Mechanisms of dCMP Transferase Reactions Catalyzed by Mouse Rev1 Protein*

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Yuji Masuda, Mamoru Takahashi, Saburo Fukuda, Masaharu Sumii, and Kenji Kamiya‡

From the Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan

The Rev1 protein, a member of a large family of translesion DNA polymerases, catalyzes a dCMP transfer reaction. Recombinant mouse Rev1 protein was found to insert a dCMP residue opposite guanine, adenine, thymine, cytosine, uracil, and an apurinic/apyrimidinic site and to have weak ability for transfer to a mismatched terminus. The mismatch-extension ability was strongly enhanced by a guanine residue on the template near the mismatched terminus; this was not the case with an apurinic/apyrimidinic site and the other template nucleotides. Kinetic analysis of the dCMP transferase reaction provided evidence for high affinity for dCTP with template G but not the other templates, whereas the template nucleotide did not much affect the $V_{\text{max}}$ value. Furthermore, it could be established that the mouse Rev1 protein inserts dGMP and dTMP residues opposite template guanine at a $V_{\text{max}}$ similar to that for dCMP.

In yeast Saccharomyces cerevisiae, the REV1 gene is required for damage-induced and spontaneous mutagenesis (1–7). A defect in the REV1 gene has in fact been found to decrease the translesion replication of apurinic/apyrimidinic (AP) sites, T-T (6–4) UV photoproducts, and N-2-acetylaminofluorene-modified guanine (8, 9). The encoded protein, containing a BRCA1 C terminus (BRCT) domain at its N terminus, possesses deoxycytidyl transferase activity (10–12) inserting dCMP residues opposite templates G, A, T, and AP sites (12). The activity of Rev1 protein could be important for the bypass of AP sites in yeast (9) because cytidine is preferentially inserted opposite these lesions in vitro (9, 13). However, a number of observations have suggested that the Rev1 protein may possess a second function. First, when the REV1 gene is required for the bypass of a T-T (6–4) UV photoproduct, dCMP incorporation occurs only very rarely in vitro (9). Second, translesion DNA synthesis and mutagenesis are greatly reduced in a rev1 mutant, rev1–1, with a BRCT domain alteration that does not affect the deoxycytidyl transferase activity in vitro (9, 11). Third, during bypass of N-2-acetylaminofluorene-modified guanine, the REV1 gene is needed only for non-slipped translesion DNA synthesis, suggesting that the uncharacterized

Rev1 activity is UmuDC-like in nature (8). Fourth, methyl methanesulfonate-induced mutagenesis was shown to be normal in a site-directed mutant lacking deoxycytidyl transferase activity (14). Fifth, +1 frameshift mutations accompanying base substitutions are dependent on the REV1 gene (15).

Recently, cloning of a human homologue of the REV1 gene (16, 17) revealed good conservation from yeast to humans. The human REV1 gene encodes a deoxycytidyl transferase, similar to the Rev1 protein of the yeast, S. cerevisiae (17, 18), with activity localized to the central domain that is conserved in the UmuC superfamily (18). Mutations in conserved residues but not the BRCT domain completely abolish the transferase activity (18).

Proteins in the UmuC superfamily, except for the Rev1 protein, are novel DNA polymerases, capable of replicating damaged DNA (19). Only for the Rev1 protein has no polymerase activity been detected so far. It has been reported that its transferase activity is limited to the insertion of a dCMP residue (12, 17). Although the enzyme is capable of incorporating a dCMP residue not only opposite G but also A, U, and AP sites, it is not clear how the Rev1 protein plays a role in mutagenesis.

In the present study, we cloned and characterized the REV1 gene of the mouse, an animal commonly used for models of human disease. We found that the mouse Rev1 protein transfers a dCMP residue not only opposite template G but also A, T, C, U, and AP sites and extends a mismatched terminus by the addition of a dCMP residue. It has been shown that mismatch-extension ability is strongly enhanced by the presence of a guanine residue (but not an AP site) on the template near the mismatched terminus. Kinetic analysis of the dCMP transferase reaction provided evidence for high affinity of dCTP with template G. Furthermore, the mouse Rev1 protein could be shown to insert dGMP and dTMP residues opposite template guanine and AP sites. The same activity was also detected with recombinant human REV1 protein but not an inactive mutant protein.

