Incorporation and Excision of 9-(2-Phosphonylmethoxyethyl)guanine (PMEG) by DNA Polymerase δ and ε in Vitro*

(Received for publication, January 21, 1998, and in revised form, April 29, 1998)

Pavel Kramata‡, Kathleen M. Downey‡, and Lisa R. Paborsky‡

From ‡Gilead Sciences, Foster City, California 94404 and the ‡Department of Medicine, University of Miami School of Medicine, Miami, Florida 33101

PMEG (9-(2-phosphonylmethoxyethyl)guanine) is an acyclic nucleotide analog being evaluated for its anti-proliferative activity. We examined the inhibitory effects of PMEG diphosphate (PMEGpp) toward DNA polymerases (pol) δ and ε and found it to be a competitive inhibitor of both these enzymes. The apparent Ki values for PMEGpp were 3–4 times lower than the Km values for dGTP. The analog was shown to function as a substrate and to be incorporated into DNA by both enzymes. Examination of the ability of pol δ and pol ε to repair the incorporated PMEG revealed that pol ε could elongate PMEG-terminated primers in both matched and mismatched positions with an efficiency equal to 27 and 85% that observed for dGMP-terminated control template-primers. Because PMEG acts as an absolute DNA chain terminator, the elongation of PMEG-terminated primers is possible only by cooperation of the 3′-5′-exonuclease and DNA polymerase activities of the enzyme. In contrast to pol ε, pol δ exhibited negligible activity on these template-primers, indicating that pol ε, but not pol δ, can repair the incorporated analog.

PMEG (9-(2-phosphonylmethoxyethyl)guanine) (Fig. 1) is a member of a new class of acyclic nucleotide analogs characterized as phosphoryl methyl ethers. Several analogs in this class have demonstrated broad spectrum antiviral activity (1). PMEG is being evaluated as an antitumor agent for cancer treatment. Its anti-proliferative activity has been demonstrated in vitro against human leukemic cells (2) as well as a number of solid tumor cell lines (3). PMEG also showed antitumor activity in two types of mouse transplantable tumors, P388 lymphocytic leukemia and B16 melanoma (4). In addition, the analog suppressed the growth of papillomavirus-induced condylomas on human foreskin xenografts in mice (5).

Once inside the cells, cellular enzymes phosphorylate the analog to PMEG mono- and diphosphate (6). The diphosphate form (PMEGpp) is an analog of dGTP. Although the mechanism of action of PMEG has not yet been determined, PMEG diphosphate has been shown to be a potent inhibitor of rat DNA polymerase α and ε (7) and to be incorporated into DNA in vitro by human DNA polymerase α and δ (8, 9). Therefore, it can potentially inhibit cellular DNA synthesis.

DNA polymerases α, δ, and ε are the enzymes cooperating in chromosomal DNA replication (10). Pol α, associated with DNA primase activity, synthesizes RNA-DNA primers for initiation of DNA replication at ori sites and for priming of Okazaki fragments on the lagging strand of DNA. Pol δ, in cooperation with proliferating cell nuclear antigen (PCNA) and other protein factors, synthesizes the leading strand of DNA. Pol ε, which is dispensable for SV40 DNA replication (11) but not for cellular DNA replication (12), may be required as a second DNA polymerase on the lagging DNA strand (13). In contrast to pol α, both pol δ and pol ε have intrinsic 3′-5′-exonuclease activity associated with a proofreading function (14). Both enzymes are necessary for the repair of cellular DNA damage after UV irradiation (15, 16), probably participating in filling the excision gaps during the final step of nucleotide excision repair (17). Moreover, pol δ is associated with DNA mismatch repair (18), and pol ε was described as a cell cycle checkpoint regulator sensing DNA damage during S phase (19). These observations suggest that pol δ and pol ε may have the potential to remove a nucleotide analog such as PMEG following its incorporation into cellular DNA.

In this study, we investigated the interaction of PMEGpp with purified bovine pol δ and human pol ε. By using oligonucleotide template-primers, we examined the inhibitory effects of PMEGpp and the ability of these enzymes to incorporate the analog into DNA as well as to excise it from 3′-ends.

