vivo (13, 19). A mutagenesis-deficient BRCT domain mutant encodes a protein with normal deoxycytidyl transferase activity (13), while methyl methane sulfonate-induced mutagenesis has been shown to be normal in a site-directed mutant lacking deoxycytidyl transferase activity (10). The second function of the Rev1 protein may be to incorporate large complexes with DNA polymerase ζ due to protein-protein interactions (5, 10, 13).

REV3 encodes the catalytic subunit of DNA polymerase ζ, which also contains Rev7 as a stabilizing and enhancing element for polymerase activity (20). DNA polymerase ζ has the ability to extend terminally mismatched primers and distorted structures (10, 21–24). It is also capable of bypass replication of T-T cyclobutane dimers (20). These properties are likely to contribute to generation of mutations. Yeast strains carrying rev3 or rev7 mutations exhibit a similar phenotype on treatment with wide variety of DNA-damaging agents, supporting the conclusion that both act in the same process (5).

The damage-induced mutagenesis pathway is evolutionarily conserved from yeast to humans. Preservation of the functions of human REV genes is supported by the finding that human cell lines expressing high levels of human REV1 or REV3 antisense RNA exhibit a much reduced frequency of 6-thioguanine-resistant mutants induced by UV (25, 26). The biochemical properties of the REV1 protein are also conserved from yeast to humans (14–16, 18). While the human DNA polymerase ζ has yet to be purified, there is no direct evidence for polymerase activity of human REV3 protein. Recently, Murakumo and coworkers found that human REV7 interacts with human REV1 and REV3 proteins in a yeast two-hybrid study, as confirmed by immunoprecipitation experiments (27, 28). With yeast proteins, a Rev1-Rev7 interaction has not been observed (20). The responsible domain in the human REV1 protein is not found in the yeast Rev1 counterpart but highly conserved in the mouse Rev1 protein (17), suggesting a specificity for higher eucaryotes (28).

In the present paper we provide several lines of evidence that human REV1 and REV7 form a stable heterodimer. In contrast to yeast Rev3, the human REV7 subunit does not affect the transreversion activity or stability of the REV1 protein. A possible molecular role of the REV7 subunit may be to help assembly of the REV1 protein to a large complex containing REV3 and/or other DNA polymerases in higher eucaryotes.
Human REV1-REV7 Complex

EXPERIMENTAL PROCEDURES

Plasmid Construction—Human REV7 cDNA was amplified from HeLa cDNA by PCR with primers (5'-CATATGGACGCTCAGCAGC-CAAGACGAC-3' and 5'-GGACGTGGAGATCTGGAGGTGTT-3') and inserted into a pCR 2.1-TOPO vector (Invitrogen) to yield plasmid pREV7. The nucleotide sequence was verified. Then a NdeI-EcoRI fragment of the pREV7 was inserted into the corresponding site of the pT20b(+) vector (Novagen) to yield plasmid pT20b-REV7, which produces intact REV7 protein under control of the T7 promoter. The pT20b-REV7 containing plasmid (pBAD-REV7-REV1S) was constructed by insertion of the Klenow-treated KpnⅠ site of pBAD-I-REV1S (16) into a pCR 2.1-TOPO vector (Invitrogen) to yield plasmid pBAD-I-REV1S (245–847) was constructed by insertion of a PCR-amplified fragment into the NdeI-HindIII site of pT715b (Novagen).

Overproduction and Purification of Human REV7—BL21 (DE3) (29) harboring pT20b-REV7 was grown in 20 liters of LB medium supplemented with ampicillin (250 μg/ml) at 15 °C with aeration until the culture reached an A600 value of 0.6. Isopropyl β-D-thiogalactopyranoside was added to 0.25 mM, and the incubation was continued for 10 h.

