

Review

## Mammalian histidine kinases

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### Abstract

Protein phosphorylation is one of the most ubiquitous and important types of post-translational modification for the regulation of cell function. The importance of two-component histidine kinases in bacteria, fungi and plants has long been recognised. In mammals, the regulatory roles of serine/threonine and tyrosine kinases have attracted most attention. However, the existence of histidine kinases in mammalian cells has been known for many years, although little is still understood about their biological roles by comparison with the hydroxyamino acid kinases. In addition, with the exception of NDP kinase, other mammalian histidine kinases remain to be identified and characterised. NDP kinase is a multifunctional enzyme that appears to act as a protein histidine kinase and as such, to regulate the activation of some G-proteins. Histone H4 histidine kinase activity has been shown to correlate with cellular proliferation and there is evidence that it is an oncodevelopmental marker in liver. This review mainly concentrates on describing recent research on these two types of histidine kinase. Developments in methods for the detection and assay of histidine kinases, including mass spectrometric methods for the detection of phosphohistidines in proteins and in-gel kinase assays for histone H4 histidine kinases, are described. Little is known about inhibitors of mammalian histidine kinases, although there is much interest in two-component histidine kinase inhibitors as potential antibiotics. The inhibition of a histone H4 histidine kinase by genistein is described and that of two-component histidine kinase inhibitors of structurally-related mammalian protein kinases. In addition, recent findings concerning mammalian protein histidine phosphatases are briefly described.

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### 1. Introduction

Protein phosphorylation is one of the most common forms of post-translational modification and one of the most important in the regulation of cellular function. Protein kinases are the enzymes that catalyse the transfer of the  $\gamma$ -phosphoryl group from a nucleoside triphosphate, usually ATP, to the side chain of an amino acid residue in the substrate protein. The best-known and most well-characterised protein kinases in

mammalian cells are the serine/threonine kinases and the tyrosine kinases, which catalyse the phosphorylation of the hydroxyl oxygen of these amino acids. There is however another type of protein kinase, the histidine kinase, which catalyses the phosphorylation of either the 1-nitrogen or the 3-nitrogen of the imidazole ring of the histidine side chain (Fig. 1). The phosphoramidate bond in phosphohistidine differs from the phosphoester bond in the phosphohydroxyamino acids in that it is unstable under acidic conditions and has a relatively high  $\Delta G^\circ$  of hydrolysis [1]. The acid-lability of phosphohistidine has meant that many techniques that are applied to the study of serine/threonine and tyrosine kinases cannot be used or have to be modified for the study of histidine kinases. The high free energy of hydrolysis of phosphohistidine facilitates phosphoryl transfer to other amino acids in intramolecular or intermolecular reactions.

The best known group of this type of protein kinase is the two-component histidine kinases which are found in bacteria,

*Abbreviations:* NDPK, nucleoside diphosphate kinase; NTP, nucleoside triphosphate; HHK, histone H4 histidine kinase;  $K_i$ , inhibitory constant; BCKDHK, branched chain  $\alpha$ -ketoacid dehydrogenase kinase; PDHK, pyruvate dehydrogenase kinase; eEF-2, eukaryotic elongation factor-2; eEF-2 kinase, eukaryotic elongation factor-2 kinase; RPTLC, reverse-phase thin layer chromatography;  $k_{cat}$ , catalytic rate constant;  $K_m$ , Michaelis constant; LHPPase, lysine/histidine protein phosphatase

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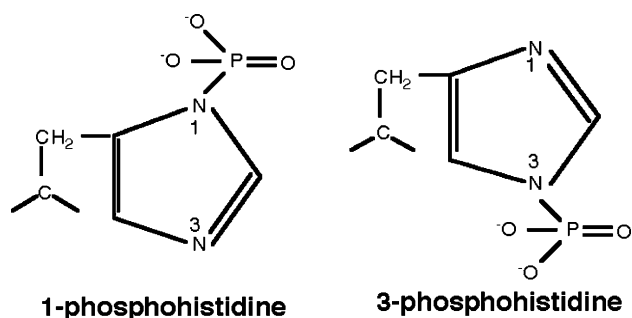


Fig. 1. Phosphorylated imidazole side chain groups of 1- and 3-phosphohistidine.

fungi and plants (for a review, see [2]). These histidine kinases are usually membrane receptor proteins, which either exist as dimers or dimerise in response to external stimuli. In response to the stimulus (e.g., change in osmolarity; ethylene concentration), a *trans*-phosphorylation reaction occurs in which one kinase of the dimer phosphorylates the other kinase on a specific, conserved histidine residue. The phosphoryl group is then transferred directly to a conserved aspartate residue on a response-regulator protein (often a transcription factor), thus activating it and initiating the cellular response. Alternatively, there are multi-component phosphorelay systems in which the phosphoryl group from phosphohistidine is firstly transferred to an aspartate residue, either in another domain of the histidine kinase or in a separate protein. The phosphoryl group is then transferred from the phosphoaspartate to a histidine residue that is again, either in another domain of the histidine kinase or in a separate protein. The phosphoryl group is then transferred to the aspartate of the response regulator protein (examples of such phosphorelay systems are given in [3]). As suggested by Besant et al. [3], this type of phosphotransfer system, which does not occur in phosphohydroxyamino acid-containing phosphoproteins, may be a reason for the occurrence of histidine kinases in mammalian cells.

The existence of histidine kinases in mammalian cells has been recognised for more than 30 years, with the early work of Smith and co-workers providing an initial impetus for further research in this area [4–7]. Although Matthews mainly worked on yeast histone H4 histidine kinase and protein histidine phosphatases, his development of assays and other methods for the investigation of protein histidine phosphorylation reinvigorated the field in the 1990s [8–16]. At the same time, Wieland and co-workers [17–22] and Kowluru and co-workers [23] were working on the histidyl phosphorylation of the  $\beta$  subunit of heterotrimeric G-proteins and the subsequent transfer of this phosphoryl group to GDP bound on the  $\alpha$  subunit. Interest was also growing in the ability of NDP kinases (human Nm23s) to act as protein kinases, in particular as protein histidine kinases [24–26]. Our interest in mammalian histone H4 histidine kinases also started in the late 1990s [27].

The aim of this review is to cover recent advances in our understanding of mammalian histidine kinases and the role of histidine phosphorylation in mammalian cells. We mainly concentrate on NDP kinases and histone H4 histidine kinases,

as there has been the most progress in these areas. We shall also refer to advances in methodologies in the investigation of protein histidine phosphorylation and the assay and detection of histidine kinases. In addition, we shall consider what is known about histidine kinase inhibitors with reference to particular mammalian enzymes.

## 2. Nucleoside diphosphate kinases (NDPKs)

In the 1990s there were a number of reports which suggested that some forms of NDPK could act as histidine kinases [24–26]. NDPK autophosphorylates on an active site histidine (using NTP as a substrate) and it was proposed that this phosphoryl group could then be transferred to histidine residues on other proteins. Wagner and Vu [25] found that rat liver NDPK could phosphorylate a histidine residue on ATP-citrate lyase. Later, Wagner et al. [26] showed that Nm23-H1, a human NDPK involved in the suppression of tumour metastasis, could also phosphorylate ATP-citrate lyase on a histidine residue. In addition, it was shown that Nm23-H1 could also phosphorylate a histidine residue on succinate thiokinase [28]. Lu et al. [24] showed that NDPK was capable of phosphorylating histidine residues in the two-component histidine kinases EnvZ and CheA in *Escherichia coli*.

More recently, doubt has been cast on the ability of NDPK to directly phosphorylate the above-mentioned proteins on histidine residues. Levit et al. [29] were able to demonstrate that CheA and EnvZ, which undergo histidyl autophosphorylation in the presence of ATP, would not autophosphorylate when incubated with GTP but could be phosphorylated using this nucleotide in the presence of NDPK. However, this phosphorylation only occurred when ADP was present in the reaction mixture and even concentrations of ADP as low as 1 nM were sufficient to promote phosphorylation of CheA and EnvZ. To explain these results, Levit et al. [29] proposed that NDPK autophosphorylated using  $[\gamma\text{-}^{32}\text{P}]\text{GTP}$  as a substrate, this phosphorylated NDPK then transferred its  $[\text{}^{32}\text{P}]$ phosphoryl group to ADP to form  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ . The  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  was then used as a substrate by CheA or EnvZ for their autophosphorylation and resulted in the re-formation of ADP, which was then re-phosphorylated by NDPK. The apparent phosphorylation of both succinate thiokinase and ATP-citrate lyase by NDPK may thus be explained as autophosphorylation events in these enzymes. Both ATP-citrate lyase and succinate thiokinase have a reactive histidine residue in their active sites which undergoes autophosphorylation in the course of their normal catalytic cycles [25,30].