EXPERIMENTAL PROCEDURES

Animals—Mice of the C57BL/6N and C3H/He strains were purchased from Charles River Laboratories Inc., Atsugi, Japan. All experiments followed the guidelines of the Animal Experimental Facility Committee of Hiroshima University.

Cloning of Mouse Rev1 cDNA and Construction of an Expression Plasmid—Poly(A)$^+$ mRNA was isolated from the liver of an inbred mouse, strain C3H/He, using a poly(A) tract mRNA isolation system (Promega). Double-stranded cDNA was synthesized using a cDNA synthesis kit (Takara), and PCR primers corresponding to the human REV1 gene were used to amplify fragments of mouse Rev1 cDNA. The amplified fragments were cloned and sequenced. From the sequence information, several primer sets were designed and tested. Consequently, mouse Rev1 cDNA was successfully amplified as three overlapping cDNA fragments using Pyrobest$^\text{TM}$ DNA polymerase (Takara). A promoter proximal fragment (fragment I) was amplified with primers

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The nucleotide sequence reported in this paper has been submitted to the DNA Data Bank of Japan (DDBJ)/GenBank$^\text{TM}$/EBI Data Bank with accession number AB057418.

‡ To whom correspondence should be addressed. Tel.: 81-82-257-5842; Fax: 81-82-257-5844; E-mail: kkamiya@hiroshima-u.ac.jp.

The abbreviations used are: AP, apurinic/apyrimidinic. BRCT, BRCA1 C terminus.
Primary Structure of Mouse Rev1 Protein — The cloned mouse Rev1 cDNA encodes a putative protein of 1249 amino acid residues with a calculated molecular mass of 137 kDa. The sequence alignment of the human and mouse Rev1 proteins is shown in Fig. 1. Comparison of the amino acid sequences of the two proteins revealed an overall amino acid identity of 84% and similarity of 90% with all of the motifs found in the human Rev1 protein conserved in the mouse counterpart (Fig. 1). The BRCT domain, motif I, and motif VIII are specific to the Rev1 family. Motifs II–VII are conserved in polymersases of the UmuC superfamily. We (18) previously showed the minimum region required for deoxycytidyl transferase activity of the human Rev1 protein (Fig. 1, boxed region). This region was highly conserved with 88% identity and 94% similarity.

Expression of the Rev1 Gene in Mouse Tissues — Expression of the mouse Rev1 gene in various tissues was examined by Northern blot analysis (Fig. 2), and the mouse Rev1 mRNA was detected in all tissues examined. Expression of the Rev1 gene was relatively high in the heart, skeletal muscle, and testis.

Purification of Recombinant Mouse Rev1 Protein and Physicochemical Properties — To purify the mouse Rev1 protein, we expressed a recombinant protein tagged with hexa-histidine at its N terminus in E. coli cells. The tagged Rev1 protein (h6-mRev1) was purified by affinity chromatography on a nickel-chelating column, and the fraction containing h6-mRev1 was applied to a gel filtration column. The h6-mRev1 protein eluted with an apparent molecular mass of 330 kDa with a Stokes’ radius of 58 Å (Table I). As shown in Fig. 3, analysis by SDS-PAGE revealed a full-length h6-mRev1 protein of 139 kDa, and smaller forms were also detected specifically when the h6-mRev1 protein was induced in E. coli cells, indicating that these bands are degradation products (data not shown). The properties were found to be identical with those of the human Rev1 protein (18). In this preparation, neither DNA polymerase activity nor deoxyribonuclease activity was detected (Fig. 4A and data not shown).

We used this fraction for further physicochemical and biochemical characterization.

To determine the molecular mass of the purified h6-mRev1 protein in solution, it was analyzed by sucrose gradient sedimentation. The determination of sedimentation coefficient was based on the method described by Siegel and Monty (22), which was calculated to be 5.7 S. Employing the method described by Siegel and Monty (22), we calculated the molecular mass at 138 kDa (Table I). These results suggested that the h6-mRev1 protein was asymmetrically shaped, existing as a monomer in solution.