The cells were harvested by centrifugation, resuspended in 2 ml of buffer B (50 mM Tris-HCl, pH 7.5/10 mM EDTA/10 mM β-mercaptoethanol/10% sucrose) containing 500 mM NaCl, were loaded at 0.1 ml/min onto a Superdex 200 HR 10/30 column (Amersham Biosciences), which had been treated with 0.1 M NiSO4, and then equilibrated with buffer D (50 mM HEPES-NaOH, pH 7.5/500 mM NaCl/10 mM β-mercaptoethanol/10% glycerol) containing 10 mM imidazole. The column was washed with 10 ml of equilibration buffer at 0.1 ml/min, and then the REV1 and REV7 proteins were eluted with 10 ml of a linear gradient of 10–100 mM imidazole in buffer D. Fractions containing both REV1 and REV7 proteins, which were eluted at about 50 mM imidazole, were pooled and dialyzed against buffer C containing 500 mM NaCl. The REV1 and REV7 proteins coeluted as a single peak with an apparent molecular mass of 410 kDa with a Stokes’ radius of 60.3 Å. The REV-REV7 complex peak fractions were pooled, frozen in liquid nitrogen, and stored at −80 °C.

Other Proteins—Intact REV1 protein and deletion derivatives of the his6-REV1 protein were purified as described previously (15, 16). Protein concentrations were determined by Bio-Rad protein assay using BSA (Bio-RAD) as the standard.

Reconstitution of the REV1-REV7 Complexes in Vitro—REV1 protein (24 μg) and REV7 protein (3 μg) were mixed in 70 μl of buffer C containing 500 mM NaCl. For truncated his6-REV1 derivatives, ΔN407 (4.2 μg) and ΔC885 (4.8 μg) proteins (15) were mixed with REV7 (1.5 μg).

Sucrose Density Gradient Sedimentation—Sucrose density gradient sedimentation was performed as described (31). The purified REV1 protein (15 μg), REV7 protein (7 μg), and REV-REV7 complex (12 μg) were sedimented through 2 ml of 10–40% sucrose gradient in buffer C for 3 h on a TLS 55 rotor (Beckman) at 4 °C.

RESULTS

In Vitro Reconstitution of the Human REV1-REV7 Complex—Recently, Murakumo and coworkers reported that the human REV1 protein directly interacts with the human REV7 protein (28). To determine whether the purified REV1 and REV7 proteins (Fig. 1A) form a stable complex, they were...
Human REV1-REV7 Complex

**Fig. 1. In vitro reconstitution of REV1-REV7 complexes.** A, 10% SDS-PAGE analysis of purified REV1 (lane 1) and REV7 (lane 2) proteins. Aliquots (0.5 μg) were electrophoresed and stained with Coomassie Brilliant Blue R-250. Lane M, molecular mass markers (Bio-Rad). B, gel filtration analysis of REV1 (panel a), REV7 (panel b), and a mixture of REV1 and REV7 proteins (panel C). Samples were loaded onto a Superdex 200 PC 3.2/30 column and 40-μl fractions were collected, analyzed by 10% SDS-PAGE, and stained with Coomassie Brilliant Blue R-250. Ferritin (440 kDa), aldolase (158 kDa), and ovalbumin (43 kDa) eluted at the positions indicated. C, gel filtration analysis of deletion derivatives of the his6-REV1 protein and REV7. Deletion mutants of his6-REV1 protein, ΔN407 (a) and ΔC885 (b), were mixed with REV7 protein and loaded on a Superdex 200 PC 3.2/30 column, and 40-μl fractions were collected, subjected to 10% SDS-PAGE, and visualized by Western blotting probed with anti REV1 (upper panels) or anti REV7 (lower panels) antisera. Positions of the respective proteins on the membrane are indicated by arrow heads.

**Fig. 2. Purification of the REV1-REV7 complex.** A, overproduction of REV1 and REV7 proteins in *E. coli* cells. Total *E. coli* proteins were analyzed by 10% SDS-PAGE before (lane 1) and after induction (lane 2) and stained with Coomassie Brilliant Blue R-250. Lane M, molecular mass markers (Bio-Rad). B, the purification scheme. C, elution profile of the REV1-REV7 complex from Superdex 200 gel filtration chromatography. Fractions eluted from the gel filtration column were analyzed by 10% SDS-PAGE and visualized by Coomassie Brilliant Blue R-250 (top panel); Western blotting probed with anti REV1 (middle panel), or anti REV7 antisera (bottom panel).

mixed and incubated on ice, and then the mixture was subjected to gel filtration chromatography in the presence of 500 mM NaCl (see “Experimental Procedures”). When the individual proteins were loaded on the column, REV1 of 138 kDa eluted at an apparent molecular mass of 380 kDa (Fig. 1B, panel a), (16) and REV7 of 24 kDa eluted as a single peak at an apparent molecular mass of 27 kDa (Fig. 1B, panel b). When the mixture of two proteins were developed on gel filtration chromatography, a limited but significant fraction of REV7 was colleted with REV1 under these conditions in the presence of 500 mM NaCl (Fig. 1B, panel c), suggesting that REV1 and REV7 form a salt-resistant complex.

