PATHOBIOLOGY IN FOCUS

The actions of NME1/NDPK-A and NME2/NDPK-B as protein kinases

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Nucleoside diphosphate kinases (NDPKs) are multifunctional proteins encoded by the *nme* (non-metastatic cells) genes, also called *NM23*. NDPKs catalyze the transfer of γ -phosphate from nucleoside triphosphates to nucleoside diphosphates by a ping-pong mechanism involving the formation of a high-energy phosphohistidine intermediate. Growing evidence shows that NDPKs, particularly NDPK-B, can additionally act as a protein histidine kinase. Protein kinases and phosphatases that regulate reversible O-phosphorylation of serine, threonine, and tyrosine residues have been studied extensively in many organisms. Interestingly, other phosphoamino acids histidine, lysine, arginine, aspartate, glutamate, and cysteine exist in abundance but remain understudied due to the paucity of suitable methods and antibodies. The N-phosphorylation of histidine by histidine kinases via the two- or multi-component signaling systems is an important mediator in cellular responses in prokaryotes and lower eukaryotes, like yeast, fungi, and plants. However, in vertebrates knowledge of phosphohistidine signaling has lagged far behind and the identity of the protein kinases and protein phosphatases involved is not well established. This article will therefore provide an overview of our current knowledge on protein histidine phosphorylation particularly the role of *nm 23* gene products as protein histidine kinases.

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PROTEIN SERINE, THREONINE, AND TYROSINE PHOSPHORYLATION

In animals the most well-recognized forms of protein phosphorylation are those in which the phosphoserine, phosphothreonine, or phosphotyrosine are formed on the target protein in reactions catalyzed by Ser/Thr or Tyr protein kinases. The phosphorus of the phosphoryl group is bonded to the hydroxyl oxygen in these phosphoamino acids, which are thus phosphoesters (Figure 1). These reactions involve the transfer of the gamma-phosphoryl group from ATP to the hydroxyl oxygen of the side chain of Ser, Thr, or Tyr. The phosphorylation of target proteins results in a change in protein activity through conformational changes in the protein structure induced by the insertion of the relatively large, negatively charged phosphoryl group. Alternatively, the phosphoamino acid acts as a docking site to allow other proteins to bind to the phosphoprotein (eg, proteins containing SH2 domains can bind via these domains to phosphoproteins containing phosphotyrosine). Many aspects of cellular function in animals are regulated by signaling pathways involving Ser/Thr and Tyr protein kinases. In addition, these pathways also involve phosphoprotein

phosphatases, which catalyze the hydrolysis of the phosphoamino acids in phosphoproteins, thus reversing the effects of phosphorylation on protein activity and protein–protein interaction. It is only relatively recently that similar protein kinases and phosphoprotein phosphatases were found to occur in bacteria (for reviews see Chao *et al*¹ and Pereira *et al*²).

PROTEIN HISTIDINE PHOSPHORYLATION Bacteria, Fungi, and Plants

Before the discovery of the occurrence of Ser/Thr and Tyr protein kinases in bacteria it was thought that the predominant form of protein phosphorylation to occur in these organisms was histidine phosphorylation, catalyzed by protein histidine kinases.

In phosphohistidine, the phosphorus of the phosphoryl group is bonded to one of the nitrogens in the imidazole ring of the side chain of histidine to form a phosphoramidate (Figure 1). As the imidazole ring of histidine contains two nitrogens, there are two possible isomers of phosphohistidine, 1-phosphohistidine and 3-phosphohistidine, both of which occur biologically (Figure 1).

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Figure 1 Structures of the side chains of (a) phosphoserine, (b) phosphothreonine, (c) phosphotyrosine, (d) 1-phosphohistidine, (e) 3-phosphohistidine, and (f) phosphoaspartate.