Whilst autophosphorylation may explain the phosphorylation of succinate thiokinase and ATP-citrate lyase, this does not explain the phosphorylation of aldolase C by Nm23-H1 and rat liver NDPK [31]. Aldolase C was found to be phosphorylated on D319 by purified, autophosphorylated NDPK and Nm23-H1 [31]. With respect to the phosphorylation of a target protein on aspartate by enzymes that autophosphorylate on histidine, NDPK and Nm23-H1 behave in a similar way to two-component histidine kinases. Mutant forms of Nm23-H1 which lack the ability to suppress cell motility also showed much

reduced ability to phosphorylate aldolase C compared wild-type Nm23-H1 [31]. It is not clear whether aldolase C is the cellular substrate of Nm23-H1 that participates in a signalling pathway that regulates cell motility or whether some other cellular proteins are involved. It has been reported that aldolase C binds to inositol-1,4,5-trisphosphate in smooth muscle cells [32] and may be involved in modulating the availability of this second messenger for its role in release of  $\text{Ca}^{2+}$  from intracellular stores. Aldolase C has also been reported to bind to phospholipase D2 in rat brain and may, like aldolase A, interact with phospholipase D2 specifically via the pleckstrin homology domain of that enzyme [33]. Binding of aldolase to phospholipase D2 inhibits that enzyme and the inhibition is enhanced by fructose-1,6-bisphosphate and glyceraldehyde-3-phosphate [33]. Aldolase also interacts with the GLUT4 glucose transporter. These interactions of aldolase with GLUT4 and phospholipase D2, both of which are involved in the insulin-regulation of glucose uptake, suggests a way that metabolites of glucose may modulate insulin action [33]. What the effects of Nm23-H1-mediated phosphorylation of aldolase C are on these signalling processes remains to be investigated.

Another intriguing kinase activity of autophosphorylated Nm23-H1 is its ability to phosphorylate the kinase suppressor of Ras, in this case on two serine residues, S392 and S434, not on histidine or aspartate [34]. Hartsough et al. [34] also produced some evidence that the MAP kinase signalling pathway was regulated by Nm23-H1 in a manner dependent on its kinase activity.

In the 1990s, a body of evidence was produced that the  $\beta$  subunit of trimeric G-proteins could be phosphorylated on a histidine residue by an unidentified histidine kinase and that this phosphoryl group could be then directly transferred to GDP bound on the  $\alpha$  subunit of the G-protein, thereby activating it [17–20,22,35,36]. Over a number of years, there have been reports of direct physical interactions between NDPKs and G-proteins resulting in the activation of the latter [37–41]. In addition, there have been a number of reports that the autophosphorylated, phospho-NDPK can directly phosphorylate GDP bound to G-proteins, thus activating them [42,43]. Recently, it was proposed that a kinase which phosphorylates a histidine residue in the  $\beta$  subunit of mammalian trimeric G-proteins ( $\text{G}\beta_1$ ) was NDPK B (Nm23-H2) [44–46]. The bases of this proposal were: co-purification of NDPK and  $\text{G}\beta$ ; co-immunoprecipitation of NDPK and  $\text{G}\beta$ ; the  $\text{G}\beta$  phosphorylating activity was enriched by partial chromatographic purification and was found to contain NDPK B but not NDPK A (Nm23-H1) [45]. Thus, the histidine kinase was not purified to homogeneity. In addition, Cuello et al. [45] state that the presence of NDPK B and heterotrimeric G-protein or  $\text{G}\beta\gamma$  subunits alone is not sufficient to promote complex formation between  $\text{G}\beta\gamma$  and NDPK B or reconstitute the phosphorylation of  $\text{G}\beta$ , although the experimental basis for this observation is not clear. Cuello et al. [45] go on to propose that another protein is involved as a scaffold to allow complex formation between NDPK and  $\text{G}\beta\gamma$ .

More indirect evidence of NDPK phosphorylation of  $\text{G}\beta$  and subsequent activation of the G-proteins comes from a

number of reports. Kowluru [35,36,47] has reported a correlation between NDPK phosphorylation and  $\text{G}\beta$  phosphorylation in islet  $\beta$  cells. Kowluru [47] showed that at concentrations up to 33  $\mu\text{M}$  arachidonic acid enhanced NDPK activity and  $\text{G}\beta$  phosphorylation. In addition, mastoparan, a known activator of G-proteins, has been reported to activate NDPK [48] and enhance phosphorylation of  $\text{G}\beta$  [35]. However, Mastoparan-17, an analogue of mastoparan which stimulates NDPK activity [49], did stimulate  $\text{G}\beta$ , but to a much lesser degree than mastoparan.

Mehta and co-workers [50–53] found that the histidine phosphorylation of annexin I (and phosphorylation of other proteins) in respiratory epithelia is part of a signalling system that is regulated by  $[\text{Cl}^-]$ . Membrane-associated NDPK has been implicated as the protein histidine kinase in this signalling system [53].

Hippe et al. [46] showed that co-overexpression of  $\text{G}_s\alpha$  and NDPK B in H10 cells resulted in enhanced adenylyl cyclase activity, enhanced accumulation of cAMP and increased phosphorylation of  $\text{G}\beta$ . These effects were dependent on the levels of overexpression of both NDPK B and  $\text{G}_s\alpha$ . However, when  $\text{G}_s\alpha$  was co-overexpressed with either NDPK A or the catalytically inactive mutant of NDPK B (H118N), no accumulation of cAMP or increased phosphorylation of  $\text{G}\beta$  occurred. This indicates that both cAMP accumulation and  $\text{G}\beta$  specifically require the presence of catalytically active NDPK B.

Although there is strong evidence that NDPK B acts as a histidine kinase to phosphorylate  $\text{G}\beta$  and hence activates the G-protein by phospho-transfer to GDP bound on  $\text{G}\alpha$ , it is not unequivocal. In relation to the action of NDPKs as protein

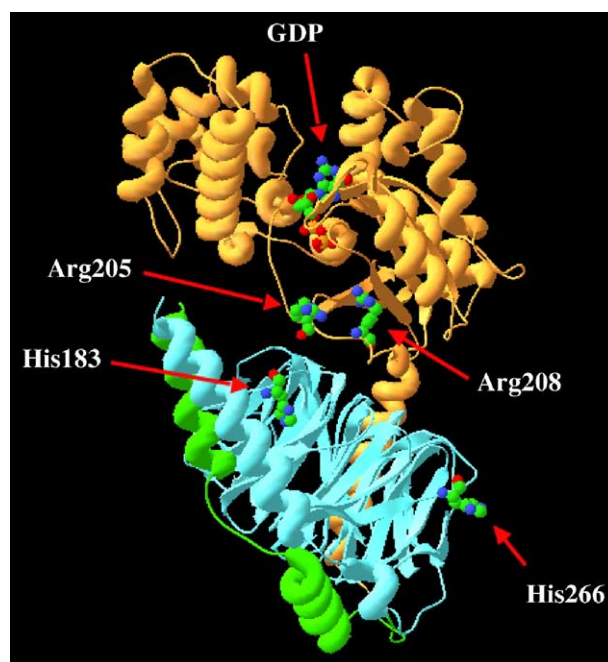


Fig. 2. Molecular model of rat heterotrimer with GDP bound in the  $\text{G}\alpha$  subunit. Shown in space-filling format are Arg205 and Arg208 in  $\text{G}\alpha$  and His183 and His266 in  $\text{G}\beta$ . The rest of the protein molecules are shown in ribbon form ( $\text{G}\alpha$ -mustard;  $\text{G}\beta$ -light blue;  $\text{G}\gamma$ -green).

