Interactions between *Escherichia coli* Nucleoside-diphosphate Kinase and DNA*

Mikhail N. Levit, Bozena M. Abramczyk, Jeffry B. Stock‡, and Edith H. Postel‡

From the Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544-1014

Nucleoside-diphosphate (NDP) kinase (NTP:nucleoside-diphosphate phosphotransferase) catalyzes the reversible transfer of γ-phosphates from nucleoside triphosphates to nucleoside diphosphates through an invariant histidine residue. It has been reported that the high-energy phosphorylated enzyme intermediate exhibits a protein phosphotransferase activity toward the protein histidine kinases CheA and EnvZ, members of the two-component signal transduction systems in bacteria. Here we demonstrate that the apparent protein phosphotransferase activity of NDP kinase occurs only in the presence of ADP, which can mediate the phosphotransfer from the phospho-NDP kinase to the target enzymes in catalytic amounts (~1 nM). These findings suggest that the protein kinase activity of NDP kinase is probably an artifact attributable to trace amounts of contaminating ADP. Additionally, we show that *Escherichia coli* NDP kinase, like its human homologue NM23-H2/PuF/NDP kinase B, can bind and cleave DNA. Previous *in vitro* functions of *E. coli* NDP kinase in the regulation of gene expression that have been attributed to a protein phosphotransferase activity can be explained in the context of NDP kinase-DNA interactions. The conservation of the DNA binding and DNA cleavage activities between human and bacterial NDP kinases argues strongly for the hypothesis that these activities play an essential role in NDP kinase function *in vivo*.

Nucleoside-diphosphate (NDP) kinase (EC 2.7.4.6) catalyzes the exchange of a γ-phosphate between nucleoside tri- and diphosphates via a ping-pong mechanism with a high-energy phosphohistidine intermediate:

\[
\begin{align*}
\text{NDPK-His} + \text{MgNTP} & \rightarrow \text{NDPK-His} \rightarrow P + \text{MgNDP} \\
\text{NDPK-His} + P + \text{MgN'DP} & \rightarrow \text{NDPK-His} + \text{MgN'TP} \\
\text{MgNTP} + \text{MgN'DP} & \rightarrow \text{MgNDP} + \text{MgN'TP}
\end{align*}
\]

**Reaction scheme**

where MgNDP and MgN'TP are the Mg\(^{2+}\) complexes with nucleoside (or 2'-deoxynucleoside) di- and triphosphates. Histidine phosphorylation by MgNTP is fully reversible with an equilibrium constant of about 0.25, and it is very efficient and has a turnover number > 1000 s\(^{-1}\). Thus, the phosphorylation and dephosphorylation steps take less than 1 ms, whereas the phosphorylated form is stable for a few hours in the absence of a nucleoside diphosphate acceptor (catalytic mechanism is reviewed in Ref. 1). NDP kinases are a large family of proteins found in many organisms with a high level of sequence and structure homology throughout the whole family. The enzyme is composed of four or six identical subunits (16–20 kDa each) with an α/β sandwich or ferredoxin fold (reviewed in Ref. 2). In both crystals and solution, NDP kinases exist in two different quaternary structures: eukaryotic enzymes are hexamers (a trimer of dimers with dihedral D3 symmetry), and some bacterial enzymes are tetramers (a pair of dimers with pyramidal D2 symmetry) (3). Each subunit in the hexamer or tetramer has an independent active center that comprises the nucleophilic histidine and the nucleotide-binding site able to bind both di- and triphosphates. The binding site forms a cleft on the protein surface about 20 Å long, 6 Å wide, and 10 Å deep, with the phosphoaccepting histidine located at the bottom of this cleft. Amino acid compositions of the active sites and their structures are almost identical in the NDP kinases from bacteria to human (3). The nucleoside-diphosphate kinase activity and the broad substrate specificity of the enzyme imply that it might play a central role in nucleotide metabolism by catalyzing the biosynthesis of nucleoside triphosphates from the corresponding diphosphates, with ATP as a phosphoryl donor. Surprisingly, however, it has been found that the *Escherichia coli* ndk gene is not essential and that deletion mutants are capable of normal growth (4). This result is consistent with the notion that the primary function of NM23/NDP kinase is not as a housekeeping nucleoside-diphosphate kinase.