EXPERIMENTAL PROCEDURES

Nucleotides—PMEGpp was synthesized at Gilead Sciences (Foster City, CA) according to a published procedure (20). The purity was checked by high pressure liquid chromatography using a Adsorbosphere SAX ion-exchange column (Alltech) and found to be greater than 97%. Deoxyribonucleotide triphosphates were purchased from Amersham Pharmacia Biotech. [γ-32P]ATP (222 TBq/nmol) and [α-32P]dGTP (111 TBq/nmol) were from Amersham Pharmacia Biotech.

Template-Primers—Poly(dA) and oligo(dT) 12–18 were obtained from Amersham Pharmacia Biotech. Oligonucleotide primers and templates were synthesized by Genosys (The Woodlands, TX). The primers were 5′-end-labeled by [γ-32P]ATP with T4 polynucleotide kinase (New England Biolabs). The labeled products were separated on Quick Spin (TE), 5′-[γ-32P]dGTP (1.89 TBq/mmol), [γ-32P]ATP (222 TBq/nmol), and [α-32P]dGTP (111 TBq/nmol) were from Amersham Pharmacia Biotech. Template-Primers—Poly(dA) and oligo(dT) 12–18 were obtained from Amersham Pharmacia Biotech. Oligonucleotide primers and templates were synthesized by Genosys (The Woodlands, TX). The primers were 5′-end-labeled by [γ-32P]ATP with T4 polynucleotide kinase (New England Biolabs). The labeled products were separated on Quick Spin (TE), 5′-[γ-32P]dGTP (1.89 TBq/mmol), [γ-32P]ATP (222 TBq/nmol), and [α-32P]dGTP (111 TBq/nmol) were from Amersham Pharmacia Biotech. Template-Primers—Poly(dA) and oligo(dT) 12–18 were obtained from Amersham Pharmacia Biotech. Oligonucleotide primers and templates were synthesized by Genosys (The Woodlands, TX). The primers were 5′-end-labeled by [γ-32P]ATP with T4 polynucleotide kinase (New England Biolabs). The labeled products were separated on Quick Spin (TE), 5′-[γ-32P]dGTP (1.89 TBq/mmol), [γ-32P]ATP (222 TBq/nmol), and [α-32P]dGTP (111 TBq/nmol) were from Amersham Pharmacia Biotech.

This paper is available on line at http://www.jbc.org
5′-TGACCTTAAACGAGG or PMEG
3′-ACTGCTATGTTGCTCTCACAACAGGCAACACACACACACACAGG-5′

IMMUNOBLOT ANALYSIS OF POL ε—I mmunoblot analysis of pol ε was performed using pol ε antibody from the hybridoma cell line SJK 132-20 as described previously (8). Aliquots of fractions eluted from the heparinagarose column (1 μl) were preincubated with 2 μg of the anti-pol ε antibody in 1.6 mg/ml BSA and 33 mM NaCl for 1 h at 4 °C. The reaction mixture (25 μl) was adjusted to 40 mM HEPES-KOH, pH 7.0, 10% glycerol, 1 mM diithiothreitol, 400 μg/ml BSA, 5 mM MgCl₂, 0.5 units/ml poly(dA)-oligo(dT)₅₄, 20 μM [³²P]dATP (18.9 GBq/mmol), and 80 μg/ml anti-pol ε antibody. After a 30-min incubation at 37 °C, the reaction mixtures were processed as described above.

Kinetic Experiments—The reaction mixture (15 μl) for the determination of kinetic constants (Vₘₐₓ, Kₘₐₓ, and Kᵦₐᵦ) was the same as for DNA polymerase unit definition except that poly(dA)-oligo(dT)₅₄ was replaced by TP-1 (0.1 μl), and dATP, dCTP, and dTTP (10 μM each) were added. The assays were performed with various concentrations of [³²P]dATP (0.05–29 TBq/mmol) and PMEG and 0.01 units of either pol δ or pol ε. After a 12-min incubation at 37 °C, aliquots were applied onto DE51 ion-exchange paper disks (Whatman). The disks were washed (3 × for 10 min) in 5% Na₂HPO₄, once in water, and once in 96% ethanol. The radioactivity on the disks was quantitated by liquid scintillation counting. The kinetic constants were calculated from the Lineweaver-Burk plots using KinetAsyst software (Think Technologies).