kinases, the question arises of the ability of their small catalytic sites (from their crystal structures, see for example [54,55]) to accommodate large protein phosphorylation sites. Possible explanations that the solution structure of NDPK may be different from the crystal structure and that it may adopt a molten globule structure were discussed in the review by Steeg et al. [56]. The second problem is how the transfer of a phosphoryl group from the phosphohistidine of phospho-G $\beta$  to GDP bound on G $\alpha$  occurs. Nederkoorn et al. [57] proposed a mechanism involving a proton relay system that destabilises the phosphohistidine and initiates a phospho-relay system involving arginine residues (R201 acting as a possible phosphoarginine intermediate and R204) in G $\alpha$ . However, the proposal was based on the assumption that H183 was the most likely histidine to be phosphorylated [57], whereas Cuello et al. [45] showed that it was H266 of G $\beta$  that was phosphorylated. Fig. 2 shows the structure of a heterotrimeric G-protein, G $i$  and the relative positions of H183 and H266 in G $\beta$ , R205 and R208 in G $i\alpha$  (equivalent to R201 and R204 [57]) and the GDP bound in G $i\alpha$ . Whilst H183 is 29 Å from the  $\beta$ -phosphate of the GDP and 15 Å from R205, H266 is 47 Å from the GDP and 35 Å from R205, thus making the proposed phosphorelay mechanism of Nederkoorn et al. [57] unlikely. There remain many questions to be answered about the link between NDPK, the phosphorylation of G $\beta$  and the activation of trimeric G-proteins.

### 3. Histone H4 histidine kinases (HHKs)

For more than 30 years, there has been a slow accumulation of evidence of the existence of one or more mammalian histidine kinases that phosphorylate histone H4. HHK activity has been detected in regenerating rat liver [4,5,7,58], in foetal rat and human liver [58], human hepatocarcinoma tissue [58], pancreatic  $\beta$  cells [35,36], thymus [27] and Walker-256 carcinosarcoma cells [6]. Better characterised than the mammalian HHKs is that from yeast [11,13] which, like HHK from *P. polycephalum* [9], phosphorylates histone H4 on H75 to form 1-phosphohistidine. HHK from both regenerating liver and Walker-256 carcinosarcoma cells appears to phosphorylate both of the histidines in histone H4 (H18 and H75), HHK from regenerating liver forms 1-phosphohistidine whilst that from the carcinosarcoma cells forms 3-phosphohistidine.

In much of the work on HHKs, the *in vitro* phosphorylation of histone H4 has been used to detect the kinase activity. However, there is some evidence that histidine phosphorylation of histone H4 is an *in vivo* phenomenon. Phosphoamino acid analysis of proteolytically digested histone H4 isolated from cell nuclei in regenerating livers revealed the presence of phosphohistidine (the rats had been injected with 2 mCi/100 g body weight  $^{32}\text{P}$ i 15 h post partial hepatectomy) [5,7]. In one of these studies Chen et al. [7] showed that newly synthesised histone H4 was not phosphorylated, whilst in *P. polycephalum*, histone in nucleosome core particles was not a substrate for HHK [9]. This led Besant et al. [3], in their 2003 review, to speculate that histidine phosphorylation of histone H4 occurs at the time histones are displaced from DNA during replication to

prevent the premature formation of nucleosome complexes during DNA synthesis.

There is much evidence of the control exerted over the cellular processes of transcription, mitosis, DNA repair and carcinogenesis by covalent modification of histones, including phosphorylation (for reviews see [59–62]). Both of the histidine residues in histone H4 are in regions of the protein that form interactions with other histones in the nucleosome [3,63]. Thus, phosphorylation of either of these histidines has the potential to disrupt these interactions and hence the nucleosome structure.

There is evidence of a correlation between HHK activity and the proliferative state of cells in the liver. The original work of Chen et al. [5] showed that HHK activity was induced in regenerating rat liver following partial hepatectomy. The induction of HHK activity started before the induction of  $^3\text{H}$ -thymidine incorporation into newly synthesised DNA [5] and more recently, HHK activity was shown to peak at about 18 h post-partial hepatectomy, ahead of the peak of cellular proliferation [58]. Tan et al. [58] went on to show that HHK activity was very low in nuclear extracts from normal adult rat and human liver and in normal post-natal rat liver. However, in both foetal rat and human liver, HHK activity was of the order of 100 fold higher than in adult liver [58]. In addition, HHK activity in human hepatocellular carcinoma tumour tissue was about 200-fold higher than in the surrounding normal tissue. Tan et al. [58] also found elevated HHK activity in liver progenitor cells that were proliferating in response to chronic injury brought about by a chemical damage regime which inhibits regeneration by hepatocytes. The HHK activity in a tumorigenic liver progenitor cell line (PIL-2) derived from a p53 knockout mouse was found to be about 25% higher than in the proliferating cells from regenerating liver [58]. On the basis of the evidence described above linking HHK activity to cellular proliferation, foetal development and cancer in liver, Tan et al. [58] suggested that HHK may be an oncodevelopmental marker. Our laboratory is currently engaged in the process of identifying and characterising the HHK in both yeast and liver.

In addition to the work described above, linking HHK activity to cellular proliferation, Huang et al. [64] found that genistein, a known tyrosine kinase inhibitor, also inhibited yeast HHK. The inhibition of HHK by genistein was non-competitive with respect to both ATP and histone H4 with  $K_i$  values of the order of 300  $\mu\text{M}$ . In contrast to the inhibition of HHK, the inhibition of tyrosine kinases by genistein was reported to be competitive with respect to ATP [65]. Although higher than the  $\text{IC}_{50}$  values reported for inhibition of three different tyrosine kinases [65], concentrations of genistein used in experiments reported in the literature to inhibit the actions of growth factors (see, for example, [66,67]) are in the range where HHK inhibition occurs [64]. Thus, some of the reported cellular effects of genistein, especially inhibition of cellular proliferation, may, at least in part, be attributed to inhibition of HHK.

The other HHK that has been described in the literature was found in islet  $\beta$  cells [35] and was shown to phosphorylate

exogenous histone H4 and G $\beta$ . The HHK activity was evenly distributed between soluble and membrane fractions of the islet  $\beta$  cell extracts and able to use both ATP and GTP as a substrate. The HHK was also reported to phosphorylate endogenous G $\beta$  in a way that was stimulated by mastoparan [35]. However, the interpretation of these results is complicated by the presence of NDPK, which is also activated by mastoparan and also reported to phosphorylate G $\beta$  on a histidine residue (see Section 2 above). The HHK phosphorylation of G $\beta$  in islet  $\beta$  cells appears to be involved in the regulation of insulin secretion [36,47], although again the presence of NDPK makes interpretation of the results less straightforward. In view of the membrane association of the HHK and that of NDPK in the reports of Cuello et al. [45] and Treharne et al. [53], it seems possible that the HHK reported by Kowluru is in fact a membrane-associated NDPK, capable of histidine phosphorylation of histone H4. However, the molecular weight of the HHK reported by Kowluru [35] was 60–70 kDa by gel filtration chromatography and hence too large to be an NDPK monomer (about 20 kDa) and too small to be a hexamer, but could be NDPK associated with another protein.

#### 4. Putative mammalian two-component histidine kinases

Although NDPK is thought to transfer a phosphoryl group from one of its histidine residues to another molecule (i.e. amino acid or GDP), to date, there is no conclusive evidence that supports the presence of bacterial-like two-component histidine kinases in mammalian cells. However, certain mammalian proteins have been found to have structural similarities to bacterial two-component histidine kinases. Two of these proteins are found in the mitochondria, suggesting a possible evolutionary link to the bacterial histidine kinases. The two mitochondrial proteins that have been studied for histidine kinase activity are branched chain  $\alpha$ -ketoacid dehydrogenase kinase (BCKDHK) and pyruvate dehydrogenase kinase (PDHK). Both proteins contain prototypical two-component histidine kinase motifs [68,69] and both are components of a mitochondrial enzyme complex involved in the regulation of oxidative decarboxylation. What is known about the kinase activity of these enzymes is that both of them have been shown to autophosphorylate on a serine residue in vitro [69,70]. However, the possibility that BCKDHK and PDHK also have intrinsic histidine kinase activity has been the subject of some debate [69,71–73]. In the case of histidine autophosphorylation of PDHK, Tuganova et al. [73] suggested an essential role for Glu-243 and His-239 of rat PDHK in the phosphotransfer reaction. They showed that a mutation of the residue His 239 to Ala resulted in a 90% decrease in activity. Conversely, Tovar-Mendez et al. [74] showed that site-directed mutagenesis of the two most likely His residues of *A. thaliana* PDHK to be involved in phosphotransfer (H121 and H168) did not abolish either PDHK autophosphorylation or the ability to transphosphorylate the substrate E1 $\alpha$ . Hence, neither autophosphorylation or serine kinase activity were affected.