The binding domain in REV1 for REV7 has been mapped to the C terminus (28). It was further examined using purified truncated his6-REV7 proteins. ΔN407 (lacking the BRCT domain of the N terminus) and ΔC885 (lacking the C terminus) (15) were mixed with purified REV7 and loaded on the gel filtration column, and then the eluted fractions were analyzed by Western blotting. Both ΔN407 and ΔC885 proteins eluted at an apparent molecular mass of around 200 kDa on this gel filtration chromatography (15). When the eluted fractions were analyzed by Western blotting, signals of REV7 were detected in fractions containing ΔN407 protein (Fig. 1C, panel a), but not ΔC885 (Fig. 1C, panel b), in agreement with the previous report that the C terminus of REV1 contains the interaction domain with REV7 (28).

**TABLE I.** Biochemical properties of REV1, REV7, and the REV1-REV7 complex

| Protein          | Stokes’ radius | Sedimentation coefficient | Molecular mass |
|------------------|----------------|---------------------------|----------------|
| REV1             | 59             | 5.6                       | 138            |
| REV7             | 25             | 1.2                       | 13             |
| REV1-REV7        | 60.3           | 5.6                       | 141            |

- Each Stokes’ radius was determined by Superdex 200 gel filtration using the size markers ferritin (61.0 Å), aldolase (48.1 Å), ovalbumin (30.5 Å), and ribonuclease A (16.4 Å), and the data were based on A$_{280}$ values monitored during the chromatography.
- Each sedimentation coefficient was determined with catalase (11.3 S), aldolase (7.4 S), albumin (4.2 S), and ribonuclease A (1.8 S) as size markers, and the data were based on the SDS-PAGE gel profile.
- Each molecular mass was calculated from the Stokes’ radius and the sedimentation coefficient assuming a partial specific volume of 0.73 (33).
- Buffer C containing 500 mM NaCl was used for the analysis.
- Buffer C containing 150 mM NaCl was used for the analysis.

**Purification of the REV1-REV7 Complex from Overproducing *Escherichia coli* Cells—With in vitro reconstitution, only a limited fraction of the proteins formed complexes. To purify a large amount, we established a system coexpressing the *REV1* and *REV7* genes in *E. coli*. The plasmid, pBAD-REV7-REV1S expressing both genes from the pBAD promoter as an operon, was employed with addition of arabinose as an inducer. SDS-PAGE analysis of the total proteins of the cells after induction revealed bands for REV1 and REV7 (Fig. 2A, lane 2) with sizes in agreement with the predicted molecular masses of 138 and 24 kDa respectively. The mobilities of the proteins in the SDS-PAGE gel profile.
the REV1 fraction for the nickel-chelating column could be due to the actions of the REV7 protein, suggesting complex formation. Whereas REV7 alone did not bind to heparin-Sepharose, as described under “Experimental Procedures,” with the mixture a significant fraction of REV7 was retained in the column and coeluted with REV1 (data not shown). The peak fraction from the heparin column was further loaded onto a gel filtration column (Fig. 2C), and SDS-PAGE analysis revealed REV1 and REV7 to coelute (Fig. 2C, top panel). Furthermore, the proteins were verified by Western blotting. The membrane was probed with anti REV7 antiserum first (Fig. 2C) and REV1-REV7 complexes (open triangles) were incubated in the standard buffer containing dCTP without template at 30 °C for the indicated times, and then the transferase reactions were started by addition of the template, P13–30G, with further incubation for 1 min. The relative residual activities were plotted. C, substrate specificity of the transferase reactions. The REV1-REV7 complex (9.3 ng) and each of primer-templates, P13–30G (panel a), P13–30A (panel b), P13–30T (panel c), P13–30C (panel d), and P13–30F (panel e) were incubated with no dNTP (–), a single dNTP (G, A, T, C), or all four dNTPs (N) under standard reaction conditions for 10 min. The reaction products were resolved on 20% polyacrylamide gels containing 8 M urea and autoradiographed at –80 °C.