Ability of the Mouse Rev1 Protein to Transfer dGMP and dTMP Residues — Using six different primer-templates, we examined the substrate specificity of the transferase activity of the h6-mRev1 protein in the presence of 100 μM dGTP, dTTP, dGTP, or dCTP (Fig. 4). In this experiment, the respective primer-templates differed only at the template nucleotide immediately downstream from the annealed primer. When a G template was incubated with the h6-mRev1 protein and each of the dNTPs, we surprisingly detected a one-base-extension product in the presence of not only dCTP but also dGTP and dTTP (Fig. 4A, panel a). We also found an ability to insert dGMP and
dTMP residues opposite the template AP site (Fig. 4A, panel f). Although the efficiency was very low, significant activity was confirmed by reactions with higher concentrations of the h6-mRev1 protein (data not shown). We could not detect dGMP insertion opposite template C or dTMP insertion opposite template A (Fig. 4A, panels b and d), and the results clearly indicated that activity from contaminating bacterial DNA polymerases was less than the detectable level. The mobility of the reaction products and defined oligonucleotide markers was compared (Fig. 4B). Those with dGTP, dTTP, and dCTP migrated exactly like the respective markers (Fig. 4B). These results indicate that the h6-mRev1 protein has a potential to transfer dGMP and dTMP residues, albeit with only a fifth of the activity with dCMP residues (Fig. 4C). In the presence of all four dNTPs, the dCMP transfer reaction predominated over the dGMP and dTMP transfer reactions (Fig. 4A, panel a, lane 6).

Only the ability to transfer dCMP has been reported for the human REV1 protein (17, 18). However, when dGMP and dTMP transferase activities of the human REV1 protein were examined (Fig. 4D), it incorporated not only dCMP but also dGTP and dTTP residues opposite template G. The activity was completely eliminated with an inactive mutant protein, D569A/E570A (18) (Fig. 4D).

Deoxycytidyl Transferase Activity and Ability to Extend the Mismatched Primer Terminus—When all of the templates were tested, the h6-mRev1 protein inserted a dCMP residue opposite not only the template AP site but also all bases examined (Fig. 4A, panels b–f, lane 5). The enzyme activity for insertion reactions opposite the template AP site was slightly higher than that opposite templates G, A, and U (1.2–1.5-fold) and six times higher than that opposite templates T and C (Fig. 4C). When G,

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**Table I**

| Property | Value |
|----------|-------|
| Stokes’ radius | 58 Å |
| Sedimentation coefficient ($S_{20,W}$) | $5.7 \times 10^{-13}$ sec |
| Molecular mass | 138 kDa |

* The Stokes’ radius was determined by 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. The sedimentation coefficient was determined with catalase (11.3 S), aldolase (7.3 S), and albumin (4.3 S) size markers, and the data were based on the both of SDS-gel profile and dCMP transferase activity. The dCMP transferase activity paralleled exactly the abundance of the h6-mRev1 protein. The molecular mass was calculated from the Stokes’ radius and the sedimentation coefficient assuming a partial specific volume of 0.73 (22).
protein might possess the ability to add a dCMP residue to the mismatched 3′ terminus resulting from the first insertion of a dCMP residue. We investigated this preliminary observation further by performing reactions using another set of primer-templates having mismatched primer-template termini (Fig. 5, A and B) differing only in the attachment of a cytidine residue at the 3′ terminus from the set of primer-templates described in Fig. 4. As illustrated in Fig. 5, A and B, the transferase extended C-A, C-T, and C-U (primer-template) mismatched termini by only one nucleotide (Fig. 5A, panels b, c, and e) but failed to extend C-C and C-AP (primer-template) termini (Fig. 5A, panels d and f). The ability did not arise from a potential of the enzyme to extend the 3′ terminus of the single-stranded DNA (Fig. 5C). In the sequence context, it should be noted that the enzyme activity for extension of the mismatched primer template (Fig. 6 and Table II). Notably, the mismatch extending ability was examined using all 16 possible combinations of primer-template termini (Fig. 6). As shown in Fig. 6, extensions of all mismatched termini except for C-C were detected. The quantitative results are summarized in Table II. Interestingly, the 3′ mismatched termini with a G template, G-G, A-G, and T-G (primer-template) tended to be extended at high efficiency (Fig. 6 and Table II). Notably, the specific activity of extension of the T-G (primer-template) mismatch was close to that of the matched termini (36% of T-A and

![Image](http://www.jbc.org/)

**Fig. 3.** SDS-PAGE analysis of the purified h6-mRev1 protein. The purified protein (0.5 μg) was loaded on an 8% polyacrylamide gel and stained with Coomassie Brilliant Blue R-250 according to the method of Sambrook et al. (27). M, molecular weight markers (Invitrogen).