In bacteria and lower eukaryotes, protein histidine kinases are parts of the two-component histidine kinase systems where the histidine kinase is usually part of a membrane-bound receptor, which is activated on receipt of an extracellular stimulus by the sensor domain. This results in the autophosphorylation of a conserved histidine residue in the dimerization domain of the receptor (see Figure 2 in Attwood³). The phosphoryl group is then transferred from the phosphohistidine to a conserved aspartate residue in the regulatory domain of a response regulator protein in a reaction catalyzed by this protein (Figure 1f). This activates the response regulator protein, which is most commonly a transcription factor, which then induces the ultimate cellular response to the original extracellular stimulus. There are more complex systems involving such histidine kinases in which there are multiple phosphoryl transfers between the histidine kinase and the response regulator protein in a phospho-relay system involving proteins/domains in which phosphoryl transfer occurs alternately to aspartate and histidine (see Figure 3 in Attwood³). These systems occur in plants and fungi, as well as bacteria. It is the thermodynamic instability of phosphohistidine (as well as that of phosphoaspartate—a phosphoanhydride) compared to the phosphoester phosphoamino acids that facilitates these phosphoryl transfer reactions (Table 1). In addition, in bacteria, Lu *et al.*⁴ showed that nucleoside diphosphate kinase (NDPK) was capable of phosphorylating histidine residues in the two-component histidine kinases EnvZ and CheA in *Escherichia coli*. However, as this phosphorylation only occurred when ADP was present in the reaction mixture, Levit *et al.*⁵ demonstrated that independent of any protein–protein phosphotransfer

Table 1

Phosphoamino acid	$\Delta G_{\text{hydrolysis}}$ (kcal/mol)
Phosphoserine	–6.5 ^a
Phosphotyrosine	–9.5 ^a
Phosphohistidine	–12 to –14 ^a
Acetyl phosphate (analogous mixed anhydride to phosphoaspartate)	–10 ^b

^aFigures given are for the hydrolysis of the phosphoamino acids in proteins taken from Stock *et al.*⁶⁷

^bFigure for the standard free energy of hydrolysis of acetyl phosphate (Carpenter).⁶⁸

mechanism by NDPK, contaminating ADP in the reactions (and NDPK) may have been responsible for generation of ³²P-ATP required for autophosphorylation of the bacterial His kinases.

Another system in bacteria that involves histidine phosphorylation is the phosphoenolpyruvate/sugar phosphotransferase system in which a phosphoryl group initially derived from phosphoenolpyruvate, and following transfer between different protein components of the system, is finally used for phosphorylation of the transported sugar molecule.⁶

Animals

This type of phosphoryl transfer between proteins does not appear to occur in animal signaling systems that use Ser/Thr and Tyr protein kinases. Nor is there any direct evidence of the occurrence of two-component histidine kinase systems in

animals, although as noted by Attwood,³ there are a number of animal proteins in sequence databases that contain the conserved amino-acid sequences associated with both histidine kinase and response regulator proteins in two-component histidine kinase systems. What little is known about protein phosphorylation by histidine kinases in animals suggests that the main purpose of this form of phosphorylation is similar to that in the Ser/Thr and Tyr kinase systems, ie, changing protein activity and possibly protein-protein interaction (see below).

If protein histidine phosphorylation is playing similar roles to serine, threonine, and tyrosine phosphorylation in animals, one would expect there to be one or more phosphatases to catalyze the dephosphorylation of phosphohistidine-containing phosphoproteins. In fact, there is some question as to the requirement for a specific protein phosphohistidine phosphatase (PHP) as it has been shown that some protein phosphoserine/phosphothreonine phosphatases are also very efficient PHPs, eg, PP1, PP2A, and PP2C.^{7,8} However, a mammalian PHP (or PHPT1) has been discovered^{9,10} that shows high specificity toward proteins and peptides containing phosphohistidine, and no activity toward peptides containing phosphoserine, phosphothreonine, or phosphotyrosine.⁶ Later though, PHPT1 was found to have a somewhat wider specificity, as it was also found to catalyze the hydrolysis of phosphoramidate¹¹ and also phospholysine, but not phosphoarginine.¹² A number of metabolic enzymes do make use of the thermodynamic instability of phosphohistidine to catalyze phosphoryl transfer reactions via an enzymic, active site phosphohistidine intermediate, eg, phosphoglycerate mutase,¹³ ATP-citrate lyase,¹⁴ and NDPK.¹⁵ It has been suggested that the apparent phosphorylation of both succinate thiokinase and ATP-citrate lyase by NDPK^{16,17} may be explained as autophosphorylation events in these enzymes as both enzymes have a reactive histidine residue in their active sites, which undergoes autophosphorylation in the course of their normal catalytic cycles.^{5,18} However, this does not explain the histidine phosphorylation of aldolase C by Nm23-H1 or rat liver NDPK¹⁹ and other mammalian proteins as detailed below.