**Fig. 3.** Transferase activity of the REV1-REV7 complex. A, time courses of deoxycytidyl transferase reactions with REV1 and the REV1-REV7 complex. The REV1 protein (open circles) and the REV1-REV7 complex (open triangles) at 18 fmol were incubated with the primer-template (P13–30G) and dCTP under standard reaction conditions for the indicated times. The amount of DNA present in each band was quantified using a Bio-Imaging Analyzer BAS2000 (Fuji Photo Film Co., Ltd.). B, stability of the REV1 protein and the REV1-REV7 complex. REV1 proteins (open circles) and REV1-REV7 complexes (open triangles) were incubated in the standard buffer containing dCTP without template at 30 °C for the indicated times, and then the transferase reactions were started by addition of the template, P13–30G, with further incubation for 1 min. The relative residual activities were plotted. C, substrate specificity of the transferase reactions. The REV1-REV7 complex (9.3 ng) and each of primer-templates, P13–30G (panel a), P13–30A (panel b), P13–30T (panel c), P13–30C (panel d), and P13–30F (panel e) were incubated with no dNTP (–), a single dNTP (G, A, T, C), or all four dNTPs (N) under standard reaction conditions for 10 min. The reaction products were resolved on 20% polyacrylamide gels containing 8 M urea and autoradiographed at –80 °C.

**Physicochemical Properties of REV1, REV7, and the REV1-REV7 Complex**—In the gel filtration chromatography final step of the purification, REV1, REV7, and the REV1-REV7 complex were eluted with apparent molecular masses of 380, 27, and 410 kDa, respectively. The respective Stokes’ radii were 59, 25, and 60.3 Å (Table I). To determine the molecular masses in solution, they were analyzed by sucrose density gradient sedimentation. They all sedimented as single peaks, and the REV1-REV7 complex a heterodimer in solution. Analysis of the stoichiometry of the REV1 and REV7 proteins in the complex by scanning of a SDS-PAGE gel visualized by Coomasie staining gave a REV1:REV7 ratio of 1:0.6, suggesting again that the REV1-REV7 complex is a heterodimer.

**Transferase Activity of the REV1-REV7 Complex**—The deoxycytidyl transferase activity of the REV1-REV7 complex was analyzed by primer extension assay using a template, with G as the nucleotide immediately downstream from the annealed primer. When equivalent amounts of REV1 and REV1-REV7 were subjected to the assay, the time course of the deoxycytidyl transferase reaction was identical (Fig. 3A). To determine the stability of the proteins in this reaction condition, incubation at 30 °C was performed for the indicated times and then residual activity was examined. The half-lives (~20 min) of both were almost the same (Fig. 3B). These results indicated that the REV7 protein did not affect the transferase activity or stability of the REV1 protein.

Then the substrate specificity of the transferase reactions was analyzed using five different primer-templates in the presence of 100 μM dNTPs (Fig. 3C). In this experiment, the respective primer-templates differed only at the template nucleotide immediately downstream from the annealed primer (16). The REV1-REV7 complex inserts dCMP opposite template G, A, T, C, and an AP site and inserts dGMP and dTMP opposite template G (Fig. 3C). The ability of the REV1-REV7 complex was essentially identical to that of REV1 alone (16). To confirm this, the kinetic parameters with the REV1-REV7 complex were...
Human REV1-REV7 Complex

determined by steady-state gel kinetic assays (Table II). The assays were all carried out with a 5-min incubation because the time course of the reactions was linear until 10 min (Fig. 3A). In the deoxycytidyl transferase reaction, the template nucleotides slightly affected $k_{\text{cat}}$ values, which were 3.7, 2.5, 1.6, 2.1, and 4.5 min$^{-1}$ opposite templates G, A, T, C, and an AP site, respectively (Table II). The template nucleotides strongly affected the $K_m$ value for dCTP (Table II), the affinity of which for template G was 53, 310, 380, and 28 times higher than for templates A, T, C, and an AP site, respectively. These properties of the REV1-REV7 complex were essentially identical to those of REV1 alone (16).