![Image](http://www.jbc.org/)

**Fig. 4.** Transferase activity of the h6-mRev1 protein. A, primer extension assays. A 5′-[32P]-labeled primer, P13, was annealed with each of the templates, 30G (panel a), 30A (panel b), 30T (panel c), 30C (panel d), and 30U (panel e). The primer-template containing an AP site (panel f) was generated as described under “Experimental Procedures.” Nucleotide sequences adjacent to the primer terminus are shown on the right of each panel. Ten ng of the h6-mRev1 protein and the indicated primer-template and dCTP at 30 °C for 30 min in 25 μl of reaction solution. The reaction products were resolved in 20% polyacrylamide gel containing 8 M urea and visualized by autoradiography. B, analysis of the products from the dGMP and dTMP transfer reactions. Reaction products from the G template with dGTP (lane 1), dGTP (lane 4), and dCTP (lane 7, shown in panel a of A, were loaded on a gel with the 5′-[32P]-labeled oligonucleotide markers P13G (lane 2), P13A (lane 3), P13C (lane 4), and P13T (lane 5). C, quantitation of results of primer extension assays. The band intensities of substrates and products were determined using a Bio-Imaging Analyzer BAS2000, and the amounts (in pmol) of products in 25-μl reaction solution were calculated.

A, T, and U templates were incubated with h6-mRev1 and dCTP, a faint two base longer band was detected (Fig. 4A, panels a–c and e), and it was concluded that the h6-mRev1 protein might possess the ability to add a dCMP residue to the mismatched 3′ terminus resulting from the first insertion of a dCMP residue. We investigated this preliminary observation further by performing reactions using another set of primer-templates having mismatched primer-template termini (Fig. 5, A and B) differing only in the attachment of a cytidine residue at the 3′ terminus from the set of primer-templates described in Fig. 4. As illustrated in Fig. 5, A and B, the transferase extended C-A, C-T, and C-U (primer-template) mismatched termini by only one nucleotide (Fig. 5A, panels b, c, and e) but failed to extend C-C and C-AP (primer-template) termini (Fig. 5A, panels d and f). The ability did not arise from a potential of the enzyme to extend the 3′ terminus of the single-stranded DNA (Fig. 5C). In the sequence context, it should be noted that the enzyme activity for extension of the mismatched primer template was 10 times lower than that of the matched C-G primer (primer-template) terminus (Fig. 5B and Table II).

**Fig. 5.** Mismatch extension ability of the h6-mRev1 protein. A, primer extension assays. A 5′-[32P]-labeled primer, P13C, was annealed with each of the templates, 30G (panel a), 30A (panel b), 30T (panel c), 30C (panel d), and 30U (panel e). A primer-template containing an AP site (panel f) was generated as described under “Experimental Procedures.” Nucleotide sequences adjacent to the primer terminus are shown on the right of each panel. Increasing amounts of protein (0, 6.3, 13, 25, 50, and 100 ng, from left to right) were incubated with the indicated primer-template and dCTP at 30 °C for 30 min in 25 μl of reaction solution. B, quantitation of results of primer extension assays. The base pairs of the primer termini are shown with symbols. C, terminal deoxynucleotidyltransferase assay. Increasing amounts of protein (0, 6.3, 13, 25, 50, and 100 ng, from left to right) were incubated with dCTP and 5′-[32P]-labeled oligonucleotide, P13C, at 30 °C for 30 min in 25 μl of reaction solution. The products were analyzed as described in the legend for Fig. 4.
Mouse Rev1 Protein