Apart from containing sequence motifs similar to bacterial-like two-component histidine kinases, it was found that the ATP-binding motif of BCKDHK is structurally similar to what is referred to as the ‘Bergerat fold’ family of proteins, of which two-component histidine kinases are members [75]. Based on this structural similarity to other members of the Bergerat fold protein family, Besant et al. [76] used the ATP mimetic radicicol as an inhibitor of both BCKDHK and a known yeast two-component histidine kinase. Although in both instances, the autophosphorylation of these enzymes was inhibited by radicicol, this only demonstrates the structural similarities of BCKDHK to the Bergerat fold family. To date, there is still no in vivo evidence of BCKDHK being involved in a signalling event similar to the well-established signalling roles of two-component histidine kinases.

In both cases, BCKDHK and PDHK raise the possibility that bacterial-like two-component histidine kinase activity may exist in mammalian cells. From an evolutionary perspective, the structural similarity of BCKDHK motifs to the Bergerat fold family of proteins, and its mitochondrial location supports the hypothesis proposed by evolutionary biologists of a primordial symbiosis of a bacterial-like cell which has evolved to what is now recognized as the mitochondrion [77].

Another interesting study involving the use of structural similarities to histidine kinases was reported for the non-mitochondrial protein, eukaryotic elongation factor-2 kinase (eEF-2 kinase). eEF-2 kinase was found to have an ATP fold structurally related to bacterial two-component histidine kinases. Inhibitors of the bacterial histidine kinases were used to demonstrate the potential of anti-microbial inhibitors as anti-cancer drugs [78]. Since eEF-2 kinase is involved in a ribosomal protein complex that modulates the protein synthesis pathways, inhibition of this protein may be a useful in preventing the growth of cancer cells. eEF-2 kinase has no known histidine kinase activity but is inhibited by members of a structurally related series of derivatives of the compound 2-methylimidazolium iodide. Arora et al. [78] demonstrated that one such derivative, NH-125, has a marked effect on the growth and viability of several cancer cell lines. The mode of action of NH-125 is thought to involve direct inhibition of the activity of eEF-2 kinase thereby decreasing the phosphorylation of its substrate eEF-2. Recognition of BCKDHK and PDHK as being structurally related to other members of the Bergerat family of proteins, together with the inhibition of eEF-2 kinase and BCKDHK by anti-microbial inhibitors of histidine kinases provides a timely cautionary note to the developers of antimicrobials intended for use in higher eukaryotes. The idea that humans and other higher order eukaryotes are devoid of two-component histidine kinases does not preclude the inhibition of proteins like BCKDHK and PDHK or other proteins that may be structural analogues of the intended bacterial target protein.

#### 5. Methods and techniques

By comparison to serine, threonine and tyrosine kinases the spotlight has seldom focused on mammalian histidine

kinases. This may be primarily due to some of the technical difficulties in detecting proteins that are phosphorylated on histidine. This is mainly attributed to the acid-labile nature of phosphohistidine and the fact that many of the classical techniques widely employed in detecting protein phosphorylation utilise acidic treatments such as trichloroacetic acid-precipitation [79]. Whilst many of the assays designed to detect phosphohistidine make use of its acid-labile nature, early methods involving phenol extraction [6] and gel-based assays [80] are difficult to reproduce and apply quantitatively. In 1990, Wei and Matthews developed a membrane-based method selective for the detection of alkali-stable protein phosphorylation [11]. In later years, this method was refined and modified to take into account the possible alkali-stable contribution of phosphotyrosine [81].

Other methods developed by Fujitaki and Smith for the detection of phosphoramidate-containing proteins include  $^{31}\text{P}$ -nuclear magnetic resonance, in addition to various methods for separation of alkali-stable phosphoamino acids involving high pressure liquid chromatography and paper electrophoresis [82]. Several methods for preparation and purification of the various isoforms of phosphohistidine have also been developed and refined [12,83]. Many of these methods have been transposed for use in phosphoamino acid analysis of proteins enzymatically phosphorylated on histidine. Techniques such as thin layer electrophoresis [27], reverse-phase thin layer chromatography (RPTLC) [84] and high performance liquid chromatography [12] have been instrumental in identifying and distinguishing biochemically derived phosphohistidine from phosphotyrosine and other phosphoamino acids.

In an attempt to increase the stability of phosphohistidine, other methods have been adopted in which the double bonded oxygen atom of the phosphate group is replaced with a sulphur atom [85]. This inherently more stable thiophosphorylated histidine can easily be synthesised using either thiophosphorylchloride ( $\text{PSCl}_3$ ) [12] or thiophosphoramidate [86]. Thiophosphohistidine is a close molecular analogue of phosphohistidine and is more stable in the acidic environments used in many classical phosphoprotein purification techniques. It may be possible to use radiolabelled  $\text{ATP}\gamma\text{S}$  as a substrate in many histidine kinase reactions, with  $\text{GTP}\gamma\text{S}$  already having been shown act as a substrate for the kinase that phosphorylates the G protein  $\beta$ -subunit [17–20,22]. However, a caveat to this is that thiophosphorylation should not be considered a panacea to solving the problem of acid-lability, as not all histidine kinases may be able to use  $\text{ATP}\gamma\text{S}$  or  $\text{GTP}\gamma\text{S}$  as a substrate and *in vivo* histidine thiophosphorylation cannot be measured.

In the age of proteomics, there are now two other techniques which potentially aid in the detection of histidine kinase activity, these are separation and detection of putative histidine kinases via *in-gel* kinase assays [27,87] and identification of phosphohistidine via mass spectrometry [88]. Providing a suitable protein or peptide substrate is available, *in-gel* kinase assays provide a means of detecting kinase activity and determining the molecular weights of the kinases. Subsequent *in-gel* digestion of the phosphorylated substrate by non-specific proteases, together with RPTLC provides a means of phos-

phoamino acid analysis to determine the nature of the phosphorylation. The yeast histone H4 histidine kinase purified by Wei and Matthews [12] was detected in this manner, using histone H4 as the substrate protein [27]. Using the similar methods, preparations of nuclear extracts from porcine thymus, regenerating rat liver, foetal human liver, human hepatocellular carcinoma and rat liver progenitor cells have all been shown to contain histone H4 histidine kinase activity and to potentially contain multiple histone H4 histidine kinases [27,89].

Mass spectrometry has proven to be useful in detecting most phosphoamino acids. Phosphohistidine is no exception, with mass spectrometry being used to detect the presence of both synthetically and enzymatically derived phosphohistidine [88,90]. Mass spectrometry was used to detect synthetic peptides containing phosphohistidine [90] and more recently metal-chelating matrices were used to selectively purify phosphohistidine-containing peptides from a digested two-component histidine kinase [91].

The development of anti-phosphoamino acid antibodies has also dramatically increased the depth of the biochemical toolbox when it comes to developing detection methods. Anti-phosphotyrosine antibodies are one particular instance where their use as a tool in the study of the involvement of tyrosine kinases in mammalian cellular signalling pathways has made an enormous impact in dissecting cellular signalling pathways. Similarly, the production of anti-phosphohistidine antibodies would be equally as significant for histidine kinases. So far, efforts to develop these antibodies have not been successful, most probably due to the lability of the phosphoramidate bond of phosphohistidine during the time it takes to elicit an immune response.