DISCUSSION

Recently, Murakumo and coworkers found that human REV1 binds to human REV7 at the C terminus (28), an interaction that has not been reported in yeast, S. cerevisiae (20). Comparison of amino acid sequences revealed the C-terminal region to be highly conserved in the human and mouse REV1 proteins but not in the yeast counterpart (17, 28). This suggests that the interaction was acquired in higher eucaryotes, although it is conceivable that it was lost in yeast during evolution and might play an important role in translesion DNA synthesis in mammalian cells (28). In this work, we provided several lines of evidence that the human REV7 forms a stable heterodimer with REV1 protein.

Purified REV1 and REV7 were found to have a considerable potential to form complexes in vitro even in the presence of 500 mM NaCl. Furthermore, both proteins co-purified after several purification steps and co-sedimented through centrifugation with constant stoichiometry. Indeed, it has been demonstrated that exogenously expressed REV1 protein co-immunoprecipitates with endogenous REV7 protein in HeLa cells (28).

The molecular masses of REV1, REV7, and the REV1-REV7 complex in solution were calculated using Stokes’ radius and sedimentation coefficients to be 138, 13, and 141 kDa, respectively. The value for REV1 agrees with the theoretical molecular mass of 138 kDa, while that of REV7 is smaller than the theoretical 25 kDa, suggesting the proteins to be monomers in solution. The ratio of hand densities of the REV1 and REV7 proteins on a SDS-PAGE gel was assigned 1:0.6, suggesting a stoichiometry of 1:1 (163 kDa) or 2:1 (301 kDa). The former value agrees well with our result of 141 kDa, suggesting that the REV1-REV7 complex is a heterodimer in solution.

Previously, it was suggested that the REV7 forms a homodimer and interacts through a common interface with REV1 (28). However, it is very likely, considering the following, that purified REV7 exists as a monomer in solution: (i) the obtained molecular mass of 13 kDa from our experiments is much smaller than the theoretical molecular mass of 24 kDa; (ii) the purified REV7 has the potential to assemble with purified REV1. If REV7 formed a stable homodimer, it could not interact with free REV1 because the interaction interface would be hidden by the homodimerization. Therefore, we suggest that REV7-REV7 interaction is transient and dynamic or tightly regulated in vivo.

Surprisingly, our results demonstrated that the REV7 did not influence the stability, substrate specificity, or kinetic parameters of the transferase reactions of REV1 protein in contrast to the case with yeast Rev3, which exhibits strongly enhanced DNA polymerase activity and stability on assembly with Rev7 (20). It is possible that such enhancement is yeast-specific or alternatively that in higher eucaryotes one of the important functions of the human REV7 is to assemble the REV1 protein to a large complex containing REV3 and/or other DNA polymerases, and this function might be required for translesion DNA replication.

Acknowledgments—We thank Dr. Yoshiki Murakumo (Nagoya University Graduate School of Medicine, Nagoya, Japan) for antisera against the human REV7 protein. We are grateful to Akiko Mizuno, Masako Okii, and Hatsue Wakayama for laboratory assistance.