We examined the potential sequence context effect on translesion synthesis activity using other sets of primer-templates (Fig. 7). With these, the template nucleotide immediately downstream from the annealed primer was cytidine (Fig. 7A). The enzyme activity for the insertion reaction of dCMP opposite template C was strongly affected by the surrounding sequence (Fig. 7, B and C). The specific activity of insertion to set a substrate was 5–8 times less than that to set b (Fig. 7, D and E). With both sets of primer-templates shown in Fig. 7A, the enzyme activity of insertion of dCMP opposite C was affected by a template nucleotide, the position of which is represented by X in Fig. 7A. The specific activity of insertion of dCMP was decreased in the order of template nucleotides of A > T > G > C at position X (Fig. 7, D and E). The C-C mismatch is the most inefficient substrate for the translesion reaction and was not extended by the Rev1 protein (Figs. 5A and 6). However, when a guanine residue was located on the template 5’ to the mismatched base pair, the C-C mismatch terminus was efficiently extended (Fig. 7, B and C, panel a) to an ~15% extent (Fig. 7C, panel a). This mismatch extension was not detected when other nucleotide residues were located in this position (Fig. 7, B and C, panels b–d).

Steady-state Kinetics of the Translesion Synthesis—We have determined kinetic parameters by steady-state gel kinetic assays (Table III). Because the reactions with mismatched templates seem to be complicated and a mixture of several pathways (see “Discussion”), we focused on the translesion reactions with matched templates. The assays were all carried out with a 5-min incubation because the time course of the reactions was linear until 10 min (data not shown). The $V_{\text{max}}$ for the dCMP insertion opposite template G, A, and U was 1.0 min$^{-1}$, and it was 0.83 and 0.85 min$^{-1}$ opposite template T and C, respectively. With the template AP site, the $V_{\text{max}}$ was a little faster (1.3 min$^{-1}$). These results indicate that the template nucleotide did not appreciably affect the velocity of the reaction. However, we found that the template nucleotide strongly affected the $K_m$ value for dCTP, revealing a specific nature for template G. The $K_m$ value with the template AP site (12 mM) was nine times higher than that with template G (1.4 mM) but still lower than that with the other templates. Because the velocity of the enzyme reaction is near to the $V_{\text{max}}$ at higher substrate concentration, values for the velocity at 100 μM of dCTP concentration were calculated using equations obtained from the Lineweaver-Burk plot, giving 1.0 min$^{-1}$ and 1.2 min$^{-1}$ with template G and the AP site, respectively (Table III). These values agree with the results of Fig. 4, for which the experiments had been performed at 100 μM of dNTP. Therefore, the higher activity of the Rev1 protein with the template AP site shown in Fig. 4 is due to the $V_{\text{max}}$ value under this condition.

The kinetic parameters for dGMP and dTMP insertion opposite template C were also determined (Table III). Affinity for dGTP and dTTP was very low, although the value of $V_{\text{max}}$ for dGTP insertion was found to be identical to that of dCTP insertion, whereas that for dTTP insertion was only half.

**DISCUSSION**

The Rev1 protein is unique in that while belonging to a large family of translesion DNA polymerases, its reported activity is restricted to the transfer of dCMP to primer termini. In this work, cloning of the mouse Rev1 gene and characterization of the translesion reaction revealed particular enzymatic properties.

We showed that the mouse Rev1 gene is expressed ubiquitously, as reported for the human REV1 gene (17, 18). Why expression in the mouse heart, skeletal muscle, and thymus was relatively high compared with that in the corresponding tissues of humans (18) remains to be clarified.

### Table II

| Primer/template$^a$ | Specific activity$^b$ | Relative activity |
|---------------------|----------------------|------------------|
| P13G/30G (G-G)      | 4.6                  | 16               |
| 30A (G-A)           | 0.9                  | 3                |
| 30T (G-T)           | 0.7                  | 2                |
| 30C (G-C)           | 28.2                 | 100              |
| P13A/30G (A-G)      | 7.2                  | 13               |
| 30A (A-A)           | 2.9                  | 5                |
| 30T (A-T)           | 55.2                 | 100              |
| 30C (A-C)           | 1.3                  | 2                |
| P13T/30G (T-G)      | 10.9                 | 36 (90)$^c$      |
| 30A (T-A)           | 30.1                 | 100              |
| 30T (T-T)           | 1.2                  | 4                |
| 30C (T-C)           | 2.3                  | 8                |
| P13C/30G (C-G)      | 12.1                 | 100 (100)$^d$    |
| 30A (C-A)           | 1.0                  | 8                |
| 30T (C-T)           | 0.7                  | 6                |
| 30C (C-C)           | <0.1                 | <1               |

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$^a$ 5'-32P-labeled P13G, P13A, P13T, and P13C primers were each annealed with the templates: 30G, 30A, 30T, and 30C. The base pairs in the primer terminus next to the primer/template are indicated in parentheses.

