Eukaryotic cells encode AMP-lysine (AMP-N-ε-(N-α-acyethyl lysine methyl ester) 5'-phosphoramidate) hydrolases related to the rabbit histidine triad nucleotide-binding protein 1 (Hint1) sequence. Bacterial and archaeal cells have Hint homologs annotated in a variety of ways, but the enzymes have not been characterized, nor have phenotypes been described due to loss of enzymatic activity. We developed a quantitative 31P NMR assay to determine whether *Escherichia coli* possesses an adenosine phosphoramidase activity. Indeed, soluble lysates prepared from wild-type laboratory *E. coli* exhibited activity on the model substrate adenosine 5'-monophosphoramidate (AMP-NH$_2$). The *E. coli* Hint homolog, which had been comprehensively designated ycfF and is here named hinT, was cloned, overexpressed, purified, and characterized with respect to purine nucleoside phosphoramidate substrates. Bacterial hinT was several times more active than human or rabbit Hint1 on five model substrates. In addition, bacterial and mammalian enzymes preferred guanosine versus adenosine phosphoramidates as substrates. Analysis of the lysates from a constructed hinT knockout strain of *E. coli* demonstrated that all of the cellular purine nucleoside phosphoramidase activity is due to hinT. Physiological analysis of this mutant revealed that the loss of hinT results in failure to grow in media containing 0.75 M KCl, 0.9 M NaCl, 0.5 M NaOAc, or 10 mM MnCl$_2$. Thus, cation-resistant bacterial cell growth may be dependent on the hydrolysis of adenylylated and/or guanylylated phosphoramidate substrates by hinT.
tides (21–25). Typically, therapeutic nucleosides must be converted to the corresponding mono-, di-, and triphosphates before exhibiting biological activity. Nevertheless, many nucleosides are poor substrates for endogenous nucleoside kinases. To overcome this hurdle, several pronucleotide approaches have been investigated, including the use of nucleoside monophosphoramidates (24, 26).

Although the enzyme responsible for phosphoramidate hydrolysis has not been determined, direct evidence of intracellular P-N bond hydrolysis by a putative phosphoramidase has been demonstrated by studies of the intracellular metabolism of fluorodeoxyuridine phosphoramidates with permeabilized cells (17) and 19F-labeled 3′-azido-3′-deoxythymidine triptophan methyl ester phosphoramidate with capillary liquid chromatograph negative mode electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) (25). Recently, Bieganowski et al. (3) reported that both S. cerevisiae Hnt1 and rabbit Hnt1 hydrolyze AMP-NH2, AMP-lysin, AMP-Mor, and AMP-ala-nine and suggested that Hnt hydrolyses might be the enzymes responsible for nucleoside monophosphoramidate prodrug activation. Targeting the specific pronucleotides of potential therapeutic utility.

In this study, we developed a simple 31P NMR assay capable of detecting total phosphoramidase activity in cell lysates. We scanned the Escherichia coli genome (27) for a Hnt homolog and discovered an open reading frame designated ycfF that is responsible for observed cellular phosphoramidase activity, we cloned, purified, and characterized the activity of the Hnt in vitro and also constructed a mutant strain in which the hinT gene was disrupted. Supporting the view that the hinT gene is entirely responsible for nucleoside monophosphoramidase hydrolysis, these substrates were completely stable in extracts of E. coli hinT knock-out mutants. Further characterization demonstrated that bacterial Hnt is homodimeric and capable of hydrolyzing adenosine and guanosine 5′-phosphoramidate monoesters significantly faster than mammalian Hnt (3). In addition, evaluation of an E. coli hinT knock-out mutant established a requirement for expression when grown on NaCl, KCl, MnCl2, and NaOAc.