RESULTS

Purification of Pol ε—Pol ε was purified from exponentially growing human CCRF-CEM cells. A heparin-agarose chroma-

| Vₘₐₓ (pmol/min/mg) | Kₘₐₓ (dGTP) | Kᵦ (PMEGpp) | K/Kᵦ |
|-------------------|-------------|-------------|-------|
| pol δ 56 ± 4       | 43 ± 6      | 13 ± 1      | 0.30  |
| pol ε 1.3 ± 0.1    | 49 ± 7      | 12 ± 1      | 0.24  |

GFA/ fiberglass disks (Whatman). The filters were washed 3 times in cold 5% trichloroacetic acid containing 20 mM Na₃PO₄, once in 96% ethanol, and then dried. The acid-insoluble radioactivity was deter-

mized by liquid scintillation counting.

Neutralization of Pol ε by Anti-pol ε Antibody—Monoclonal anti-pol ε antibody from the hybridoma cell line SJK 132-20 was purified as described previously (8). Aliquots of fractions eluted from the heparinagarose column (1 μl) were preincubated with 2 μg of the anti-pol ε antibody in 1.6 mg/ml BSA and 33 mM NaCl for 1 h at 4 °C. The reaction mixture (25 μl) was adjusted to 40 mM HEPES-KOH, pH 7.0, 10% glycerol, 1 mM diithiothreitol, 400 μg/ml BSA, 5 mM MgCl₂, 0.5 units/ml poly(dA)-oligo(dT)₅₄, 20 μM [³²P]dATP (18.9 GBq/mmol), and 80 μg/ml anti-pol ε antibody. After a 30-min incubation at 37 °C, the reaction mixtures were processed as described above.

Kinetic Experiments—The reaction mixture (15 μl) for the determination of kinetic constants (Vₘₐₓ, Kₘₐₓ, and Kᵦᵦ) was the same as for DNA polymerase unit definition except that poly(dA)-oligo(dT)₅₄ was replaced by TP-1 (0.1 μl), and dATP, dCTP, and dTTP (10 μM each) were added. The assays were performed with various concentrations of [³²P]dATP (0.05–29 TBq/mmol) and PMEG and 0.01 units of either pol δ or pol ε. After a 12-min incubation at 37 °C, aliquots were applied onto DE51 ion-exchange paper disks (Whatman). The disks were washed (3 × for 10 min) in 5% Na₂HPO₄, once in water, and once in 96% ethanol. The radioactivity on the disks was quantitated by liquid scintillation counting. The kinetic constants were calculated from the Lineweaver-Burk plots using KinetAsyst software (Think Technologies).

RESULTS

Purification of Pol ε—Pol ε was purified from exponentially growing human CCRF-CEM cells. A heparin-agarose chroma-

| Vₘₐₓ (pmol/min/mg) | Kₘₐₓ (dGTP) | Kᵦ (PMEGpp) | K/Kᵦ |
|-------------------|-------------|-------------|-------|
| pol δ 56 ± 4       | 43 ± 6      | 13 ± 1      | 0.30  |
| pol ε 1.3 ± 0.1    | 49 ± 7      | 12 ± 1      | 0.24  |

GFA/ fiberglass disks (Whatman). The filters were washed 3 times in cold 5% trichloroacetic acid containing 20 mM Na₃PO₄, once in 96% ethanol, and then dried. The acid-insoluble radioactivity was deter-

mized by liquid scintillation counting.

Neutralization of Pol ε by Anti-pol ε Antibody—Monoclonal anti-pol ε antibody from the hybridoma cell line SJK 132-20 was purified as described previously (8). Aliquots of fractions eluted from the heparinagarose column (1 μl) were preincubated with 2 μg of the anti-pol ε antibody in 1.6 mg/ml BSA and 33 mM NaCl for 1 h at 4 °C. The reaction mixture (25 μl) was adjusted to 40 mM HEPES-KOH, pH 7.0, 10% glycerol, 1 mM diithiothreitol, 400 μg/ml BSA, 5 mM MgCl₂, 0.5 units/ml poly(dA)-oligo(dT)₅₄, 20 μM [³²P]dATP (18.9 GBq/mmol), and 80 μg/ml anti-pol ε antibody. After a 30-min incubation at 37 °C, the reaction mixtures were processed as described above.