During the last 10 years, NM23/NDP kinases have been implicated in the regulation of tumor metastasis (5), cell differentiation (6), and cell motility (7). In addition, a wide variety of new activities of the enzyme have been discovered, such as DNA binding, transcriptional regulation (8–12), a mutator function (4), and DNA cleavage (13, 14). It has also been reported that the high-energy phosphorylated NDP kinase intermediate exhibits a protein phosphotransferase activity toward a number of important regulatory proteins, leading to the proposal that NDP kinases can participate in phosphorelay networks that regulate gene expression and metabolism. Histidine (15–18), serine/threonine (19), and aspartate (18, 20) have been identified as the phosphoaccepting residues in these proteins.

Among the reported targets of the phosphotransferase activity of NDP kinase were the histidine protein kinases CheA and EnvZ (15), members of the two-component signal transduction systems in bacteria (21). In these systems the activity of the first component, a histidine kinase, is regulated by different environmental factors. The kinases utilize ATP to phosphoryl-
ate their own histidine residue; the phosphoryl group is then transferred to an aspartyl residue in the molecule of the second, response regulator, component. The cytoplasmic histidine protein kinase CheA is the key enzyme in the system controlling bacterial chemotaxis. CheA activity is regulated by transmembrane chemoreceptors. EnvZ is a membrane receptor kinase that serves as an osmosensor in bacteria. It phosphorylates a cognate response regulator, OmpR, which activates porin gene transcription. The other proteins reportedly phosphorylated by NDP kinase were the energy metabolism enzymes succinic thiokinase (17, 18) and ATP-citrate lyase (16, 18). The common feature of all of the reported targets of the phosphotransferase activity of NDP kinase is the presence of a histidine residue in the active center of the enzyme that is phosphorylated by ATP during their normal catalytic cycle in the absence of NDP kinase.

Here we demonstrate that the apparent protein phosphotransferase activity of NDP kinase in two-component systems is an artifact resulting from the presence of catalytic amounts of nucleoside di- or triphosphates in the reaction mixtures, which mediate the phosphotransfer from the phospho-NDP kinase to the target enzymes. Part of the evidence supporting a role for E. coli NDP kinase in EnvZ function was the demonstration that ndk overexpression resulted in the transcriptional activation of genes that are controlled by the histidine kinase osmosensor (15). There is an alternative explanation for this result, however. It has been shown that changes in DNA structure associated with increases in osmotic pressure act cooperatively with OmpR phosphorylation to activate transcription (22). Given the recently established role of human NM23-H2/NDP kinase in DNA structural changes (9, 10, 13, 14) and transcription (8–12), it seemed likely that NDP kinase overproduction in some way mimicked the effect of high osmolarity on DNA to enhance expression of the OmpR-dependent genes. In light of this possibility, we examined whether E. coli NDP kinase, like its human counterpart NM23-H2/NDP kinase B (13, 14), is capable of binding and cleaving DNA.

In addition to being an NDP kinase, NM23-H2 is also a transcriptional activator (8–12) that can form a covalent lysine-phosphodiester bond with DNA and catalyze DNA cleavage, suggesting that it is involved in changing the DNA structure (13, 14). The covalent bond between NM23-H2 and DNA is formed through an evolutionarily conserved lysine residue (Lys22), located in the nucleotide-binding site, which is also required for the NDP kinase activity (14). Whether and how the nuclease and NDP kinase activities might be integrated for in vivo function are not known. Whereas E. coli encodes only one ndpk gene, there are eight orthologous NM23/NDP kinase genes in humans (23). NM23-H2 and the E. coli enzyme are 45% identical and, in addition to the catalytic lysine, share all of the important active site residues for NDP kinase.