EXPERIMENTAL PROCEDURES

Evaluation of Phosphoramidase Activity in E. coli Cell Lysates—AMP-NH2 was used to investigate the phosphoramidase activity in E. coli cell lysates because it was earlier shown to be a specific substrate for rabbit and yeast Hint hydrodases (3). Phosphoramidase activity in cell lysates was measured by the 31P-decoupling mode of 31P NMR spectroscopy (Varian VAC-300 spectrometer), which can clearly distinguish the substrate, AMP-NH2, and the product, AMP. NMR spectrometry is suitable for quantitation by integration of the area under the peak, which is proportional to the amount of nuclei present. The relative ratio of peak areas can be used to calculate the amount of turnover based on a standard curve (supplemental data Fig. 1). Chemical shift information is described in Fig. 1 of the supplemental data. Cell lysates from E. coli Tuner(DE3)pLacI cells (Novagen) were obtained by treatment with lysozyme (1 mg/ml), and the DNA was precipitated by pronase digestion followed by centrifugation to remove cell debris. The supernatant was dialyzed extensively against Buffer A (20 mM Tris-HCl, pH 7.0, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 10% glycerol, and 0.5% Triton X-100) and the DNA was precipitated by protein precipitation. The product of this reaction was cloned into Bluescript SK+ using EcoRI and BamHI sites included in the sequences of the primers. The resulting plasmid was named pH429. Mutagenesis (29) of phagemid DNA with primer 7330 resulted in plasmid pH431 containing hinT with an H101A substitution. Plasmids pH432 and pH433 were obtained by cloning HindIII-EcoRI fragments containing wild-type and H101A mutant forms of hinT from plasmids pH429 and phosphoramidase PACY184. The expression construct pH70D contains the E. coli dihydrofolate reductase (DHFR) gene followed by a thrombin cleavage site and the protein of interest (30). The fusion proteins are purified with a methotrexate affinity chromatography followed by cleavage with human thrombin to release the native protein. Plasmids were constructed by replacing the NAT2 (hamster polymorphic N-acetyltansferase 2) cDNA in pPH70D with the desired open reading frame. After PCR amplification from the chromosomal DNA of the E. coli K12 strain, EMG2, with the primers 101 and 102 containing XhoI and XbaI sites, the product was cloned into the T/A cloning vector, pSTBlue 1 (Novagen). Double digestion of pTFCY-TA followed by subcloning into pH70D resulted in the plasmid pTFCF15DmY. The plasmid pJLCF15DmY, containing human HINT cDNA was obtained by a similar procedure after PCR amplification with primers 101 and 104 from cDNA prepared from the human T-lymphoblast leukemia cell line, CEM. E. coli hinT-pSAG02 was subjected to site-directed mutagenesis using primer 105 and 106 to create E. coli hinT-H101A mutant.

Expression and Purification of Recombinant Proteins—The cell growth and cell lysate extraction were described previously except that 0.5 mM isopropyl-β-D-thiogalactopyranoside was used for induction (30). Blotting was carried out using human Hint-DHFR fusion protein purified with a methotrexate-agarose (Sigma) using a 12.5-mg column, washed with 40 column volumes of buffer A, 60 column volumes of buffer A with 1 M NaCl, and followed by folate elution with 5 mM folate, 32 mM Tris, pH 9.0, 1 mM EDTA, 1 mM DTT, and 0.01 mM phenylmethylsulfonyl fluoride. Fractions (8 ml) were collected at a flow rate of 3 ml/min, and an aliquot (10 μl) was assayed for protein with the Bradford dye reagent (Bio-Rad). Fractions containing more than 0.1 mg/ml protein were analyzed by 12% SDS-PAGE, and DHFR activities were determined (31, 32). The standard DHFR assay mixture contained 50 μM DHF, 100 mM NADPH, and 1 mM DTT in the assay buffer (50 mM morpholinethanesulfonic acid, 25 mM tris(hydroxymethyl)aminomethane, 25 mM ethanolamine, and 100 mM NaCl, pH 7.0) and the enzyme in a final volume of 1.0 ml. The reaction was started by the addition of DHF (32). Fractions containing pure Hint-DHFR fusion proteins were pooled and concentrated to 2 mg/ml, and the buffer was exchanged with 50 mM Tris, pH 8, 100 mM NaCl, and 2.5 mM CaCl2. The fusion protein was digested at 4 °C for 19 h with 8 units of human thrombin (Sigma/mg of protein). To separate Hint hydrodases from DHFR, reaction mixtures were applied onto a 10-ml AMP-agarose column (Sigma) and washed with 16 column volumes of buffer A with 1 mM NaCl and 4 column volumes of buffer A and then eluted with adenosine buffer (2 mM adenosine, 20 mM Tris, pH 7.0, 1 mM EDTA; 1 mM DTT). Fractions (8 ml) were collected at a flow rate of 3 ml/min, and aliquots (10 μl) from each fraction were used to determine the protein concentration. Fractions containing more than 0.1 mg/ml protein were pooled and concentrated to 2.6 mg/ml. Rabbit Hint1 and E. coli hinT-H101A were purified by AMP-agarose affinity chromatography as described previously (3, 33) with the exception that expression was carried out in the E. coli hinT disruption mutant (BB1) described in this study.