Kinetic Experiments—The reaction mixture (15 μl) for the determination of kinetic constants (Vₘₐₓ, Kₘₐₓ, and Kᵦᵦ) was the same as for DNA polymerase unit definition except that poly(dA)-oligo(dT)₅₄ was replaced by TP-1 (0.1 μl), and dATP, dCTP, and dTTP (10 μM each) were added. The assays were performed with various concentrations of [³²P]dATP (0.05–29 TBq/mmol) and PMEG and 0.01 units of either pol δ or pol ε. After a 12-min incubation at 37 °C, aliquots were applied onto DE51 ion-exchange paper disks (Whatman). The disks were washed (3 × for 10 min) in 5% Na₂HPO₄, once in water, and once in 96% ethanol. The radioactivity on the disks was quantitated by liquid scintillation counting. The kinetic constants were calculated from the Lineweaver-Burk plots using KinetAsyst software (Think Technologies).

RESULTS

Purification of Pol ε—Pol ε was purified from exponentially growing human CCRF-CEM cells. A heparin-agarose chroma-

| Vₘₐₓ (pmol/min/mg) | Kₘₐₓ (dGTP) | Kᵦ (PMEGpp) | K/Kᵦ |
|-------------------|-------------|-------------|-------|
| pol δ 56 ± 4       | 43 ± 6      | 13 ± 1      | 0.30  |
| pol ε 1.3 ± 0.1    | 49 ± 7      | 12 ± 1      | 0.24  |
Incorporation of PMEG by pol δ (A) and pol ε (B). Pol δ or pol ε (0.07 units) was incubated for 15 min at 37 °C in 15-μl reaction mixtures containing buffer δ or ε, 0.1 μM 32P-labeled TTP-2, and various concentrations of PMEGpp or dGTP as indicated. Reactions were stopped by the addition of an equal volume of 98% formamide containing 10 mM EDTA, 0.2% bromphenol blue, and 0.2% xylene cyanole FF. Products were separated by 20% denaturing PAGE; the gels were scanned, and the radioactivity was quantitated using a PhosphorImager (Molecular Dynamics). The $K_m$ and $V_{max}$ values were calculated based on the Michaelis-Menten equation using Lineweaver-Burk plots and the KinetAsyst computer program.
A photography step was added to the published procedure outlined for HeLa cells (22) to ensure the separation of polα and polε. The separation was verified using a monoclonal anti-polα antibody (SJK 132). As shown in Fig. 2A, the antibody neutralized polα activity (fractions 2–20) but not polε activity (fractions 51–60). To confirm the presence of polε, fractions 51–60 were collected and immunoblotted against an anti-polε monoclonal antibody (3C5.1). As shown in Fig. 2B, our purified human polε has a molecular mass of ~250 kDa, which is consistent with previously published observations (21, 22).

Inhibitory Potency of PMEGpp toward Pol δ and Pol ε—To kinetically characterize the competition between PMEGpp and dGTP, an oligonucleotide template-primer (TP-1) containing 13 incorporation sites for dGMP was used. Kinetic analysis utilizing Lineweaver-Burk plots confirmed that PMEGpp was a competitive inhibitor of both polδ and polε with respect to dGTP (plots are not shown). The apparent Ki values of 1.36 ± 1.60 nM were calculated for polδ and polε, respectively. The inhibitory potency, expressed as a Ki(PMEGpp)/Km(dGTP) ratio (Table I) indicated that the affinity of both enzymes was approximately 3–4 times higher for the analog than for dGTP. These results indicated that PMEGpp was a strong competitive inhibitor of both polδ and polε.

Incorporation of PMEGpp into DNA in Vitro—A primer extension assay utilizing an oligonucleotide template-primer (TP-2) was used to determine the ability of polδ and polε to incorporate the analog into DNA. The Km and Vmax for PMEGpp and dGTP were obtained from Lineweaver-Burk plots as shown in Fig. 3, A (polδ) and B (polε). In agreement with the affinity data derived from the Ki studies, both polδ and polε had 3–4 times lower Km values for PMEGpp as compared with dGTP. However, this was combined with proportionally lower Vmax values for PMEGpp compared with dGTP.