Here we show that, like the human enzyme, E. coli NDP kinase can indeed bind and cleave both supercoiled plasmid and duplex DNA oligonucleotides. The conservation of these enzymatic activities and the active site residues between human and E. coli enzymes indicates strongly that the NM23/NDP kinase family of proteins performs similar functions in all organisms that most likely involve both the NDP kinase and the DNA cleavage activities.

MATERIALS AND METHODS

Proteins

CheA was purified as described previously (24). A truncated EnvZ protein (EnvZ115) in which the first 38 amino acids of EnvZ are replaced with 8 amino acids of LacZ (25), EnvZ N342K mutant protein (26), and OmpR fused to the maltose-binding protein (OmpR-MBP) were all gifts from T. Silhavy (Princeton University). Human NM23-H2/NDP kinase B was purified by overexpressing plasmid p19PET3c in BL21 (DE3) E. coli cells, by ammonium sulfate fractionation, and passage through DEAE ion exchange and hydroxyapatite columns, as described previously (8). NDP kinase from E. coli was prepared using the expression vector ndhK (a gift of M. Konrad, Max-Planck-Institute, Goettingen, Germany) and BL21 (DE3) E. coli cells. The enzyme was purified by ammonium sulfate fractionation and passage through DEAE ion exchange column, from which it was eluted with a 0–1 M NaCl gradient (peak fractions were eluted at ~100 mM NaCl). E. coli NDP kinase provided by Ioan Lascu (University of Bordeaux-2) was prepared by Blue-Sepharose affinity chromatography. The purity of all NDP kinase preparations was assessed as >99% by SDS-PAGE analysis.

Protein Phosphorylation Assays

The assays were conducted according to the method of Lu et al. (15). Reactions were started by adding [γ-32P]ATP or [γ-32P]GTP to 20 μl of reaction buffer 1 (50 mM Tris-Cl, pH 8.0, 50 mM KCl, 2 mM dithiothreitol, and 5 mM MgCl2) containing proteins as indicated in the figure legends. After incubation at 30 °C for 30 min, 5 μl of 5× SDS sample buffer (60 mM Tris-Cl, pH 6.8, 25% glycerol, 2% SDS, 7.2 mM 2-mercaptoethanol, and 0.1% bromphenol blue) were added to each reaction mixture, and aliquots were subjected to 15% SDS-PAGE analysis. Gels were dried and autoradiographed. [γ-32P]GTP and [γ-32P]ATP (4500 Ci/mmol, 10 μCi/ml on the reference day) were obtained from ICN.

DNA Cleavage Analyses

Cleavage of Supercoiled Plasmid DNA—Ten-μl reaction mixtures were assembled in reaction buffer 2 (50 mM Tris–HCl, pH 7.9, 100 mM KCl, 1.5 mM MgCl2, 50 μg/ml bovine serum albumin, and 2% (v/v) glycerol) with negatively supercoiled plasmid DNA (pUC19MYC) containing a 57-bp c-MYC NHE sequence as described previously (13, 14), and NM23-H2 or E. coli NDP kinase as indicated in the figure legends. After incubation for 30 min at 30 °C, the reactions were terminated with 2% sarkosyl and 10 mM EDTA and further treated with proteinase K (200 μg/ml) for 1 h at 55 °C before electrophoresis on 1% agarose gels (13).

Cleavage of 32P 5′-End-labeled Duplex RNA Oligodeoxyribonucleotide—Reaction mixtures were assembled in 14 μl of reaction buffer 2 with the indicated amounts of 32P-end-labeled 45-bp duplex NHE oligonucleotide with the sequence 5′-AGTCTCTCCACCCATGCTCCACCCATGCTCCACCCTCCACCCCATAGC-3′ and NM23-H2 or E. coli NDP kinase as indicated in the figure legends. After incubation at 0 °C for 20 min, the samples were analyzed by electron photophoresis mobility shift assay (13, 14).