Substrate Specificity—AMP-NH2, AMP-Mor, and GMP-Mor (10 mM) were incubated with human Hint1-DHFR, E. coli hint-DHFR, human
HinT1, rabbit HinT1, and E. coli hinT (2.5 or 10 μg) in the reaction buffer (500 μl, 0.5 mM MgCl₂, 20 mM HEPES, pH 7.2) at 22 °C for intervals of 36 and 66 min. To determine the residual phosphoramidase activity in E. coli hinT-H101A, AMP-NH₂ (10 mM) was incubated with E. coli hinT-H101A (1200 and 2700 μg) in the reaction buffer at 22 °C for 14 h. Reactions were quenched by adding 5 μl NaOH (20 μl) followed by snap-freezing in a dry ice/acetone slush bath and then lyophilized. AMP-lysine and GMP-lysine (2.5 mM) were added to the flask containing AMP (0.15 g, 0.42 mmol), 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide HCl (0.24 g, 1.25 mmol), and H₂O (15 ml, pH 7.0 adjusted with triethylamine). The product was then removed under reduced pressure. The residue was dissolved in a mixture of chloroform/methanol/water (5:3:0.5, v/v) with 0.5% ammonium hydroxide and subjected to chromatography on a silica gel column. The column was eluted first with the same solvent mixture and followed by increasing the polarity to a solvent mixture of chloroform/methanol/water (5:4:0.5, v/v) with 0.5% ammonium hydroxide. The product was isolated as a white solid (90 mg, yield 39%). ¹H NMR (D₂O): δ 8.53 (1H, s), 8.05 (1H, s), 5.95 (1H, d), 4.36 (1H, m), 4.20 (1H, m), 3.94 (1H, m), 3.84 (2H, m), 3.51 (3H, s), 2.45 (2H, m), 1.82 (3H, s), 1.40–0.85 (6H, m). ³¹P NMR (D₂O): δ 10.37. ESI-MS m/z [M+Na]+ 532.2 and [M-H]− 530.1.

To synthesize AMP-lysine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (0.15 g, 0.78 mmol) was added to the flask containing GMP-N-((N-acetyl lysine methyl ester) 5'-phosphoramidates were prepared in a single-step condensation reaction employing the carbodiimide-mediated coupling method of monophosphate to amine with minor modification (34). To synthesize AMP-lysine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (0.24 g, 1.25 mmol) was added to the flask containing AMP (0.15 g, 0.42 mmol), N-acetyl-lysine methyl ester (0.2 g, 0.84 mmol), and H₂O (15 ml, pH 7.0, adjusted with triethylamine). The reaction mixture was stirred and heated to 60 °C for 7 h. After cooling to room temperature, water was then removed under reduced pressure. The residue was dissolved in a mixture of chloroform/methanol/water (5:3:0.5, v/v) with 0.5% ammonium hydroxide and subjected to chromatography on a silica gel column. The column was eluted first with the same solvent mixture and followed by increasing the polarity to a solvent mixture of chloroform/methanol/water (5:4:0.5, v/v) with 0.5% ammonium hydroxide. The product was isolated as a white solid (90 mg, yield 39%). ¹H NMR (D₂O): δ 8.53 (1H, s), 8.05 (1H, s), 5.95 (1H, d), 4.36 (1H, m), 4.20 (1H, m), 3.94 (1H, m), 3.84 (2H, m), 3.51 (3H, s), 2.45 (2H, m), 1.82 (3H, s), 1.40–0.85 (6H, m). ³¹P NMR (D₂O): δ 10.37. ESI-MS m/z [M+Na]+ 532.2 and [M-H]− 530.1.