$^b$ An appropriate amount of h6-mRev1 was incubated with the indicated primer-template and dCTP at 30 °C for 30 min in 25 μl of reaction solution. The reaction products were resolved in 20% polyacrylamide gels containing 8 M urea, and the band intensities of substrates and products were determined using a Bio-Imaging Analyzer BAS2000 to give amounts in picomoles.

$^c$ Specific activity of the T-G (primer-template) mismatch-extension reaction was compared with that for the C-G primer terminus.

90% of C-G (primer-template) matched termini. This property of the T-G (primer-template) mismatch is not due to a property of the base pairing of T and G because the enzyme activity for extension of the G-T (primer-template) mismatch was very low (Fig. 6 and Table II). While a fraction of the mismatched primer terminus could have been misaligned and the dCMP transferred from a dCTP pairing with template G, as shown in Fig. 8A, if this was the case, the template nucleotide immediately downstream from the new primer terminus could have become A (Figs. 6 and 8A). Because template A is a better substrate than template C (Fig. 4), h6-mRev1 could extend one more nucleotide by the insertion of dCMP opposite the template A (Fig. 8A).

**Fig. 6.** Systematic analysis of the mismatch extension ability of the h6-mRev1 protein. 5'-32P-labeled P13G (panel a), P13A (panel b), P13T (panel c), and P13C (panel d) primers were each annealed with templates: 30G (lane G), 30A (lane A), 30T (lane T), and 30C (lane C). Nucleotide sequences adjacent to the primer terminus are shown on the right of each panel. One hundred ng of the h6-mRev1 protein was incubated with the indicated primer-template and dCTP at 30 °C for 30 min in 25 μl of reaction solution. The products were analyzed as described in the legend for Fig. 4.
We (18) previously suggested, based on the results of gel filtration chromatography, that the human REV1 protein might be a dimer in solution. However, the present results with sucrose gradient sedimentation strongly suggest that the h6-hmRev1 protein is an asymmetrically shaped molecule with a molecular mass of 138 kDa calculated from the Stokes’ radius and a sedimentation coefficient very close to the value of 139 kDa calculated from the amino acid sequence. These data imply the monomeric status of the human REV1 protein in solution.

The present experiments revealed that the mouse Rev1 protein inserts dGMP and dTMP residues opposite template G and AP sites, whereas no incorporation was detected opposite templates C and A. The \( V_{\text{max}} \) for dGMP insertion is identical to that for dCMP insertion, and that for dTMP insertion is only half. Therefore, the low efficiency of the reactions is due to the low

**FIG. 7.** Sequence context effect on deoxycytidyl transferase activity of the h6-mRev1 protein. A, nucleotide sequences of primer-templates. Sets a and b consisted of five and four different primer-templates, respectively. X represents the position of the different nucleotide residues or AP sites.

**FIG. 8.** Model for the enhancement of mismatch extension by a guanine residue on the template. A 3’-terminus mispairing with template G is efficiently extended by the Rev1 protein. The incorporating dCMP residue is paired with template G, and the new 3’-terminus of the resulting product may be extended by a second dCMP insertion opposite template (A). The Rev1 protein does not extend 3’-terminus mispairing with a template AP site (B). When a dCMP residue is incorporated opposite template C and a guanine residue is located on the template immediately downstream from the mismatched 3’-terminus, a second dCMP residue is efficiently incorporated, but other nucleotides and an AP site do not assist such mismatch extension (C and D). This pathway might not assist mismatch extension and is not prevalent (E). Dashes represent AP sites.

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**TABLE III**

Kinetic analysis of nucleotide incorporation on various DNA templates

| Template | Substrate | \( V_{\text{max}}^{a} \) | \( K_{m}^{a} \) | \( v_{o}^{b} \) (100 \( \mu \)M) |
|----------|-----------|----------------|-------------|------------------|
| G        | dCTP      | 1.0            | 1.4         | 1.0              |
| A        | dCTP      | 1.0            | 26          | 0.76             |
| T        | dCTP      | 0.83           | 320         | 0.20             |
| C        | dCTP      | 0.85           | 500         | 0.15             |
| U        | dCTP      | 1.0            | 38          | 0.72             |
| AP       | dCTP      | 1.3            | 12          | 1.2              |
| G        | dGTP      | 1.0            | 1300        | 0.070            |
| G        | dTTP      | 0.55           | 640         | 0.074            |

**a** The \( K_{m} \) and \( V_{\text{max}} \) values were determined using equations obtained from a Lineweaver-Burk plot. Data from two to four independent experiments were plotted together, and the correlation coefficients (R²) of the straight lines were 0.9 for dCTP and dGTP and 0.8 for dTTP.