**Table II**

|        | PMEGpp | dGTP |
|--------|--------|------|
|        | Vmax   | Km   | Vmax   | Km   |
|        | pmol/min/mg | nM   | pmol/min/mg | nM   |
| polδ   | 1.94 ± 0.25 | 75 ± 16 | 5.88 ± 0.94 | 282 ± 40 |
| polε   | 0.054 ± 0.006 | 56 ± 12 | 0.169 ± 0.013 | 140 ± 16 |

**Fig. 4.** The excision of PMEG incorporated at matched (A) or mismatched (B) position by polε. Polε (0.07 units) was incubated for the indicated times in a 15-μl reaction mixture containing buffer ε and 0.02 μM 32P-labeled matched or mismatched template-primer. When added, dNTPs were at 10 μM concentration. Reaction products were separated by 20% denaturing PAGE, and the gels were autoradiographed. The radioactivity was quantitated using a PhosphorImager (Molecular Dynamics). As a control, the extent of DNA synthesis without the removal of the 3’-terminal mismatched nucleotide was determined in the absence of dATP (the only position for dAMP incorporation in the mismatched TP is the one replacing the terminal G-T mismatch). These values were then subtracted from the values generated using all four dNTPs.
values for the analog (Table II). When the incorporation efficiency of PMEGpp toward dGTP ($f_{inc} = (V_{max}/K_m)_{PMEGpp}/(V_{max}/K_m)_{dT}$) was calculated, the resulting $f_{inc}$ values were close to 1 for both enzymes (1.2 and 0.8 for pol δ and pol ε, respectively). This result would suggest that the analog and the natural substrate have equal incorporation efficiencies only if there was not a substantial difference in their excision following incorporation under “standing start” reaction conditions.

**Excision and Repair of the Incorporated PMEG**—To examine the ability of the DNA polymerases to excise and repair the incorporated PMEG, template-primers were generated with PMEG incorporated in matched (with dC) or mismatched (with dT) positions. The results were compared with those generated with dGMP-terminated primers. To quantify the difference, the products of the DNA polymerase reactions were scanned and the activity of pol δ and pol ε on templates with dGMP-terminated primers was taken as 100%. As shown in Fig. 4, A and B, and Table III, pol ε not only excised the PMEG from PMEG-terminated primers (lanes -dNTPs, 23 and 68% of the control dGMP matched and mismatched primers, respectively) but was able to use the PMEG-terminated oligonucleotide as a primer for elongation in the presence of dNTPs (27 and 83% of the control dGMP matched and mismatched primers, respectively). Because PMEG lacks a 3’-OH group, the elongation was possible only following the cleavage of PMEG from the 3’ terminus. The observation that the mismatched PMEG-terminated primer was elongated more efficiently than the matched one is consistent with the known preference of the 3’-5’-exonuclease activity of pol ε for mismatched ends of DNA (28). In contrast to pol ε, pol δ excised and elongated the PMEG-terminated primers poorly (Table III). Pol δ excised matched PMEG-terminated primers with 9% of the efficiency compared with the control. In the presence of dNTPs, the efficiency decreased to less than 1%, compared with the control. This result indicated that the 3’-5’-exonuclease activity of pol δ, in contrast to pol ε, was inhibited by the presence of dNTPs in the reaction mixture. This inhibition, however, was not found for the mismatched PMEG-terminated primer, in which 4% of the efficiency compared with the control was observed both in the exonuclease and polymerase reactions. These results demonstrated that pol ε, but not pol δ, has the ability to repair PMEG from the 3’ terminus by cooperation of 3’-5’-exonuclease and DNA polymerase activities.

**DISCUSSION**

This study demonstrated the strong inhibitory potential of PMEG diphosphate toward DNA polymerase δ and ε. PMEGpp was found to be a competitive inhibitor of pol δ and ε with respect to dGTP incorporation. The apparent $K_i$ values for the analog (13 and 12 nM for pol δ and pol ε, respectively) were 3–4 times lower than the $K_m$ values for the natural substrate. In addition, both enzymes can recognize PMEGpp as a substrate and incorporate PMEG into DNA. Due to the lack of a 3’-OH moiety, the nucleotide analog functions as an absolute DNA chain terminator. The prevention of DNA chain elongation as well as the competition between PMEGpp and dGTP for the nucleotide-binding site of the polymerases are probably the main mechanisms of PMEGpp inhibition.