RESULTS

E. coli Cell Lysates Possess Adenosine Phosphoramidase Activity—AMP-NH₂ hydrolysis by E. coli cell lysates (pH 7.2, 37 °C) was observable by ³¹P NMR with a rate of 76.3 (± 20.9) nmol mg⁻¹ min⁻¹ over the course of 30 min (Fig. 1, A and B). Partial conversion of the hydrolysis product, AMP, to Pi, presumably by E. coli phosphatases, could be detected after incubation at 22 °C for 12 h (Fig. 1C). To verify that the formation of AMP was due to an enzymatic process and not chemical hydrolysis, AMP-NH₂ was incubated for 12 h under acidic conditions (pH 2.0, 37 °C) (Fig. 1D). The pH 2.0 test conditions were chosen due to the reported acid lability of the phosphorous-nitrogen bond (35). AMP-NH₂ and TMP signals were visible by ³¹P NMR, with no detectable AMP or Pi, confirming that AMP-NH₂ is stable at pH 2.0 for at least 12 h. "hinT is the Only Bacterial Source of Adenosine Phosphoramidase Activity"—To determine whether hinT is solely responsible for the observed cellular phosphoramidase activity, a mutant strain, BB1, in which the hinT gene was replaced with the chloramphenicol resistance marker, was constructed. The phosphoramidase activities of the wild-type E. coli BL21 Star strain and the mutant strain lysates were determined (supplemental data, Fig. 3). When using AMP-NH₂ as a substrate, the specific activity of BL21 Star wild-type cell lysates was found to be 20.1 (± 7.7) nmol mg⁻¹ min⁻¹ (pH 7.2, 22 °C). However, no AMP formation could be observed with lysates derived from the hinT disruption strain, BB1 (supplemental data, Fig. 3 and Table II). Therefore, we conclude that hinT is fully responsible for the soluble adenosine phosphoramidase activity of E. coli.
Nucleoside and Leaving Group Specificity of Bacterial and Mammalian Hint Phosphoraminidases—Both *E. coli* hinT and human Hint1 were purified to homogeneity as DHFR fusion proteins by methotrexate affinity chromatography (see supplemental data, Fig. 4, lanes 1, 2, and 6). Removal of the DHFR affinity handle by thrombin digestion (supplemental data, Fig. 4, lane 7) and AMP affinity chromatography afforded the purified recombinant enzymes (supplemental data, Fig. 4, lanes 3–5). The apparent molecular sizes of recombinant purified *E. coli* hinT and *E. coli* hinT-DHFR fusion protein were analyzed by gel filtration chromatography on a Superdex G-75 size exclusion column and strongly indicated, as has been observed for rabbit and humanHint1, the formation of a stable homodimer in solution (supplemental data, Fig. 5) (2, 36). Based on our $^{31}$P NMR assay, we found that *E. coli* hinT and human Hint1 are adenosine phosphoraminidases with specific activities of 526 (±27) and 196 (±33) nmol nmol$^{-1}$ min$^{-1}$, respectively. The representative $^{31}$P NMR spectra of *E. coli* hinT incubation with AMP-NH$_2$ are shown in Fig. 3A.