NDP Kinase Assay

NDP kinase activity was measured with ATP as a phosphate donor and DTDP as an acceptor nucleotide, in a coupled pyruvate kinase lactate dehydrogenase assay for measurements of ADP and AMP at 20°C, as described previously (14). The specific activities of the human NM23-H2 enzyme and E. coli NDP kinase were similar (620–800 units/mg, respectively).

RESULTS

Examination of the Protein Phosphotransferase Activity of E. coli NDP Kinase—To examine whether the phosphoryl group can be transferred directly from NDP kinase to the bacterial protein histidine kinases CheA or EnvZ, we incubated preparations of the purified human NM23-H2 or E. coli NDP kinase with the purified target proteins, CheA and EnvZ, in the presence of [γ-32P]GTP as described by Lu et al. (15). Because GTP can serve as a substrate for autophosphorylation of NDP kinase but not for CheA and EnvZ, appearance of the phosphorylated forms of the latter would be evidence of direct phosphotransfer from the phospho-NDP kinase. Products of the reaction were separated by SDS-PAGE and visualized by autoradiography (Fig. 1). We used a wild type CheA protein (Fig. 1A) and an EnvZ115 mutant (Fig. 1B), a truncated EnvZ protein in which the first 38 amino acids, including the first transmembrane sequence and a part of the extracellular region, are replaced with 8 amino acids of LacZ (25). As a positive control, CheA and EnvZ115 were phosphorylated in the presence of [γ-32P]ATP (Fig. 1A, lane 1 and Fig. 1B, lane 1, respectively). As an additional control for the intactness of the phosphoacceptor-phos-
FIG. 1. Phosphorylation of the histidine kinases CheA (A) and EnvZ (B) in the presence of NDP kinases. Reactions were started by adding [γ-32P]ATP or [γ-32P]GTP to the mixtures composed as indicated in the tables in a final volume of 20 μl of reaction buffer 1. After incubation at 30 °C for 30 min, 5 μl of 5× SDS sample buffer were added to each reaction mixture, and 2-μl (A) or 5-μl (B) aliquots were subjected to 15% SDS-PAGE. Autoradiograms of the dried gels are shown. Reaction mixtures contained the following concentrations of components: A, 1 μM CheA, 3 μM human NM23-H2 (lane 3) or E. coli (lanes 4–11) NDP kinase, 0.14 μCi/μl [γ-32P]ATP (620 Ci/mmol), 0.15 μCi/μl [γ-32P]GTP (2600 Ci/mmol), and ADP as indicated; B, 1 μM EnvZ, 1 μM OmpR-MBP, 3 μM human NM23-H2 (lane 13) or E. coli (lanes 4–8, 11, and 12) NDP kinase, 1.1 μCi/μl [γ-32P]ATP (5000 Ci/mmol), 1.1 μCi/μl [γ-32P]GTP (5000 Ci/mmol), and ADP as indicated. The EnvZ115 (lanes 1–3, 5–8, and 13) or EnvZ N343K mutants (lanes 9–12) were used. The OmpR-MBP protein (lane 2) was added after the mixture containing EnvZ115 and [γ-32P]ATP was preincubated for 15 min, and the incubation was continued for an additional 15 min. CheA, EnvZ, and NDP kinases are expressed in terms of their monomeric concentrations. Molecular masses of the protein species are indicated to the right of the gels. Small fractions of CheA and E. coli NDP kinase proteins migrated as dimers of 146 and 30 kDa, respectively. The presence of these dimers is probably due to insufficient denaturation because the samples were not boiled before SDS-PAGE in order to preserve the phosphorylated state of the polypeptides.

and Fig. 1B, lanes 6–8). Phosphorylation was not observed with EnvZ N342K, a kinase-negative mutant that is inactive (26) (Fig. 1B, lanes 9–12).