The inhibition of DNA polymerases *in vivo* depends on the intracellular concentration ratio of PMEGpp to dGTP. The size of the dGTP pool, which is usually the smallest of the dNTP pools (29), differs considerably among cell lines, ranging between 3 and 50 μM (30, 31). The level of PMEGpp will also differ among cell lines depending on the transport and phosphorylation efficiency of the analog. Following the incubation of Vero cells with PMEG, intracellular PMEGpp was found in micromolar concentrations (6). Due to the higher affinity of both pol δ and pol ε for PMEGpp than for dGTP, DNA replication may be significantly slowed down not only in cells with equimolar PMEGpp and dGTP ratios but also in cells containing an excess of the natural substrate. The analog may also successfully compete with dGTP during DNA repair synthesis, because both pol δ and pol ε also participate in nucleotide excision repair (17).

PMEG molecules incorporated into DNA may be repaired by the cooperation of the 3’-5’-exonuclease and the DNA polymerase activities of pol δ and/or pol ε. We determined that pol ε, but not pol δ, was able to repair PMEG incorporated into the 3’ terminus of DNA. Pol ε may recognize incorporated PMEG and remove it prior to cells undergoing mitosis as a part of its checkpoint role in S phase (19) or during homologous recombination (32). In contrast to PMEG, dFdCMP, and F-ara-AMP, the active forms of anticancer drugs gemcitabine and fludarabine were not effectively excised by pol ε from the 3’ terminus of DNA (33, 34). The pol ε-catalyzed reduction in the number of PMEG molecules in DNA may have certain limits depending on the extent of DNA damage and on the intracellular concentration ratio of PMEGpp to dGTP. Therefore, the significance of our observation *in vivo* has to be determined. Pol δ was unable to repair PMEG efficiently even on the template-primer terminated by PMEG in the mismatched position. This may be due either to the decreased ability of pol δ to recognize PMEG-terminated primers or to cleave the phosphodiester bond between PMEG and the adjacent nucleotide.

The mechanism responsible for the cytotoxic activity of nucleoside analogs such as gemcitabine (2’,2’-difluorodoxycytidine, Ref. 33), fludarabine (9-β-D-arabinofuranosyl-2-fluoroadenine, Ref. 35), cladribine (2-chlorodeoxycytosine, Ref. 36), CI-F-ara-A (2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine, Ref. 37), and dFdG (2’,2’-difluorodeoxyguanosine, Ref. 30) is believed to be their incorporation into DNA. In addition, these nucleoside analogs inhibit ribonucleotide reductase activity resulting in the imbalance of the cellular deoxynucleotide pools. Currently, studies are underway to determine whether PMEG inhibits ribonucleotide reductase and other enzymes participating in the *de novo* and salvage synthesis of nucleotides.

### Table III

| Matched | Mismatched |
|---------|------------|
|         |            |
| dG-dC   |            |
| pol δ   | 11.7 ± 3.7 | 3.1 ± 0.5 | 0.9 ± 0.2 | 8.7 ± 0.9 |
| pol ε   | 5.9 ± 1.6  | 8.9 ± 2.7 | 27.0 ± 4  | 22.5 ± 3.0 |
| dG-dT   |            |
| pol δ   | 3.3 ± 1.0  | 5.4 ± 1.2 | 3.6 ± 1.8 | 4.3 ± 0.6 |
| pol ε   | 5.8 ± 1.4  | 9.3 ± 3.0 | 82.9 ± 5.2| 67.9 ± 8.9 |
REFERENCES