ANIMAL PROTEIN HISTIDINE KINASES

Histone H4 Histidine Kinase(s)

Amongst the earliest described animal (mammalian) histidine kinases is histone H4 histidine kinase. This was discovered in regenerating rat liver nuclei^{20,21} and in Walker 256 carcinosarcoma cells.²² Later, it was shown that a similar histone H4 histidine kinase was present in porcine thymus nuclei²³ and in human hepatocellular carcinoma tissue and human fetal liver.¹⁷ Histone H4 histidine kinase activity was also found in *Physarum polycephalum*²⁴ and *Saccharomyces cerevisiae*.²⁵ Thus, this histidine kinase activity is associated with cellular proliferation in normal and cancerous cells, however it is not clear how histone H4 histidine phosphorylation is linked to

Figure 2 The reaction mechanism of the normal metabolic reaction catalyzed by NDPK. This shows the initial transfer of gamma-phosphoryl group from a nucleoside triphosphate (XTP) to a histidine residue in the active site of NDPK (NDPK-His) to form NDPK-phosphohistidine (NDPK-His-P) and XDP. The nucleoside diphosphate (YDP) then binds in the active site of NDPK and the phosphoryl group is transferred to it from the phosphohistidine to YDP to form YTP.

these process. In addition, a histone H4 histidine kinase has never been fully characterized and identified from any source.

Nucleoside Diphosphate Kinase

NDPK is normally regarded to fulfill a metabolic role in cells by maintaining the balance of nucleoside triphosphates by catalyzing the transfer of a phosphoryl group from a nucleoside triphosphate (most commonly ATP) to a nucleoside diphosphate via an active site phosphohistidine intermediate (Figure 2). Recently however, the -A and -B isoforms of NDPK have been reported to act protein histidine kinases, in which the phosphoryl group from the active site phosphohistidine of NDPK is transferred to a histidine residue in another protein. NDPK-A has been reported to phosphorylate ATP-citrate lyase²⁶ and annexin A1 (refs 27,28) whilst NDPK-B phosphorylates the beta subunit of trimeric G proteins,²⁹ the potassium channel—K_{Ca}3.1,³⁰ and the calcium channel—TRPV5.³¹

HISTIDINE PHOSPHORYLATION AND TRPV5 CALCIUM CHANNELS

Cai *et al*³¹ provided evidence that TRPV5 is regulated by reversible histidine phosphorylation. The authors demonstrated that NDPK-B, but not NDPK-A, regulates the epithelial Ca²⁺ channel the transient receptor potential-vanilloid-5 (TRPV5); activation of TRPV5 requires phosphorylation of His711 in the C terminus by NDPK-B, whereas dephosphorylation by PHPT1 inactivated the channel. Furthermore, knockdown of NDPK-B by siRNA resulted in decreased TRPV5 channel activity, and in addition, NDPK-B^{-/-} mice fed a high-Ca²⁺ diet showed increased urinary Ca²⁺ excretion. The findings that NDPK-B and PHPT1 regulate TRPV5 and Ca²⁺ reabsorption support the idea that reversible histidine phosphorylation has many important but as yet-unknown roles in mammalian cellular function.