**b** Velocity at 100 \( \mu \)M substrate was calculated using the equations obtained from the Lineweaver-Burk plot.
affinity of dGTP and dTTP but not due to the $V_{\text{max}}$. These results suggest that the template G is not a true substrate for the transferase reaction and that activity of the Rev1 protein might be required for bypassing some DNA lesions that could increase the affinity for dGTP and dTTP. With the yeast Rev1 protein but not the human protein, a weak ability to incorporate a dCMP opposite template G has been detected (12). The fact that incorporation on dCMP and dTMP was detected for the mouse Rev1 protein might result from differences in the sequence context of the primer-templates used. Examination of the human REV1 protein (18) using our substrates was therefore conducted. We found that the properties of the human and mouse enzymes were essentially identical. It is possible that the nucleotide sequence of our primer-templates used in the primer extension assay may be serendipitously suitable for detecting such activity. Importantly, the activity was completely eliminated with the D569A/E570A, providing evidence that transferase activity is intrinsic to mammalian Rev1 proteins.

Our systematic analysis of the transferase activity of the mouse Rev1 protein using various primer-templates revealed a unique property. We showed that the mouse Rev1 protein inserts a dCMP opposite templates G, A, T, C, U, and AP sites. The yeast Rev1 protein has only weak ability to insert a dCMP opposite template C (12), and no such activity has been reported for the human REV1 protein, but we showed the reaction efficiency to be strongly affected by the surrounding nucleotide sequence so that a direct comparison of the results is difficult. We found that the Rev1 protein has a weak ability to extend the mismatched 3' terminus and that this was strongly enhanced by the presence of a guanine residue on the template near the primer terminus but not an AP site or the other nucleotides. Kinetic analysis provided evidence for a high affinity of dCTP with template G and low affinity with the other templates, whereas the template nucleotide did not appreciably affect the $V_{\text{max}}$ value. These properties might explain the specific nature of template G enhancement of mismatch extension.

We propose a model to account for this enhancement (Fig. 8). In Fig. 8A, the mispaired 3' terminus with a guanine residue on the template could be misaligned and ligated to a dCMP paired with the guanine residue (Fig. 8A). Even though the Rev1 protein efficiently inserts dCMP opposite a template AP site, an AP site does not assist mismatch extension (Fig. 8B). When a guanine residue is located on the template 5' close to the mismatched base pair, the mismatched 3' terminus may be efficiently ligated to the dCMP residue paired with the guanine residue on the template (Fig. 8C). Again, a template AP site does not assist under this condition (Fig. 8D). Alternatively, the first insertion of dCMP might be the result of misalignment with template G as shown in Fig. 8E. However, we suspect that this process is not prevalent because a guanine base at this position did not enhance the dCMP insertion reaction (Fig. 7). Currently, we cannot distinguish the two reaction pathways (Fig. 8, C and E) and are not able to quantitate their respective efficiencies. Recently, Harfe and Jinks-Robertson (15) found complex mutations with frameshifts accompanied by base substitutions accumulating in a yeast strain defective for nucleotide excision repair. These events were dependent on the REV1 and REV3 genes and triggered by a misincorporation opposite template G, similar to the pathway shown in Fig. 8. The properties described in this study for the Rev1 protein might imply a possible direct role of this protein in inducing frameshift mutations.

The kinetic analysis revealed that the velocity of the transferase reaction was restricted by the concentration of dCTP but not template nucleotides. It is known that a second ribonucleotide reductase is induced by DNA damage in yeast and humans (23, 24). It is very likely that the concentration of dNTPs is increased in the nucleus in response to DNA damage (25, 26) and could affect the substrate specificity of the Rev1 protein.

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Yuji Masuda, Mamoru Takahashi, Saburo Fukuda, Masaharu Sumii and Kenji Kamiya

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