Whilst a seemingly large array of techniques has been developed to detect phosphohistidine, the purification and identification of a mammalian histidine kinase (other than the mammalian NDP kinase) has not been accomplished. Consequently, although we are capable of detection and quantitation of histidine kinase activity in mammalian cells, the identity of most mammalian histidine kinases remains elusive. With the continued development, improvement in sensitivity and refinement of methodologies for the detection and assay of protein histidine kinases and their phosphorylated protein products, the identities of mammalian histidine kinase must soon be revealed.

## 6. Histidine phosphatases

The detection of histidine kinases in mammalian systems raises the question of the existence of complementary protein histidine phosphatases. Evidence of mammalian histidine phosphatases gives rise to the possibility of histidine kinase/phosphatase regulatory mechanisms akin to the regulation of cellular signalling pathways involving the phosphorylation/dephosphorylation of serine, threonine and tyrosine. There have been numerous reports of histidine phosphatase activity, amongst which Wong et al. [92] and Ohmori et al. [93] have both identified an enzyme with dual histidine/lysine phosphatase activity isolated from rat tissue extracts. In both cases,

phosphatase activity was assayed by measuring the dephosphorylation of a synthetic polyphosphohistidine or 3-phosphohistidine substrate. In the study by Wong et al. [92], two polyphosphohistidine phosphatases were identified, one of which was stimulated up to 3.5-fold by  $Mg^{2+}$ , suggesting that this was similar to a type 2C phosphatase.

Unlike the enzyme reported by Wong et al., the 150-kDa dual 6-phospholysine/3-phosphohistidine phosphatase identified by Ohmori et al. [93] was not dependent on  $Mg^{2+}$  for activity and was later shown to possess characteristics similar to an acid phosphatase [94]. In both cases the phosphatases described by Wong et al. [92] and Ohmori et al. [93] have not been fully identified and characterized and although there was speculation that the phosphatases might act as phosphoprotein phosphatases, studies to verify their activity using non-synthetic substrates were not tested.

Other phosphohistidine phosphatases also reported to have been identified in rat liver [16,95] include a soluble 45 kDa protein phosphatase that dephosphorylates a p36 phosphoprotein. This phosphatase displayed a requirement for  $Mg^{2+}$  and was resistant to inhibition by okadaic acid (a known type 2A and 2B phosphatase inhibitor) suggesting that it too belonged to the protein phosphatase 2C (PP2C) family [96].

In a study of serine/threonine phosphatases in yeast, protein phosphatases 1, 2A and 2C were found to also act as protein histidine phosphatases [14]. In particular the  $k_{cat}/K_m$  of protein phosphatases 1 and 2A for the substrate histone H4 phosphorylated on histidine by the yeast histidine kinase were far better than for the serine-phosphorylated substrate, phosphorylase a. This is indicative of the phosphohistidine hydrolase function of these phosphatases being a major component of their activity [14]. The phosphohistidine phosphatase activity of protein phosphatases 1 and 2A were studied further by demonstrating their histidine phosphatase activity in rat liver and spinach cell extracts [16]. Although these two sources of enzyme are represented by two divergent eukaryotic species, protein phosphatases 1 and 2A were both shown to dephosphorylate histone H4 phosphorylated specifically on histidine. The presence of histidine phosphatase activity in such evolutionarily divergent sources supports the premise of phosphohistidine being widely utilised in eukaryotic phosphorylation events and underlies the potential importance of protein histidine phosphorylation [97–99].

More recently, several groups have made a significant impact on identifying and characterizing mammalian histidine phosphatases. Several publications by Klumpp and co-workers have significantly enhanced the profile of histidine phosphatases in vertebrates. In many of their studies, they describe the detection and implementation of several histidine phosphatase detection methods [97–99]. Of the histidine phosphatases that have been well characterised, Ek et al. [100] have reported a 14-kDa mammalian histidine phosphatase isolated from porcine liver cytosol that is capable of dephosphorylating a synthetic phosphohistidine containing peptide. This phosphatase was found to be unlike phosphatase 1, 2A and 2C and subsequent cloning and expression revealed an EDTA-insensitive phosphatase that appears to be specific for phosphohis-

tidine when compared to phosphatase activity tested against peptides phosphorylated on hydroxy amino acids. Another well-characterised phosphatase was first identified by Hiraishi et al. [101,102] in bovine liver and later cloned from a human cDNA library and expressed by Yokoi et al. [103]. This phosphatase is a 56-kDa inorganic pyrophosphatase named lysine/histidine protein phosphatase (LHPPase) because of its capacity to dephosphorylate phospholysine and phosphohistidine. The recombinant human LHPPase was shown to be highly expressed in liver and kidney and enzymatically is similar to the 56-kDa histidine phosphatase initially identified and purified from bovine liver.

Even though protein histidine phosphatases clearly exist, the need for histidine phosphatases to dephosphorylate phosphoproteins containing phosphohistidine *in vivo* remains to be explored. It is possible that, as in the ‘two component’ histidine kinase systems, the phosphoryl group is removed by an intramolecular transfer to an aspartate residue (or other amino acid) on the same or another protein. Additionally, it is also possible that the phosphoryl group is used to phosphorylate GDP, or other small molecules, similar to the phosphorylated G protein  $\beta$ -subunit described elsewhere in this review. The acid-lability of phosphohistidine may obviate any need for a specific phosphatase given the right intracellular conditions, where the pH of a particular microenvironment within the cell is acidic. However, the presence of phosphohistidine, along with evidence of histidine phosphatases supports its potential importance in eukaryotic signal transduction pathways.

## 7. Conclusions and perspectives

The field of mammalian histidine kinase research is still very much in its infancy, but as methods for detection of phosphohistidine-containing proteins and assays for histidine kinases improve, the true importance of these enzymes in mammalian cell regulation is likely to be revealed in the near future. Perhaps, there are more enzymes like NDP kinase which was thought to be essentially a housekeeping enzyme, but which now appears to have many roles different roles such as a protein kinase, a nuclease and as a transcriptional regulator (for a review, see [104]). The evidence that a histone H4 histidine kinase is an oncodevelopmental marker in liver opens up new possibilities for anti-cancer drug development, with HHK as the therapeutic target. The finding that two-component histidine kinase inhibitors also inhibit some structurally related mammalian protein kinases indicates that the use of these inhibitors may be a two-edged sword. On the one hand, they may be useful as therapeutic drugs targeting these mammalian enzymes, but on the other hand, these very interactions may affect their usefulness as antibiotics.