Phosphorylation of the protein histidine kinases CheA and EnvZ in the mixtures with NDP kinase and [γ-32P]GTP was not a result of direct phosphotransfer from the phospho-NDP kinase but rather a consequence of generation of [γ-32P]ATP from the added (or contaminating) ADP (Fig. 2). The generated [γ-32P]ATP is used by the protein histidine kinase as its natural substrate in the autophosphorylation reaction, and the ADP that is produced is recycled at the expense of GTP by the nucleoside-diphosphate kinase reaction. Thus, ADP functions as a catalyst (i.e., it is not consumed), and even trace amounts of ADP can mediate the phosphotransfer. The concentration of ADP in the reaction mixture does affect the rate of phosphotransfer (compare lanes 5–11 in Fig. 1A with lanes 6–8 in Fig. 1B), however, so that one’s ability to register the phosphotransfer depends on the sensitivity of the analytical method and reaction parameters (autoradiographic exposure time, incubation condition, and so forth). ADP or ATP in a catalytic concentration that can promote the phosphotransfer cascade may not be easily detected. In our experiments, these concentrations (~1 nM) were approximately 1 order of magnitude lower than the detection limit of the thin layer chromatography assay, which was used by Lu et al. (15) to demonstrate the absence of trace amounts of ADP/ATP in their reaction mixtures. In the experiments where the protein phosphotransferase activity of NDP kinase was reportedly observed, the enzyme was not in catalytic but rather in stoichiometric (micromolar) concentrations comparable with those of the target protein kinases. Concentrations of ADP/ATP in the reaction mixture 3 orders of magnitude lower than the concentration of these nucleotide-binding proteins would be sufficient to promote the phosphotransfer reactions that were observed (15).

DNA Binding and Cleavage Activity of E. coli NDP Kinase—We have recently identified the human homologue of E. coli NDP kinase, NM23-H2, as a nuclease capable of covalently binding and cleaving DNA (13). The active site amino acid residue through which NM23-H2 covalently binds to DNA is an evolutionarily conserved lysine (Lys12). Mutation of Lys12 to Ala or Gln, amino acids that do not have the functional amino group, abolished the covalent DNA binding and cleavage but not the noncovalent DNA binding by NM23-H2 (14). Because Lys12 (Lys11 in E. coli) and other residues in the catalytic pocket of NDP kinase are fully conserved, we hypothesized that E. coli NDP kinase should also be able to bind and cleave DNA. This was confirmed experimentally. E. coli NDP kinase, like its human counterparts (13, 14), can convert negatively supercoiled plasmid DNA to nicked circular and linear cleaved products as a consequence of single-stranded and double-stranded cuts (Fig. 3).

We also compared by electrophoretic mobility shift assay the DNA binding and cleaving activities of the two enzymes using oligonucleotides comprising the NM23-H2 natural target sequence, the c-MYC promoter termed NHE (8). As shown in Fig. 4 with a 45-bp 32P-end-labeled duplex DNA, E. coli NDP kinase was also active toward this substrate, although, as in the case of the plasmid DNA cleavage, its activity was less than that of the human enzyme. The lower activity of the E. coli enzyme may be explained on the basis that the sequence-dependent DNA binding by E. coli NDP kinase and NM23-H2 is different. Indeed, even the DNA sequences recognized by NM23-H1,
NDP Kinase Is Not a Protein Phosphotransferase—NDP kinases were originally proposed to be pivotal as housekeeping enzymes largely responsible for shuttling $\gamma$-phosphoryl groups from ATP to all the various NTPs and deoxynucleotide triphosphates. During the last decade, a large body of evidence has been collected regarding their involvement in the regulation of gene expression during development, tumorigenesis, and tumor metastasis (reviewed in Ref. 9). Additionally, NDP kinases were proposed by Lu et al. (15) to participate in signal transduction by functioning as protein phosphotransferases. These authors reported the possibility of phosphotransfer from E. coli NDP kinase to the active site histidine in the E. coli EnvZ and CheA histidine protein kinases. This observation indicated that NDP kinase could provide a general bypass mechanism for the global activation of this important class of regulatory kinases.