The co-crystal structure of rabbit Hint1 with GMP (2) and the fact that GMP can elute rabbit Hint1 from an adenosine affinity column (33) imply that guanosine phosphoraminidases may also be substrates for Hint hydrolases. To evaluate the substrate specificity with respect to the different purine nucleoside phosphoraminidates, the purified Hint hydrolases were evaluated with AMP-Mor and GMP-Mor (Fig. 2). AMP-Mor has previously been shown to be a reasonable substrate for rabbit Hint1 and yeast Hint1 (3). All three Hint hydrolases were fully capable of utilizing both compounds as substrates with a 2-fold preference for the GMP-Mor. Since AMP-lysine was shown to be a substrate for rabbit Hint1, yeast Hint1, and chicken Hint (3, 5), both AMP-lysine and GMP-lysine were also evaluated as substrates for *E. coli* hinT and human Hint1. As observed for the morpholino compounds, GMP-lysine was approximately a 2-fold better substrate than AMP-lysine for both enzymes (4). Hydrolysis rates for the three Hint hydrolases with each substrate are summarized in Table I.

**TABLE I**

| Substrate | *E. coli* hinT-DHFR | Human Hint1-DHFR | *E. coli* hinT | Human Hint1 | Rabbit Hint1 |
|-----------|---------------------|------------------|---------------|-------------|--------------|
| AMP-NH$_2$ | 753 ± 2             | 388 ± 45         | 526 ± 27      | 196 ± 33    | 70.0 ± 1.3   |
| AMP-Mor   | 411 ± 4             | 43.4 ± 4.0       | 360 ± 1       | 45.0 ± 0.1  | 26.6 ± 0.6   |
| GMP-Mor   | 675 ± 17            | 87.2 ± 11.8      | 669 ± 1       | 78.7 ± 1.3  | 48.6 ± 0.4   |
| AMP-lysine| ND                  | ND               | 529 ± 7       | 102 ± 19    | ND           |
| GMP-lysine| ND                  | ND               | 636 ± 262     | 292 ± 68    | ND           |

**FIG. 3. Phosphoraminidase activity of the wild-type *E. coli* hinT and Hint10A mutant by $^{31}$P NMR Assay**. A. *E. coli* hinT protein (2.5 µg) incubated with AMP-NH$_2$ (10 mM) at 22 °C for 36 and 60 min; B. *E. coli* hinT-H101A, 1200 and 2700 µg were incubated with AMP-NH$_2$ (10 mM) at 22 °C for 14 h.

**DISCUSSION**

Nucleoside phosphoraminidases have been exploited for the delivery of nucleoside monophosphate antiviral and anticancer agents. Extensive analysis of their metabolism by cell extracts and whole cells has provided supporting evidence that the hydrolysis of the P-N bond necessary for subsequent phosphoraminidations is enzyme-mediated. Recently, yeast Hint1, rabbit Hint1, and chicken Hint have been shown to be AMP-lysine hydrolases, suggesting that this class of enzymes may be responsible for nucleoside phosphoraminidase activities (3–5). A test of this hypothesis required development of an assay that could measure total cellular nucleoside monophosphoraminidate hydrolase activity and a genetic system to knock out candidate genes. Here we accomplished this in *E. coli*.

The $^{31}$P NMR assay requires little sample preparation and is capable of accurately determining the concentration of substrates and products because the chemical shifts of the phosphoraminidate and the monophosphate are generally separated by 4–6 ppm. In addition, we have demonstrated that this assay is independent of the structure of either the nucleoside or the amine that compose the nucleoside phosphoraminidate.

Applying our assay to the investigation of the phosphoraminidase hydrolase of bacteria cells, we found that AMP-NH$_2$ was rapidly hydrolyzed by *E. coli* cell extracts. Purified recombinant *E. coli* hinT and human Hint1 were shown to be adenosine/guanosine phosphoraminidases. In addition, rabbit Hint1, known to be an adenosine phosphoraminidase, was shown to be a guanosine phosphoraminidase as well. We can also conclude, based on gene replacement in the BB1 cell lysate studies, that hinT is the only purine phosphoraminidase expressed by *E. coli*. Conversion of the specific activity of the purified *E. coli* hinT (526 nmol/nmol/min) to units used for cellular lysates (39,000
studies with transgenic mice, in which the Cdk7 was observed in a yeast-two hybrid assay (38). However, lated experiments. An interaction between human Hint1 and controversial, with a mixture of conflicting evidence and iso-

phenotype. Given the wide distribution of orthologous genes in bacteria, the expression of these proteins may be a general requirement for cation-resistant bacterial cell growth.