NDPK AND TUMOR METASTASIS SUPPRESSION

In the early 1990s, it became evident that enhanced cancer metastasis was linked to reduced expression of a gene named *nm23*, which turned out to be identical to human Nme1, also called Nm23-H1 or NDPK-A. Differential expression of Nme1 was found in experimental melanoma metastasis whereby Nme1 was more highly expressed in poorly metastatic sublines of the K-1735 murine melanoma than in related, highly metastatic sublines.³² Transfection of Nme1

into a highly metastatic melanoma line significantly reduced metastasis but had no effect on primary tumor size.³³ The metastasis suppressor activity of Nme1 has been demonstrated in many cancers except leukemias, lymphomas, and neuroblastoma.^{34,35} Multiple aspects of metastasis have been implicated in Nme1 suppression, including cell adhesion,^{36–38} motility and signaling,^{39–45} and proteolysis.⁴⁶ As discussed by Kaetzel *et al*,³⁸ Nme1 promotes genomic stability in melanoma through direct interactions with at least two DNA repair pathways, nucleotide excision repair and double-strand break repair. However, to date, the mechanism and function of Nme1 underlying its tumor metastasis suppressor activity remains to be established.

The question of which of the biological activities of Nme1 is associated with suppression of metastasis has been the subject of investigation over a number of years. Freije *et al*⁴⁷ and Wagner *et al*⁴⁸ used the approach of examining the effects of overexpression of wild-type and site-directed mutant forms of Nme1 on human breast carcinoma cell motility. Freije *et al*⁴⁷ showed that the loss of protein kinase activity in the two mutants (P96S and S120G) correlated with the inability of these mutants to suppress cell motility. Both of these mutant forms of Nme1 retained normal autophosphorylating activity.⁴⁷ Wagner *et al*⁴⁸ showed that the failure of these two mutant forms of Nme1 to suppress motility was most strongly correlated with a reduced ability to phosphorylate glutamate or aspartate residues on a 43 kDa protein from bovine brain rather than the ability to phosphorylate histidine residues on succinate thiokinase or ATP-citrate lyase. Later work by Hartsough *et al*⁴⁰ associated the metastasis suppressor activity of Nme1 with its ability to phosphorylate the kinase suppressor of Ras on serine residues. More recently, Wu *et al*⁴⁹ showed that two serine mutants of Nme1 (S44A and S120G) were unable to suppress tyrosine phosphorylation of STAT3, which is involved in the upregulation of expression of metalloproteinase 9, an important metastatic factor. However, the H118F mutant of Nme1 still maintained the ability to suppress STAT3 phosphorylation. In this instance at least, the autophosphorylation ability and protein kinase function of Nme1 does not appear to be directly connected with the effect on STAT3 phosphorylation. Thus, much remains to be investigated concerning the relationship between the protein kinase activity of Nme1 in general, and its protein histidine kinase activity in particular, and its anti-metastatic effect.

HISTIDINE PHOSPHORYLATION AND Ca^{2+} -ACTIVATED POTASSIUM CHANNELS

$\text{K}_{\text{Ca}}3.1$ is a Ca^{2+} -activated K^+ channel of an intermediate conductance, which is expressed in T and B cells, smooth muscle, and epithelial cells. The channel functions to keep a negative membrane potential by mediating the efflux of K^+ , which is required to maintain a favorable electrochemical gradient for Ca^{2+} influx. Activation of $\text{K}_{\text{Ca}}3.1$ requires not only binding of phosphatidylinositol 3-phosphate PI(3)P and Ca^{2+} /calmodulin to 14 amino acids in the carboxyl terminus