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## References

- [1] J.B. Stock, A.M. Stock, J.M. Mottonen, Signal transduction in bacteria, *Nature* 344 (1990) 395–400.
- [2] A.M. Stock, V.L. Robinson, P.N. Goudreau, Two-component signal transduction, *Annu. Rev. Biochem.* 69 (2000) 183–215.
- [3] P.G. Besant, E. Tan, P.V. Attwood, Mammalian protein histidine kinases, *Int. J. Biochem. Cell Biol.* 35 (2003) 297–309.
- [4] D.L. Smith, B.B. Bruegger, R.M. Halpern, R.A. Smith, New histone kinases in nuclei of rat tissues, *Nature* 246 (1973) 103–104.
- [5] C.C. Chen, D.L. Smith, B.B. Bruegger, R.M. Halpern, R.A. Smith, Occurrence and distribution of acid-labile histone phosphates in regenerating rat liver, *Biochemistry* 13 (1974) 3785–3789.
- [6] D.L. Smith, C.C. Chen, B.B. Bruegger, S.L. Holtz, R.M. Halpern, R.A. Smith, Characterization of protein kinases forming acid-labile histone phosphates in Walker-256 carcinosarcoma cell nuclei, *Biochemistry* 13 (1974) 3780–3785.
- [7] C.C. Chen, B.B. Bruegger, C.W. Kern, Y.C. Lin, R.M. Halpern, R.A. Smith, Phosphorylation of nuclear proteins in rat regenerating liver, *Biochemistry* 16 (1977) 4852–4855.
- [8] L. Carlomagno, V.D. Huebner, H.R. Matthews, Rapid separation of phosphoamino acids including the phosphohistidines by isocratic high-performance liquid chromatography of the orthophthalaldehyde derivatives, *Anal. Biochem.* 149 (1985) 344–348.
- [9] V.D. Huebner, H.R. Matthews, Phosphorylation of histidine in proteins by a nuclear extract of *Physarum polycephalum* plasmodia, *J. Biol. Chem.* 260 (1985) 16106–16113.
- [10] K.H. Pesis, Y. Wei, M. Lewis, H.R. Matthews, Phosphohistidine is found in basic nuclear proteins of *Physarum polycephalum*, *FEBS Lett.* 239 (1988) 151–154.
- [11] Y.F. Wei, H.R. Matthews, A filter-based protein kinase assay selective for alkali-stable protein phosphorylation and suitable for acid-labile protein phosphorylation, *Anal. Biochem.* 190 (1990) 188–192.
- [12] Y.F. Wei, H.R. Matthews, Identification of phosphohistidine in proteins and purification of protein-histidine kinases, *Methods Enzymol.* 200 (1991) 388–414.
- [13] J.M. Huang, Y.F. Wei, Y.H. Kim, L. Osterberg, H.R. Matthews, Purification of a protein histidine kinase from the yeast *Saccharomyces cerevisiae*. The first member of this class of protein kinases, *J. Biol. Chem.* 266 (1991) 9023–9031.
- [14] Y. Kim, J. Huang, P. Cohen, H.R. Matthews, Protein phosphatases 1, 2A, and 2C are protein histidine phosphatases, *J. Biol. Chem.* 268 (1993) 18513–18518.
- [15] Y. Kim, H.R. Matthews, Protein phosphatase assay suitable for acid-labile substrates, *Anal. Biochem.* 211 (1993) 28–33.
- [16] H.R. Matthews, C. Mackintosh, Protein histidine phosphatase activity in rat liver and spinach leaves, *FEBS Lett.* 364 (1995) 51–54.
- [17] T. Wieland, I. Ulibarri, P. Gierschik, K.H. Jakobs, Activation of signal-transducing guanine-nucleotide-binding regulatory proteins by guanosine 5'-[gamma-thio]triphosphate. Information transfer by intermediately thiophosphorylated beta gamma subunits, *Eur. J. Biochem.* 196 (1991) 707–716.
- [18] T. Wieland, M. Ronzani, K.H. Jakobs, Stimulation and inhibition of human platelet adenylate cyclase by thiophosphorylated transducin beta gamma-subunits, *J. Biol. Chem.* 267 (1992) 20791–20797.
- [19] T. Wieland, B. Nurnberg, I. Ulibarri, S. Kaldenberg-Stasch, G. Schultz, K.H. Jakobs, Guanine nucleotide-specific phosphate transfer by guanine nucleotide-binding regulatory protein beta-subunits. Characterization of the phosphorylated amino acid, *J. Biol. Chem.* 268 (1993) 18111–18118.
- [20] S. Kaldenberg-Stasch, M. Baden, B. Fesseler, K.H. Jakobs, T. Wieland, Receptor-stimulated guanine-nucleotide-triphosphate binding to guanine-nucleotide-binding regulatory proteins. Nucleotide exchange and beta-subunit-mediated phosphotransfer reactions, *Eur. J. Biochem.* 221 (1994) 25–33.
- [21] T. Wieland, K. Liedel, S. Kaldenberg-Stasch, D. Meyer zu Heringdorf, M. Schmidt, K.H. Jakobs, Analysis of receptor-G protein interactions in permeabilized cells, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 351 (1995) 329–336.
- [22] B. Nurnberg, R. Harhammer, T. Exner, R.A. Schulze, T. Wieland, Species- and tissue-dependent diversity of G-protein beta subunit phosphorylation: evidence for a cofactor, *Biochem. J.* 318 (1996) 717–722.
- [23] A. Kowluru, S.E. Seavey, C.J. Rhodes, S.A. Metz, A novel regulatory mechanism for trimeric GTP-binding proteins in the membrane and secretory granule fractions of human and rodent beta cells, *Biochem. J.* 313 (Pt. 1) (1996) 97–107.
- [24] Q. Lu, H. Park, L.A. Egger, M. Inouye, Nucleoside-diphosphate kinase-mediated signal transduction via histidyl-aspartyl phosphorelay systems in *Escherichia coli*, *J. Biol. Chem.* 271 (1996) 32886–32893.
- [25] P.D. Wagner, N.D. Vu, Phosphorylation of ATP-citrate lyase by nucleoside diphosphate kinase, *J. Biol. Chem.* 270 (1995) 21758–21764.
- [26] P.D. Wagner, P.S. Steeg, N.D. Vu, Two-component kinase-like activity of nm23 correlates with its motility-suppressing activity, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 9000–9005.
- [27] P.G. Besant, P.V. Attwood, Detection of a mammalian histone H4 kinase that has yeast histidine kinase-like enzymic activity, *Int. J. Biochem. Cell Biol.* 32 (2000) 243–253.
- [28] J.M. Freije, P. Blay, N.J. MacDonald, R.E. Manrow, P.S. Steeg, Site-directed mutation of Nm23-H1. Mutations lacking motility suppressive capacity upon transfection are deficient in histidine-dependent protein phosphotransferase pathways in vitro, *J. Biol. Chem.* 272 (1997) 5525–5532.
- [29] M.N. Levit, B.M. Abramczyk, J.B. Stock, E.H. Postel, Interactions between *Escherichia coli* nucleoside-diphosphate kinase and DNA, *J. Biol. Chem.* 277 (2002) 5163–5167.
- [30] M.E. Fraser, M.A. Joyce, D.G. Ryan, W.T. Wolodko, Two glutamate residues, Glu 208 alpha and Glu 197 beta, are crucial for phosphorylation and dephosphorylation of the active-site histidine residue in succinyl-CoA synthetase, *Biochemistry* 41 (2002) 537–546.
- [31] P.D. Wagner, N.D. Vu, Histidine to aspartate phosphotransferase activity of nm23 proteins: phosphorylation of aldolase C on Asp-319, *Biochem. J.* 346 (2000) 623–630.
- [32] C.B. Baron, P. Greeley, R.F. Coburn, Smooth muscle aldolase C-bound inositol 1,4,5-trisphosphate studied in vitro under physiological conditions, *Biochim. Biophys. Acta* 1401 (1998) 81–92.
- [33] J.H. Kim, S. Lee, T.G. Lee, M. Hirata, P.G. Suh, S.H. Ryu, Phospholipase D2 directly interacts with aldolase via its PH domain, *Biochemistry* 41 (2002) 3414–3421.
- [34] M.T. Hartsough, D.K. Morrison, M. Salerno, D. Palmieri, T. Ouatas, M. Mair, J. Patrick, P.S. Steeg, Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway, *J. Biol. Chem.* 277 (2002) 32389–32399.
- [35] A. Kowluru, Identification and characterization of a novel protein histidine kinase in the islet beta cell: evidence for its regulation by mastoparan, an activator of G-proteins and insulin secretion, *Biochem. Pharmacol.* 63 (2002) 2091–2100.
- [36] A. Kowluru, Defective protein histidine phosphorylation in islets from the Goto-Kakizaki diabetic rat, *Am. J. Physiol. Endocrinol. Metab.* 285 (2003) E498–E503.
- [37] H. Uesaka, M. Yokoyama, K. Ohtsuki, Physiological correlation between nucleoside-diphosphate kinase and the enzyme-associated guanine nucleotide binding proteins, *Biochem. Biophys. Res. Commun.* 143 (1987) 552–559.
- [38] N. Kimura, N. Shimada, Direct interaction between membrane-associated nucleoside diphosphate kinase and GTP-binding protein(Gs), and its regulation by hormones and guanine nucleotides, *Biochem. Biophys. Res. Commun.* 151 (1988) 248–256.
- [39] N. Kimura, N. Shimada, Evidence for complex formation between GTP binding protein(Gs) and membrane-associated nucleoside diphosphate kinase, *Biochem. Biophys. Res. Commun.* 168 (1990) 99–106.
- [40] S. Kikkawa, K. Takahashi, N. Shimada, M. Ui, N. Kimura, T. Katada, Conversion of GDP into GTP by nucleoside diphosphate kinase on the GTP-binding proteins, *J. Biol. Chem.* 265 (1990) 21536–21540.
- [41] F. Niroomand, R. Mura, K.H. Jakobs, B. Rauch, W. Kubler, Receptor-