A possible unique role for Hint proteins has been proposed in regulating the nucleotidylation of protein substrates (1, 3). The nucleotidylation post-translational modification has been observed for the regulation of the glutamine synthetase activity by two enzymes: uridylyl transferase and adenylyl transferase (39). Our data also indicate that both AMP-lysine and GMP-lysine are excellent substrates for E. coli HintT and human Hint1 (Table I). Based on these observations, it is likely that Hint proteins maybe involved in reversing either protein adenylylation and/or protein guanylylation. Ongoing studies should define the natural substrates and physiological role of Hint proteins and their relationship to cation resistance. In addition, the potential differences in substrate specificity observed between the bacterial and mammalian enzymes suggest that the design of bacteria-specific pronucleotides may be achievable.

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REFERENCES

1. Brenner, C. (2002) Biochemistry 41, 9003–9014
2. Brenner, C., Garrison, P., Gilmour, J., Peisach, D., Ringo, D., Petsko, G. A., and Lowenstein, J. M. (1997) Nat. Struct. Biol. 4, 231–238
3. Bieganowski, P., Garrison, P. N., Hodawadekar, S. C., Faye, G., Barnes, L. D., and Brenner, C. (2002) J. Biol. Chem. 277, 10852–10860
4. Krakowiak, A., Pace, H. C., Blackburn, G. M., Adams, M., Mekhalfia, A., Kaczmarek, R., Baraniak, J., Stec, W. J., and Brenner, C. (2004) J. Biol. Chem. 279, 18711–18716
5. Parks, K. P., Siddle, H., Wright, N., Sperry, J. B., Bieganowski, P., Howitz, K., Wright, D. L., and Brenner, C. (2004) Physiol. Genomics 20, 12–14
6. Pace, H. C., and Brenner, C. (2003) Genome Biol. 4, R18
7. Korissiaari, N., Rossi, D. J., Laxiko, K., Huebner, K., Henkmeyer, M., and Makela, T. P. (2003) Mol. Cell. Biol. 23, 3929–3935
8. Su, T., Suzuki, M., Wang, L., Lin, C. S., Xing, W. Q., and Weinstein, I. B. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7824–7829
9. Date, H., Onodera, O., Tanaka, H., Iwabuchi, K., Uekawa, K., Igarashi, S.,
Koike, R., Hiroi, T., Yusa, T., Awaya, Y., Sakai, T., Takahashi, T., Nagatomo, H., Sekijima, Y., Kawachi, I., Takiyama, Y., Nishizawa, M., Fuku- hara, N., Saito, K., Sugano, S., and Tsuji, S. (2001) Nat. Genet. 29, 184–188

10. Moreira, M. C., Barbot, C., Tachi, N., Kozuka, N., Uchida, E., Gibson, T., Mendonca, P., Costa, M., Barros, J., Yanagisawa, T., Watanabe, M., Ikeda, Y., Aoki, M., Nagata, T., Coutinho, F., Sequeiros, J., and Koenig, M. (2001) Nat. Genet. 29, 189–193

11. Liu, H., Rodgers, N. D., Jiao, X., and Kiledjian, M. (2002) EMBO J. 21, 4699–4708

12. Salehi, Z., Geffers, L., Vilela, C., Birkenhager, R., Ptushkina, M., Berthelot, K., Ferro, M., Gaskell, S., Hagan, I., Stapley, B., and McCarthy, J. E. (2002) Mol. Microbiol. 46, 49–62