but also phosphorylation by NDPK (NDPK-B) at His358 in the same cytoplasmic domain.^{30,50} Among the four calcium-activated K_{Ca} potassium channels ($\text{K}_{\text{Ca}}2.1$, $\text{K}_{\text{Ca}}2.2$, $\text{K}_{\text{Ca}}2.3$, and $\text{K}_{\text{Ca}}3.1$; also called SK1–4), $\text{K}_{\text{Ca}}3.1$ is unique as it possesses a His residue at 358, which requires phosphorylation for activation. Using a yeast two-hybrid library screen, Srivastava *et al*³⁰ demonstrated binding of NDPK-B to the 14 amino-acid carboxyl terminus of $\text{K}_{\text{Ca}}3.1$ and showed that phosphorylation of H358 of $\text{K}_{\text{Ca}}3.1$ regulates its activation in CD4^+ T cells. The specificity and relevance of the NDPK-B/ $\text{K}_{\text{Ca}}3.1$ interaction was confirmed by biochemical, molecular, and functional approaches: (a) immunoprecipitation involving endogenous NDPK-B and $\text{K}_{\text{Ca}}3.1$ proteins in human CD4^+ T cells; (b) whole-cell patch-clamp experiments showing increased amplitude in CHO cells overexpressing NDPK-B and $\text{K}_{\text{Ca}}3.1$ proteins; and (c) NDPK-B knockdown showed inhibition of $\text{K}_{\text{Ca}}3.1$ channel activity, Ca^{2+} influx, and proliferation of human CD4^+ T cells. Srivastava *et al*⁵¹ have also demonstrated that the protein histidine phosphatase PHPT1 dephosphorylates pHis358 of $\text{K}_{\text{Ca}}3.1$ leading to negative regulation of CD4^+ T cells. Interestingly, that PHPT1 failed to dephosphorylate autophosphorylated NDPK-B (pHis118)⁵² provided evidence for the potential existence of other PHPs apart from Ser/Thr phosphatases that can also randomly dephosphorylate pHis proteins as shown by a previous report.⁷ In this regard, Panda *et al*.⁵³ recently provided confirmation that other phosphatases exist that may specifically interact with and target phosphohistidine residues on particular proteins via specific binding motifs; they showed that phosphoglycerate mutase 5 (PGAM5) dephosphorylates NDPK-B autophosphorylated on H118, thereby inhibiting subsequent phosphorylation and activation of $\text{K}_{\text{Ca}}3.1$ and resulting in decreased TCR-mediated Ca^{2+} influx and proinflammatory cytokine production in CD4^+ T cells. These authors also observe that PGAM5 function as a histidine phosphatase is restricted to NDPK-B pHis118 and that a 12 aa motif N-terminal to the PGAM domain mediates binding to NDPK-B, but PGAM is unable to dephosphorylate NDPK-A pHis118 or the $\text{K}_{\text{Ca}}3.1$ pHis358 and inhibit its activity; both NDPK-A and $\text{K}_{\text{Ca}}3.1$ lack the cognate motif for PGAM5 (conversely NDPK-B lacks the binding motif for PHPT1).⁵¹ Although counterintuitive at first glance, as the 3D structure of human NDPK-A/ADP shows only a single NTP/NDP binding,⁵⁴ this suggests that phosphorylated His118 in the NDPK active site is exposed and accessible to PGAM5. It is worth noting that PGAM5 belongs to the histidine phosphatase superfamily, a large functionally diverse group of proteins that share a common catalytic domain, designated as the PGAM domain and functions as phosphotransferases and/or phosphohydrolases; and share a conserved catalytic core centered on a histidine, which becomes phosphorylated during the course of the reaction.⁵⁵ Most members of the superfamily possess phosphoglycerate mutase activity and are involved in regulating glucose metabolism. On the other hand, some family members such as PGAM5, which do not

possess PGAM activity, have been shown to function as protein phosphatases specific for phospho-Ser/Thr (pSer/pThr) residues. Thus, Panda *et al*⁵³ provide evidence for transfer of phosphate from the autophosphorylated NDPK-B H118 active site and subsequent phosphorylation of His105 within the PGAM5 active site. However, the reaction stoichiometry is unclear from this study and whether the transferred phosphate is retained for further relay to yet-unknown target(s) or finally released as Pi; nonetheless, the study provides more evidence for His/His phospho-relay signaling networks, largely underappreciated in eukaryotic systems. In addition, it is also as yet unclear whether PGAM5 activity against pSer/Thr results in similar phosphorylation of PGAM at H105 or Pi release, as phosphoester bonds have low Delta G degrees of hydrolysis, compared to the phosphoramidate (P–N) bond of phosphohistidine (Table 1).