- independent activation of cardiac adenylyl cyclase by GDP and membrane-associated nucleoside diphosphate kinase. A new cardiotoxic mechanism? *J. Mol. Cell Cardiol.* 29 (1997) 1479–1486.
- [42] K. Ohtsuki, M. Yokoyama, Direct activation of guanine nucleotide binding proteins through a high-energy phosphate-transfer by nucleoside diphosphate-kinase, *Biochem. Biophys. Res. Commun.* 148 (1987) 300–307.
- [43] K. Ohtsuki, M. Yokoyama, H. Uesaka, Physiological correlation between nucleoside-diphosphate kinases and the 21-kDa guanine-nucleotide binding proteins copurified with the enzymes from the cell membrane fractions of Ehrlich ascites tumor cells, *Biochim. Biophys. Acta* 929 (1987) 231–238.
- [44] J.F. Klinker, R. Seifert, Nucleoside diphosphate kinase activity in soluble transducin preparations biochemical properties and possible role of transducin-beta as phosphorylated enzyme intermediate, *Eur. J. Biochem.* 261 (1999) 72–80.
- [45] F. Cuello, R.A. Schulze, F. Heemeyer, H.E. Meyer, S. Lutz, K.H. Jakobs, F. Niroomand, T. Wieland, Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and Gbeta subunits. Complex formation of NDPK B with Gbeta gamma dimers and phosphorylation of His-266 IN Gbeta, *J. Biol. Chem.* 278 (2003) 7220–7226.
- [46] H.J. Hippe, S. Lutz, F. Cuello, K. Knorr, A. Vogt, K.H. Jakobs, T. Wieland, F. Niroomand, Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and Gbeta subunits. Specific activation of Galpha by an NDPK B.Gbetagamma complex in H10 cells, *J. Biol. Chem.* 278 (2003) 7227–7233.
- [47] A. Kowluru, Differential regulation by fatty acids of protein histidine phosphorylation in rat pancreatic islets, *Mol. Cell Biochem.* 266 (2004) 175–182.
- [48] S. Kikkawa, K. Takahashi, N. Shimada, M. Ui, N. Kimura, T. Katada, Activation of nucleoside diphosphate kinase by mastoparan, a peptide isolated from wasp venom, *FEBS Lett.* 305 (1992) 237–240.
- [49] A. Kowluru, S.E. Seavey, M.E. Rabaglia, S.A. Metz, Non-specific stimulatory effects of mastoparan on pancreatic islet nucleoside diphosphokinase activity: dissociation from insulin secretion, *Biochem. Pharmacol.* 49 (1995) 263–266.
- [50] K.J. Treharne, L.J. Marshall, A. Mehta, A novel chloride-dependent GTP-utilizing protein kinase in plasma membranes from human respiratory epithelium, *Am. J. Physiol.* 267 (1994) L592–L601.
- [51] R. Muimo, S.J. Banner, L.J. Marshall, A. Mehta, Nucleoside diphosphate kinase and Cl(–)-sensitive protein phosphorylation in apical membranes from ovine airway epithelium, *Am. J. Respir. Cell Mol. Biol.* 18 (1998) 270–278.
- [52] R. Muimo, Z. Hornickova, C.E. Riemen, V. Gerke, H. Matthews, A. Mehta, Histidine phosphorylation of annexin I in airway epithelia, *J. Biol. Chem.* 275 (2000) 36632–36636.
- [53] K.J. Treharne, C.E. Riemen, L.J. Marshall, R. Muimo, A. Mehta, Nucleoside diphosphate kinase-a component of the [Na(+)]- and [Cl(–)]-sensitive phosphorylation cascade in human and murine airway epithelium, *Pflugers Arch.* 443 (Suppl. 1) (2001) S97–S102.
- [54] S. Morera, M. Chiadmi, G. LeBras, I. Lascu, J. Janin, Mechanism of phosphate transfer by nucleoside diphosphate kinase: X-ray structures of the phosphohistidine intermediate of the enzymes from *Drosophila* and *Dictyostelium*, *Biochemistry* 34 (1995) 11062–11070.
- [55] S. Morera, I. Lascu, C. Dumas, G. LeBras, P. Briozzo, M. Veron, J. Janin, Adenosine 5'-diphosphate binding and the active site of nucleoside diphosphate kinase, *Biochemistry* 33 (1994) 459–467.
- [56] P.S. Steeg, D. Palmieri, T. Ouatas, M. Salerno, Histidine kinases and histidine phosphorylated proteins in mammalian cell biology, signal transduction and cancer, *Cancer Lett.* 190 (2003) 11–12.
- [57] P.H.J. Nederkoom, H. Timmerman, D. Timms, A.J. Wilkinson, D.R. Kelly, K.J. Broadley, R.H. Davies, Stepwise phosphorylation mechanisms and signal transmission within a ligand-receptor–Gabg-protein complex, *J. Mol. Struct.* 452 (1998) 25–47.
- [58] E. Tan, P.G. Besant, X.L. Zu, C.W. Turck, M.A. Bogoyevitch, S.G. Lim, P.V. Attwood, G.C. Yeoh, Histone H4 histidine kinase displays the expression pattern of a liver oncogene marker, *Carcinogenesis* 25 (2004) 2083–2088.
- [59] H. He, N. Lehming, Global effects of histone modifications, *Brief Funct. Genomic Proteomic.* 2 (2003) 234–243.
- [60] G. Pascreau, Y. Arlot-Bonnemains, C. Prigent, Phosphorylation of histone and histone-like proteins by aurora kinases during mitosis, *Prog. Cell Cycle Res.* 5 (2003) 369–374.
- [61] M. Iizuka, M.M. Smith, Functional consequences of histone modifications, *Curr. Opin. Genet. Dev.* 13 (2003) 154–160.
- [62] S.B. Hake, A. Xiao, C.D. Allis, Linking the epigenetic ‘language’ of covalent histone modifications to cancer, *Br. J. Cancer* 90 (2004) 761–769.
- [63] K. Luger, T.J. Richmond, The histone tails of the nucleosome, *Curr. Opin. Genet. Dev.* 8 (1998) 140–146.
- [64] J. Huang, M. Nasr, Y. Kim, H.R. Matthews, Genistein inhibits protein histidine kinase, *J. Biol. Chem.* 267 (1992) 15511–15515.
- [65] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami, Genistein, a specific inhibitor of tyrosine-specific protein kinases, *J. Biol. Chem.* 262 (1987) 5592–5595.
- [66] T.D. Hill, N.M. Dean, L.J. Mordan, A.F. Lau, M.Y. Kanemitsu, A.L. Boynton, PDGF-induced activation of phospholipase C is not required for induction of DNA synthesis, *Science* 248 (1990) 1660–1663.
- [67] J. Zwiller, P. Sassone-Corsi, K. Kakazu, A.L. Boynton, Inhibition of PDGF-induced c-jun and c-fos expression by a tyrosine protein kinase inhibitor, *Oncogene* 6 (1991) 219–221.
- [68] K.M. Popov, Y. Zhao, Y. Shimomura, M.J. Kuntz, R.A. Harris, Branched-chain alpha-ketoacid dehydrogenase kinase. Molecular cloning, expression, and sequence similarity with histidine protein kinases, *J. Biol. Chem.* 267 (1992) 13127–13130.
- [69] J.R. Davie, R.M. Wynn, M. Meng, Y.S. Huang, G. Aalund, D.T. Chuang, K.S. Lau, Expression and characterization of branched-chain alpha-ketoacid dehydrogenase kinase from the rat. Is it a histidine-protein kinase? *J. Biol. Chem.* 270 (1995) 19861–19867.
- [70] J.J. Thelen, J.A. Miernyk, D.D. Randall, Pyruvate dehydrogenase kinase from *Arabidopsis thaliana*: a protein histidine kinase that phosphorylates serine residues, *Biochem. J.* 349 (2000) 195–201.
- [71] B.P. Mooney, N.R. David, J.J. Thelen, J.A. Miernyk, D.D. Randall, Histidine modifying agents abolish pyruvate dehydrogenase kinase activity, *Biochem. Biophys. Res. Commun.* 267 (2000) 500–503.
- [72] M.V. Lasker, P. Thai, P.G. Besant, C.D. Bui, S. Naidu, C.W. Turck, Branched-chain a-ketoacid dehydrogenase kinase: a mammalian enzyme with histidine kinase activity, *J. Biomol. Tech.* 13 (2002) 1–9.
- [73] A. Tuganova, M.D. Yoder, K.M. Popov, An essential role of Glu-243 and His-239 in the phosphotransfer reaction catalyzed by pyruvate dehydrogenase kinase, *J. Biol. Chem.* 276 (2001) 17994–17999.
- [74] A. Tovar-Mendez, J.A. Miernyk, D.D. Randall, Histidine mutagenesis of *Arabidopsis thaliana* pyruvate dehydrogenase kinase, *Eur. J. Biochem.* 269 (2002) 2601–2606.
- [75] R. Dutta, M. Inouye, GHKL, an emergent ATPase/kinase superfamily, *Trend Biochem. Sci.* 25 (2000) 24–28.
- [76] P.G. Besant, M.V. Lasker, C.D. Bui, C.W. Turck, Inhibition of branched-chain alpha-keto acid dehydrogenase kinase and Sln1 yeast histidine kinase by the antifungal antibiotic radicicol, *Mol. Pharmacol.* 62 (2002) 289–296.
- [77] T.M. Embley, M. van der Giezen, D.S. Horner, P.L. Dyal, S. Bell, P.G. Foster, Hydrogenosomes, mitochondria and early eukaryotic evolution, *IUBMB Life* 55 (2003) 387–395.
- [78] S. Arora, J.M. Yang, T.G. Kinzy, R. Utsumi, T. Okamoto, T. Kitayama, P.A. Ortiz, W.N. Hait, Identification and characterization of an inhibitor of eukaryotic elongation factor 2 kinase against human cancer cell lines, *Cancer Res.* 63 (2003) 6894–6899.
- [79] J.J. Witt, R. Roskoski Jr., Rapid protein kinase assay using phosphocellulose-paper absorption, *Anal. Biochem.* 66 (1975) 253–258.
- [80] Y.F. Wei, J.E. Morgan, H.R. Matthews, Studies of histidine phosphorylation by a nuclear protein histidine kinase show that histidine-75 in histone H4 is masked in nucleosome core particles and in chromatin, *Arch. Biochem. Biophys.* 268 (1989) 546–550.
- [81] E. Tan, X. Lin Zu, G.C. Yeoh, P.G. Besant, P.V. Attwood, Detection of