1. De Clercq, E. (1991) *Biochem. Pharmacol.* **42**, 963–972
2. Robbins, B. L., Connelly, M. C., Marshall, D. R., Srinivas, R. N., and Fridland, A. (1985) *Mol. Pharmacol.* **47**, 391–397
3. Paborsky, L. R., Mao, C. T., and Toole, J. J. (1997) *Proc. Am. Assoc. Cancer Res.* **38**, 100
4. Rose, W. C., Crosswell, A. R., Bronson, J. J., and Martin, J. C. (1995) *Mol. Pharmacol.* **47**, 391–397
5. Krieder, J. W., Balogh, K., Olson, R. O., and Martin, J. C. (1990) *Antiviral Res.* **14**, 51–58
6. Ho, H.-T., Woods, K. L., Konrad, S. K., DeBoeck, H., and Hitchcock, M. J. M. (1992) *Innovations in Antiviral Development and the Detection of Virus Infection*, pp. 159–166, Plenum Publishing Corp., New York
7. Kramata, P., Votruba, I., Otová, B., and Holy, A. (1996) *Mol. Pharmacol.* **49**, 1005–1011
8. Chihlar, T., and Chen, M. S. (1997) *Antiviral Chem. Chemother.* **8**, 187–195
9. Pisarev, V. M., Lee, S.-H., Connelly, M. C., and Fridland, A. (1997) *Mol. Pharmacol.* **52**, 63–68
10. Bambara, R. A., Murante, R. S., and Henricksen, L. A. (1997) *J. Biol. Chem.* **272**, 4647–4650
11. Waga, S., and Stillman, B. (1994) *Nature* **369**, 207–212
12. Zlotkin, T., Kaufmann, G., Jiang, Y., Lee, M. Y. W. T., Uitto, L., Syvaoja, J., Donreiter, I., Fanning, E., and Nethanel, T. (1996) *EMBO J.* **15**, 2298–2305
13. Podust, V. N., and Hubscher, U. (1993) *Nucleic Acids Res.* **21**, 841–846
14. Kunkel, T. (1990) *BioEssays* **14**, 303–308
15. Huang, P., Chubb, S., Hertel, L. W., and Plunkett, W. (1995) *J. Biol. Chem.* **269**, 13748–13751
16. Nishida, C., Reinhard, P., and Linn, S. (1989) *J. Biol. Chem.* **264**, 2489–2497
17. Sancar, A. (1995) *Annu. Rev. Genet.* **29**, 69–105
18. Longley, M. J., Pierce, A. J., and Modrich, P. (1997) *J. Biol. Chem.* **272**, 10917–10921
19. Navas, T. A., Zhou, Z., and Elledge, S. J. (1995) *Cell* **80**, 29–39
20. Holy, A., and Rosenberg, I. (1987) *Collect. Czech. Chem. Commun.* **52**, 2801–2809
21. Chui, G., and Linn, S. (1995) *J. Biol. Chem.* **270**, 7799–7808
22. Syvaoja, J., and Linn, S. (1989) *J. Biol. Chem.* **264**, 2489–2497
23. Mozzherin, D. J., and Fisher, P. A. (1998) *Biochemistry* **37**, 3572–3577
24. Ng, L., Tan, C.-K., Downey, K. M., and Fisher, P. A. (1991) *J. Biol. Chem.* **266**, 11699–11704
25. Tan, C.-K., Castillo, C., So, A. G., and Downey, K. M. (1996) *J. Biol. Chem.* **271**, 12310–12316
26. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
27. Petrakova, J., Goodman, M. F., Boosalis, M. S., Sowers, L. C., Cheong, C., and Tinoco, I. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 6252–6256
28. Sabatino, R. D., Myers, T. W., and Bambara, R. A. (1990) *Cancer Res.* **50**, 5340–5344
29. Reichard, P. (1988) *Annu. Rev. Biochem.* **57**, 349–374
30. Gandhi, V., Mineishi, S., Huang, P., Chapman, A. J., Yang, Y., Chen, F., Nowak, R. C., Chubb, S., Hertel, L. W., and Plunkett, W. (1995) *Cancer Res.* **55**, 1517–1524
31. Heinemann, V., Xu, Y.-Z., Chubb, S., Sen, A., Hertel, L. W., Grindey, G. B., and Plunkett, W. (1990) *Mol. Pharmacol.* **38**, 567–572
32. Jessberger, R., Podust, V., Hubscher, U., and Berg, P. (1993) *J. Biol. Chem.* **268**, 15070–15079
33. Huang, P., Chubb, S., Hertel, L. W., Grindey, G. B., and Plunkett, W. (1990) *Mol. Pharmacol.* **38**, 567–572
34. Kunkel, T., 1990 *BioEssays* **14**, 303–308
35. Zlotkin, T., Kaufmann, G., Jiang, Y., Lee, M. Y. W. T., Uitto, L., Syvaoja, J., Donreiter, I., Fanning, E., and Nethanel, T. (1996) *EMBO J.* **15**, 2298–2305
36. Podust, V. N., and Hubscher, U. (1993) *Nucleic Acids Res.* **21**, 841–846
37. Xie, K. C., and Plunkett, W. (1996) *Cancer Res.* **56**, 3030–3037