NDPK AND CFTR CHLORIDE CHANNELS

In epithelia, chloride (Cl[−]) channels have a prominent role in fluid and electrolyte transport. Of particular importance is the cystic fibrosis (CF) transmembrane conductance regulator Cl[−] channel (CFTR, a cAMP/ATP, and/or GTP-regulated chloride channel) with mutations of the CFTR-encoding gene causing CF. Using a two-hybrid screen and immunoprecipitation analysis, Klein *et al*⁵⁶ recently provide evidence that CFTR and K_{Ca}3.1 can exist in a dynamic complex in epithelia and demonstrate that physical interaction occurs between cytosolic domains of both proteins; both the N-terminal fragment M1–M40 and part of the calmodulin-binding domain (residues L345–A400) of K_{Ca}3.1 were found to interact with the nucleotide-binding domain NBD2 segment (G1237–Y1420) and C-terminal region of CFTR (residues T1387–L1480).

Previous work demonstrated that in apical membranes from airway epithelia, NDPK histidine phosphorylation is regulated by both chloride and cation concentration *in vitro* and provided evidence not only for NDPK involvement but for potential His/His phospho-relay signaling in chloride secretion and epithelial function.^{27,28,57}

Although NDPK-A and -B have previously been shown to exist as a heterohexamer, increasing evidence suggests that despite their highly homologous nature (and their genes lie adjacent to one another), the cellular actions of NDPK-A and -B isoforms differ substantially in airway epithelia. For example, NDPK-B is predominantly cytosolic in airway epithelia while NDPK-A is present in both the cytosol and membrane fractions.⁵⁸ The precise distinction between cytosolic and particulate NDPK-A is as yet-unknown but recent evidence also shows that membrane-bound NDPK-A interacts with AMP-activated protein kinase (AMPK).^{59,60} Although both NDPK-A and AMPK exist in the cytosol and membrane compartments, the two proteins do not always exist in a complex in epithelia suggesting the existence of a specific mechanism or post-translational modification and/or binding partner (unpublished observations).

Interestingly, both NDPK-A and -B interact with CFTR either directly or indirectly through associated proteins.^{58,60} This interaction with CFTR occurs independently of each isoform, by different mechanisms and with different cellular end points. King *et al* provided evidence that NDPK-A, AMPK, and CFTR exist in a plasma membrane-associated complex in airway epithelia, whereby AMPK phosphorylates CFTR and inhibits PKA-stimulated CFTR channel gating. Although the precise mechanisms remain unclear, it was observed that (a) AMPK catalytic function enhances NDPK-A autophosphorylation at His118 *in vitro*, (b) *Xenopus* oocytes co-expressing AMPK and CFTR with wild type, but not the NDPK-A catalytically inactive H118F mutant or various Ser-120 NDPK-A mutants, inhibit CFTR conductance in two-electrode voltage clamp studies indicating that NDPK-A catalytic function has an integral role in the inhibition of CFTR by AMPK.⁶⁰ The observation that AMPK enhances NDPK-A autophosphorylation is in agreement with a previous work in human bronchial epithelial cell line 16HBE14o[−] (enhancement does not occur in CF bronchial epithelial cells (CFBE41o[−]) cell lines.^{59,61} In contrast, others have provided evidence that during nutrient stress, AMPK phosphorylates NDPK at S120, thereby mediating inhibition of NDPK activity. AMPK is a key energy sensor that regulates metabolism to maintain cellular energy balance.⁶² The functional interaction between CFTR, AMPK α 1, and NDPK-A, is independent of NDPK-B.

Following cAMP/PKA activation, NDPK-B translocates from the cytosol to the apical membrane in airway epithelia to form a functionally relevant complex with CFTR. The precise mechanism leading to NDPK-B relocation to the apical membrane is still unclear but interaction with CFTR occurs through a distinct domain of NDPK-B involving amino acids 36–56 while the nucleotide-binding domain 1 (NBD1, aa 351–727) of CFTR constitutes an NDPK-B interaction site. Interestingly, although both CFTR and NDPK-B exist in other cellular compartments, the cAMP/PKA-induced interaction is restricted to the apical membrane and highlights a particular role for NDPK-B in epithelial cell chloride secretion. A peptide derived from NDPK-B 36–54 aa sequence, but not its NDPK-A equivalent, not only disrupts the interaction between NDPK-B and CFTR but also reduces chloride currents in epithelia.