- histidine kinases via a filter-based assay and reverse-phase thin-layer chromatographic phosphoamino acid analysis, *Anal. Biochem.* 323 (2003) 122–126.
- [82] J.M. Fujitaki, R.A. Smith, Techniques in the detection and characterization of phosphoramidate-containing proteins, *Methods Enzymol.* 107 (1984) 23–36.
- [83] P.G. Besant, L. Byrne, G. Thomas, P.V. Attwood, A chromatographic method for the preparative separation of phosphohistidines, *Anal. Biochem.* 258 (1998) 372–375.
- [84] P.G. Besant, M.V. Lasker, C.D. Bui, C.W. Turck, Phosphohistidine analysis using reversed-phase thin-layer chromatography, *Anal. Biochem.* 282 (2000) 149–153.
- [85] M. Lasker, C.D. Bui, P.G. Besant, K. Sugawara, P. Thai, G. Medzihradzsky, C.W. Turck, Protein histidine phosphorylation: increased stability of thiophosphohistidine, *Protein Sci.* 8 (1999) 2177–2185.
- [86] M.C. Pirrung, K.D. James, V.S. Rana, Thiophosphorylation of histidine, *J. Org. Chem.* 65 (2000) 8448–8453.
- [87] I. Kameshita, H. Fujisawa, Detection of protein kinase activities toward oligopeptides in sodium dodecyl sulfate-polyacrylamide gel, *Anal. Biochem.* 237 (1996) 198–203.
- [88] M. Wind, A. Wegener, R. Kellner, W.D. Lehmann, Analysis of CheA histidine phosphorylation and its influence on protein stability by high-resolution element and electrospray mass spectrometry, *Anal. Chem.* 77 (2005) 1957–1962.
- [89] E. Tan, P.G. Besant, X. Lin Zu, C.W. Turck, M.A. Bogoyevitch, S. Gee Lim, P.V. Attwood, G.C. Yeoh, Histone H4 histidine kinase displays the expression pattern of a liver oncodevelopmental marker, *Carcinogenesis* (2004).
- [90] K.F. Medzihradzsky, N.J. Phillipps, L. Senderowicz, P. Wang, C.W. Turck, Synthesis and characterization of histidine-phosphorylated peptides, *Protein Sci.* 6 (1997) 1405–1411.
- [91] S. Napper, J. Kindrachuk, D.J. Olson, S.J. Ambrose, C. Dereniwsky, A.R. Ross, Selective extraction and characterization of a histidine-phosphorylated peptide using immobilized copper(II) ion affinity chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *Anal. Chem.* 75 (2003) 1741–1747.
- [92] C. Wong, B. Faiola, W. Wu, P.J. Kennelly, Phosphohistidine and phospholysine phosphatase activities in the rat: potential protein-lysine and protein-histidine phosphatases? *Biochem. J.* 296 (1993) 293–296.
- [93] H. Ohmori, M. Kuba, A. Kumon, Two phosphatases for 6-phospholysine and 3-phosphohistidine from rat brain, *J. Biol. Chem.* 268 (1993) 7625–7627.
- [94] H. Ohmori, M. Kuba, A. Kumon, 3-Phosphohistidine/6-phospholysine phosphatase from rat brain as acid phosphatase, *J. Biochem.* 116 (1994) 380–385.
- [95] K. Motojima, S. Goto, Histidyl phosphorylation and dephosphorylation of P36 in rat liver extract, *J. Biol. Chem.* 269 (1994) 9030–9037.
- [96] P.T. Cohen, Novel protein serine/threonine phosphatases: variety is the spice of life, *Trends Biochem. Sci.* 22 (1997) 245–251.
- [97] S. Klumpp, J. Hermesmeier, D. Selke, R. Baumeister, R. Kellner, J. Krieglstein, Protein histidine phosphatase: a novel enzyme with potency for neuronal signaling, *J. Cereb. Blood Flow Metab.* 22 (2002) 1420–1424.
- [98] S. Klumpp, J. Krieglstein, Phosphorylation and dephosphorylation of histidine residues in proteins, *Eur. J. Biochem.* 269 (2002) 1067–1071.
- [99] V. Dombradi, J. Krieglstein, S. Klumpp, Regulating the regulators. Conference on protein phosphorylation and protein phosphatases, *EMBO Rep.* 3 (2002) 120–124.
- [100] P. Ek, G. Pettersson, B. Ek, F. Gong, J.P. Li, O. Zetterqvist, Identification and characterization of a mammalian 14-kDa phosphohistidine phosphatase, *Eur. J. Biochem.* 269 (2002) 5016–5023.
- [101] H. Hiraishi, F. Yokoi, A. Kumon, 3-phosphohistidine and 6-phospholysine are substrates of a 56-kDa inorganic pyrophosphatase from bovine liver, *Arch. Biochem. Biophys.* 349 (1998) 381–387.
- [102] H. Hiraishi, F. Yokoi, A. Kumon, Bovine liver phosphoamidase as a protein histidine/lysine phosphatase, *J. Biochem. (Tokyo)* 126 (1999) 368–374.
- [103] F. Yokoi, H. Hiraishi, K. Izuhara, Molecular cloning of a cDNA for the human phospholysine phosphohistidine inorganic pyrophosphate phosphatase, *J. Biochem.* 133 (2003) 607–614.
- [104] E.H. Postel, Multiple biochemical activities of NM23/NDP kinase in gene regulation, *J. Bioenerg. Biomembr.* 35 (2003) 31–40.