REFERENCES

1. Lemontt, J. F. (1971) Genetics 68, 21–23
2. Lawrence, C. W., Das, G., and Christensen, R. B. (1985) Mol. Gen. Genet. 200, 80–85
3. Lawrence, C. W., and Christensen, R. (1976) Genetics 82, 207–232
4. Xiao, W., Chow, B. L., Broughfield, S., and Hanna, M. (2000) Genetics 155, 1633–1641
5. Lawrence, C. W. (2002) DNA Repair 1, 425–435
6. Burgers, P. M., Koonin, E. V., Bruford, E., Blanco, L., Burris, K. C., Christman, M. F., Cupeland, W. C., Friedberg, E. C., Hanako, F., Hinkle, D. C., Lawrence, C. W., Nakashiki, M., Ohnmi, H., Prakash, L., Prakash, S., Reynaud, C. A., Sugino, A., Todo, T., Wang, Z., Weil, J. C., and Woodgate, R. (2001) J. Biol. Chem. 276, 43487–43490
7. Ohmori, H., Friedberg, E., Fuchs, R. P., Goodman, M. F., Hanako, F., Hinkle, D., Kinkel, T. A., Lawrence, C. W., Livneh, Z., Nomhi, T., Prakash, L., Prakash, S., Todo, T., Walker, G. C., Wang, Z., and Woodgate, R. (2001) Mol. Cell 7, 1–8
8. Caleforni, T., and Mornon, J. P. (1997) FEBS Lett. 400, 25–30
9. Nelson, J. R., Lawrence, C. W., and Hinkle, D. C. (1996) Nature 382, 729–731
10. Harasaka, L., Ung, I., Johnson, R. E., Johansson, E., Burgers, P. M., Prakash, S., and Prakash, L. (2001) Genes Dev. 15, 945–954
11. Harasaka, L., Prakash, S., and Prakash, L. (2002) J. Biol. Chem. 277, 15546–15551
12. Gibbs, P. E., and Lawrence, C. W. (1995) J. Mol. Biol. 251, 229–236
13. Nelson, J. R., Gibbs, P. E., Nowicka, A. M., Hinkle, D. C., and Lawrence, C. W. (2000) Mol. Microbiol. 37, 549–554
14. Lin, W., Xin, H., Zhang, Y., Wu, X., Yuan, F., and Wang, Z. (1999) Nucleic Acids Res. 27, 4468–4475
15. Masuda, Y., Takahashi, M., Tsubekuni, N., Minami, T., Sumii, M., Miyagawa, K., and Kamiya, K. (2001) J. Biol. Chem. 276, 10515–10558
16. Masuda, Y., and Kamiya, K. (2003) FEBS Lett. 520, 88–92
17. Masuda, Y., Takahashi, M., Fukuda, S., Sumii, M., and Kamiya, K. (2002) J. Biol. Chem. 277, 3040–3046
18. Zhang, Y., Wu, X., Reckosh, D., Geacintov, N. E., Taylor, J. S., and Wang, Z. (2002) Nucleic Acids Res. 30, 1630–1638
19. Otsuka, C., Kobayashi, K., Kawaguchi, N., Kunitome, N., Morigaya, K., Hata, Y., Iwaki, S., Leodke, D., Nuske, V. N., Pavlov, Y., and Negishi, K. (2002) Mutat. Res. 502, 53–60
20. Nelson, J. R., Lawrence, C. W., and Hinkle, D. C. (1996) Science 272, 1646–1649
21. Lawrence, C. W., and Hinkle, D. C. (1996) Cancer Surv. 28, 21–31
22. Johnson, R. E., Washington, M. T., Harasaka, L., Prakash, S., and Prakash, L. (2000) Nature 406, 1013–1019
23. Johnson, R. E., Harasaka, L., Prakash, S., and Prakash, L. (2001) Mol. Cell. Biol. 21, 3558–3563
24. Gao, D., Wu, X., Rajpal, D. K., Taylor, J. S., and Wang, Z. (2001) Nucleic Acids Res. 29, 2875–2883
25. Gibbs, P. E., McGregor, W. G., Maher, V. M., Niessen, P., and Lawrence, C. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 85, 6876–6880
26. Gibbs, P. E., Wang, X. D., Li, Z., McManus, T. P., McGregor, W. G., Lawrence, C. W., and Maher, V. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4186–4191
27. Murakumo, Y., Roth, T., Ishii, H., Baso, D., Numata, S., Croce, C. M., and Fishel, R. (2000) J. Biol. Chem. 275, 4391–4397
28. Murakumo, Y., Oyura, Y., Ishii, H., Numata, S., Ichihara, M., Croce, C. M., Fishel, R., and Takashashi, M. (2001) J. Biol. Chem. 276, 35644–35651
29. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1999) Methods Enzymol. 185, 60–89
30. Mayer, M. F. (1995) Gene 163, 41–46
31. Maki, H., and Kornberg, A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4389–4392
32. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
33. Siegel, L. M., and Monty, K. J. (1996) Biochem. Biophys. Acta 112, 346–362