HISTIDINE PHOSPHORYLATION AND HETEROTRIMERIC G PROTEIN SIGNALING

Stimulated G protein-coupled receptors catalyze the exchange of GTP for GDP and the dissociation of the G protein heterotrimer, allowing both the GTP-bound G α subunit and free $\beta\gamma$ complexes to signal to downstream effectors including adenylate cyclase, phosphodiesterase, phospholipase C, or ion channels to bring about a wide variety of physiological processes. Recent evidence shows that His/His phospho-relay signaling provides an alternative receptor-independent way of heterotrimeric G protein activation.^{29,63} NDPK-B, but not

NDPK-A, forms complexes with $G\beta\gamma$ resulting in phosphorylation of histidine residue His266 on the G protein β subunit of heterotrimeric G proteins and leading to formation of GTP and to an increase in cAMP synthesis. Cells expressing an inactive NDPK-B mutant (H118N) exhibit decreased cAMP formation. In cardiomyocytes, heterotrimeric G proteins are key regulators of myocardial contractility. SiRNA-mediated NDPK-B knockdown and overexpression of a $\beta\gamma$ -dimer mutant carrying a single amino-acid substitution at His266 in the $\beta 1$ subunit ($G\beta 1H266L_{\gamma 2}$) led to reduced receptor-independent $G_{\alpha s}$ activation, suppressed basal cAMP formation, and basal contractility in intact cardiomyocytes.⁶⁴ However, the β -adrenoceptor (βAR) agonist isoproterenol-induced increase in cAMP formation was not affected by $G\beta 1H266L_{\gamma 2}$.

Mäurer *et al*⁶⁵ have also demonstrated that eukaryotic PHP (also called PHPT1) specifically dephosphorylates $G\beta$ (pHis266), but not NDPK-B (pHis118), in reconstituted systems and in H10 cells membranes suggesting that PHPT1 might be a candidate for an endogenous regulator of basal NDPK-dependent G protein activation. It has also been observed that in plasma membranes of hearts from patients with severe congestive heart failure, but not in patients treated with a βAR antagonist, a threefold elevated content and activity of NDPK is detected and that chronic treatment of rats with isoproterenol, induces an increase in plasma membrane-bound NDPK-B.^{66,67} This suggests that NDPK-B membrane content may be under the control of β -adrenergic signaling and that NDPK may have an important role in heart failure and hypertrophy. Abu-Taha *et al*⁶⁸ show that NDPK-C mediates targeting of NDPK-A and -B isoforms to the plasma membrane and formation of the NDPK-B/G proteins complex in cardiomyocytes and is thus a critical regulator of β -adrenoceptor/cAMP signaling and cardiac contractility.

In conclusion, currently the well-studied cellular functions of NDPKs as protein kinases is the regulation of G protein activation and of ion channels by NDPK-B. Among the important questions that remain to be addressed is how NDPK can function both as a nucleoside phosphotransferase and a histidine protein kinase. The crystal structure of human NDPK-A/ADP shows a single NTP/NDP-binding pocket that in this conformation does not appear to be accessible to protein substrates.⁵⁴ It has been suggested that residues of the substrate proteins of NDPK may have a role in catalyzing phosphoryl transfer from pHis118 of NDPK-B and in this regard, Attwood and Wieland⁶ have recently suggested various models of structures of NDPK-B and target proteins. However, it is also clear that other as yet uncharacterized protein factors are required for interaction and phosphoryl transfer to occur.^{29,30,68}

In view of the high sequence similarity between NDPK-A to -C (66–88%),²⁶ protein–protein interactions most likely form the basis for target selection and distinction between the various NDPKs. As demonstrated for CFTR, $K_{Ca}3.1$, and PGAM5 binding with NDPK-B, the notion that

protein–protein interactions modulate the specific molecular actions of NDPK⁵⁹ remains current and is likely to grow in importance particularly as more targets for NDPKs are identified, eg, by new advances in phosphohistidine proteomics.^{69,70}

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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