13. Kwasnicka, D. A., Krakowiak, A., Thacker, C., Brenner, C., and Vincent, S. R. (2003) J. Biol. Chem. 278, 39051–39058

14. Trapasso, F., Krakowiak, A., Cesari, R., Arkles, J., Yendamuri, S., Ishii, H., Vecchione, A., Kuroki, T., Bieganowski, P., Pace, H. C., Huebner, K., Croce, C. M., and Brenner, C. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1592–1597

15. Ledneva, R. K., Preobrazhenskaya, N. N., Shinskii, N. G., Shabarova, Z. A., and Prokof’ev, M. A. (1970) Dokl. Akad. Nauk SSSR 193, 1308–1310

16. Dudkin, S. M., Ledneva, R. K., Shabarova, Z. A., and Prokofiev, M. A. (1971) FEBS Lett. 16, 48–50

17. Abraham, T. W., Kalman, T. I., McIntee, E. J., and Wagner, C. R. (1996) J. Med. Chem. 39, 4569–4575

18. McIntee, E. J., Remmel, R. P., Schinazi, R. F., Abraham, T. W., and Wagner, C. R. (1997) J. Med. Chem. 40, 3223–3231

19. Chang, S. L., Griesgraber, G. W., Southern, P. J., and Wagner, C. R. (2001) J. Med. Chem. 44, 223–231

20. Abraham, T. W., McIntee, E. J., Vidhya, V. I., Schinazi, R. F., and Wagner, C. R. (1997) Nucleos. Nucleot. Nucl. 16, 2079–2092

21. McGuigan, C., Kinchington, D., Nicholas, S. R., Nickson, C., and O’Connor, T. J. (1993) Bioorg. Med. Chem. Lett. 3, 1207–1210

22. Balzarini, J., Karlsson, A., Aquaro, S., Perno, C. F., Cahard, D., Naesens, L., De Clercq, E., and McGuigan, C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7295–7299

23. McGuigan, C., Cahard, D., Sheeka, H. M., De Clercq, E., and Balzarini, J. (1996) Bioorg. Med. Chem. Lett. 6, 1183–1186

24. Wagner, C. R., Iyer, V. V., and McIntee, E. J. (2000) Med. Res. Rev. 20, 417–451

25. Kim, J., Chou, T.-I., Griesgraber, G. W., and Wagner, C. R. (2004) Mol. Pharmacol. 1, 102–111

26. Drexl, D. P., and Wagner, C. R. (2004) Mini-reviews Med. Chem. 4, 409–419

27. Brenner, C., Bieganowski, P., Pace, H. C., and Huebner, K. (1999) J. Cell. Physiol. 181, 179–187

28. Datsenko, K. A., and Wanner, B. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6640–6645

29. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382

30. Sticha, K. R., Sieg, C. A., Bergstrom, C. P., Hanna, P. E., and Wagner, C. R. (1997) Protein Expression Purif. 10, 141–153

31. Stone, S. R., and Morrison, J. F. (1982) Biochemistry 21, 3757–3765

32. Morrison, J. F., and Stone, S. R. (1988) Biochemistry 27, 5499–5506

33. Gilmour, J., Liang, N., and Lowenstein, J. M. (1997) Biochem. J. 326, 471–477

34. Moffatt, J. G. a. K., H. G. (1961) J. Am. Chem. Soc. 83, 649–658

35. Song, H., Johns, R., Griesgraber, G. W., Wagner, C. R., and Zimmerman, C. L. (2003) Pharm. Res. (N. Y.) 20, 448–451

36. Lima, C. D., Klein, M. G., Weinstein, I. B., and Hendrickson, W. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5257–5262

37. Busta, S. A., Schoefer, M. R., and Golden, S. S. (1990) J. Bacteriol. 172, 1998–2004

38. Korsisara, N., and Makela, T. P. (2000) J. Biol. Chem. 275, 34837–34840

39. Jaggi, R., van Heerwijk, W. C., Westerhoff, H. V., Ollis, D. L., and Vaeudean, S. G. (1997) EMBO J. 16, 5562–5571