It has also been reported that the phosphoryl group can be transferred from the phospho-NDP kinase to the histidine residue in the catalytic site of several other proteins, the energy metabolism enzymes succinic thiokinase (succinyl-CoA synthetase, EC 6.2.1.4) (17, 18) and ATP-citrate lyase (16, 18) and NDP kinase itself (via transphosphorylation) (17). In these experiments, phospho-NDP kinase was generated by incubation with $[\gamma-^{32}P]ATP$ and followed by removal of the bulk free $[\gamma-^{32}P]ATP$ by either size exclusion chromatography or successive rounds of dilution and ultrafiltration. By means of these procedures, the content of $[\gamma-^{32}P]ATP$ in the preparations of phospho-NDP kinase was decreased to the molar ratios (ATP/NDP kinase) of 1:40 (16), 1:1000 (17), or 1:100 (18), as stated in the publications. To demonstrate that phosphorylation of the target proteins is a result of direct phosphotransfer from the phospho-NDP kinase and not a consequence of their phosphorylation by the residual $[\gamma-^{32}P]ATP$, in the negative control experiments the target proteins were incubated with $[\gamma-^{32}P]ATP$ taken in the low “residual” concentrations, some-
times in the presence of unlabeled NDP kinase (16) or the kinase with mutated histidine residue in the active center (17). However, the absence of detectable amounts of the phosphorylated target proteins in these controls may result from the fact that the amount of the labeled phosphoryl groups present in the reaction was at least 1 order of magnitude lower here than in the reactions where the phospho-NDP kinase was present. In the experiments described above, it was the phospho-NDP kinase that served as a major source of the phosphoryl groups, and the contaminating ATP functioned more as a catalyst in the phosphotransfer cascade than a substrate supplying the phosphoryl groups.

NDP kinase has been found to phosphorylate the serine/threonine residues in the proteins in cell extracts (19) and the aspartate residue in aldolase C (18, 20). These reactions required stoichiometric rather than catalytic amounts of the phospho-NDP kinase, and they were greatly accelerated by the presence of urea (19) or sodium cholate (20). It is likely that reactions in this case were essentially nonenzymatic processes, i.e. the nucleophilic, phosphoaecrepting residues were interacting with the phosphohistidine, a strong phosphorylating agent, in the unfolded proteins. Crystal structures of NDP kinase show that the catalytic histidine is located at the bottom of the nucleotide-binding cleft (2), which is not compatible with the possibility of direct contact between the phosphohistidine and a residue in another protein in the absence of major conformational changes.

\textbf{E. coli NDP Kinase Is a DNA-binding Protein with DNA Cleaving Activity—}Lu et al. (15) also reported that NDP kinase overexpression caused EnvZ-mediated transcriptional activation of gene expression. There is an explanation for this result that is unrelated to EnvZ function, however. It has been shown that changes in DNA structure associated with increases in osmotic pressure act cooperatively with EnvZ-mediated phosphorylation events to activate transcription (22). Given the recently established role of NM23-H2/NDP kinase B in DNA structural changes (13, 14), it seems likely that NDP kinase overproduction in some way mimicked the effects of high osmolarity on DNA to enhance the expression of EnvZ-regulated genes.

We have previously identified human NM23-H2/NDP kinase as a DNA-binding transcriptional activator (8–12) and, more recently, as a DNA-cleaving enzyme (13). Because of the high level of conservation of residues in the catalytic pocket, we hypothesized that other members of the NM23/NDP kinase family, including the \textit{E. coli} enzyme, should have a similar DNA cleaving activity. Using duplex DNA oligonucleotides and supercoiled plasmid DNA (13), we have confirmed that the \textit{E. coli} enzyme does indeed bind and cleave DNA.

The degree of conservation of structure and function between human and \textit{E. coli} NDP kinase and NM23-H2 is a strong indicator that members of the NM23/NDP kinase family of proteins perform similar biological roles that most likely involve both NDP kinase and DNA cleavage activities. There is evidence suggesting that NM23/NDP kinases are involved in DNA repair (4, 14) as well as transcription. This type of activity would fit nicely with the function of NM23/NDP kinases in development and cancer.

\textbf{Acknowledgments—}We thank I. Lascu for the gift of \textit{E. coli} NDP kinase, M. Konrad for the ndke plasmid, and T. Silhavy for the EnvZ and OmpR-MBP proteins.

\textbf{REFERENCES}

1. Lascu, I., and Gonin, P. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 327–346
2. Janin, J., Dumas, C., Morera, S., Xu, Y., Meyer, P., Chaidimi, M., and Cherfils, J. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 215–225
3. Lascu, I., Giartosio, A., Ransac, S., and Erent, M. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 227–236
4. Lu, Q., Zhang, X., Almazua, N., Mathews, C. K., and Inouye, M. (1995) \textit{J. Mol. Biol.} \textbf{254}, 337–341
5. Hartsough, M. T., and Steeg, P. S. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 301–308
6. Timmons, L., and Shearn, A. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 293–300
7. MacDonald, N. J., Freije, J. M. P., Stracke, M. L., Manrow, R. E., and Steeg, P. S. (1996) \textit{J. Biol. Chem.} \textbf{271}, 25107–25116
8. Postel, E. H., Berberich, S. J., Flint, S. J., and Ferrone, C. A. (1993) Science \textbf{261}, 478–480
9. Postel, E. H. (1998) \textit{Int. J. Biochem. Cell Biol.} \textbf{30}, 1295–1298
10. Postel, E. H., Berberich, S. J., Rooney, J. W., and Kaezelt, D. M. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 277–284
11. Ma, D., Xiang, Z., Liu, B., Pedigo, N. G., Zimmer, S. G., Bai, Z., Postel, E. H., and Kaezelt, D. M. (2002) \textit{J. Biol. Chem.} \textbf{277}, 1560–1567
12. Berberich, S. J., and Postel, E. H. (1995) Oncogene \textbf{16}, 2343–2347
13. Postel, E. H. (1999) \textit{J. Biol. Chem.} \textbf{274}, 22821–22829
14. Postel, E. H., Abranzysh, B. M., Levit, M. N., and Kyin, S. (2000) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{97}, 14194–14199
15. Lu, Q., Park, H., Egger, L. A., and Inouye, M. (1996) \textit{J. Biol. Chem.} \textbf{271}, 32886–32893
16. Wagner, P. D., and Vu, N. D. (1995) \textit{J. Biol. Chem.} \textbf{270}, 21758–21764
17. Freije, J. M., Blay, P., MacDonald, N. J., Manrow, R. E., and Steeg, P. S. (1997) \textit{J. Biol. Chem.} \textbf{272}, 5525–5532
18. Wagner, P. D., Steeg, P. S., and Vu, N. D. (1997) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{94}, 9000–9005
19. Engel, M., Veron, M., Theisinger, B., Lacombe, M. L., Seib, T., Dooley, S., and Welte, C. (1995) \textit{Eur. J. Biochem.} \textbf{234}, 200–207
20. Wagner, P. D., and Vu, N. D. (2000) \textit{Biochem. J.} \textbf{346}, 623–630
21. Stock, A. M., Robinson, V. L., and Goudreau, P. N. (2000) \textit{Annu. Rev. Biochem.} \textbf{69}, 183–215
22. Higgins, C. F., Dormon, C. J., Stirling, D. A., Waddell, L., Booth, I. R., May, G., and Brenner, E. (1998) \textit{Cell} \textbf{52}, 569–584
23. Lacombe, M. L., Milon, L., Munier, A., Melhus, J. G., and Lambeth, D. O. (2000) \textit{J. Bioenerg. Biomembr.} \textbf{32}, 247–258
24. Stock, A., Chen, T., Welsh, D., and Stock, J. (1998) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{85}, 1405–1407
25. Igo, M. M., and Silhavy, T. J. (1988) \textit{J. Bacteriol.} \textbf{170}, 5971–5973
26. Hsing, W., Russo, F. D., Bernd, K. K., and Silhavy, T. J. (1998) \textit{J. Bacteriol.} \textbf{180}, 4538–4546