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REVIEW

# Interaction of nucleoside diphosphate kinase B with heterotrimeric G protein βγ dimers: consequences on G protein activation and stability

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**Abstract** It is generally accepted that G protein coupled receptors (GPCR) activate heterotrimeric G proteins by inducing a GDP/GTP exchange at the G protein α subunit. In addition, the transfer of high energetic phosphate by nucleoside diphosphate kinase (NDPK) and/or the β subunit of G proteins (Gβ) can induce G protein activation. Recent evidence suggests that the NDPK isoform B (NDPK B) forms a complex with Gβγ dimers. In this complex, NDPK B acts as a protein histidine kinase phosphorylating Gβ at histidine residue 266 (His266). The high energetic phosphoamidate bond on His266 allows for a phosphate transfer specifically onto GDP and thus local formation of GTP, which binds to and thereby activates the respective G protein α subunit. Apparently, this process occurs independent of the classical GPCR-induced GDP/GTP exchange at least for members of the $G_s$ and $G_i$ subfamilies of heterotrimeric G proteins. By using a mutant of $Gβ_1$ in which His266 was replaced by Leu, it was recently demonstrated that NDPK B/Gβγ-mediated $G_s$ activation contributes by about 50% to basal cAMP formation and contractility in rat cardiac myocytes. Besides its apparent role in G protein activation, the complex formation of NDPK B with Gβγ dimers might be essential for G protein stability. Depletion of either the NDPK B orthologue or $Gβ_1$ isoforms in zebrafish embryos led to a similar phenotype displaying contractile dysfunction in the heart accompanied by a complete loss of heterotrimeric G protein expression. In conclusion, the interaction of NDKP B with Gβγ dimers might play an important role in signal transduction, and alterations in this novel pathway might be of pathophysiological importance.

**Keywords** Heterotrimeric G proteins · Nucleoside diphosphate kinase · nm23-H2 · Gβγ dimers · Signal transduction

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## Introduction

Nucleoside diphosphate kinase (NDPK) is a ubiquitous enzyme that catalyses the transfer of the γ-phosphate from nucleoside 5′-triphosphates (NTP) to nucleoside 5′-diphosphates (NDP) by a ping-pong mechanism involving the formation of a high energy phosphate intermediate on His118 (Morera et al. 1995; Tepper et al. 1994). Eight isoforms of NDPK encoded by the nm23 genes in humans have been identified (Lacombe et al. 2000). In mammalian tissues, the cytosolic enzyme forms heterohexamers of 17–21 kDa subunits (Gilles et al. 1991; Janin et al. 2000). These are composed of different combinations of the three major isoforms, NDPK A, B and C. In addition to NTP synthesis, NDPKs are involved in a variety of processes in cellular physiology, including tumour metastasis (Steeg et al. 1988), development (Rosengard et al. 1989), gene regulation (Postel 2003), apoptosis (Fan et al. 2003), endocytosis (Krishnan et al. 2001; Palacios et al. 2002), vesicular transport from the endoplasmatic reticulum (Kapetanovich et al. 2005) and regulation of the cystic fibrosis transmembrane conductance regulator (Crawford et al. 2006a).

The activity of heterotrimeric G proteins (Gαβγ) is controlled by G protein coupled receptors (GPCRs), and upon activation, they transduce extracellular signals to

intracellular effectors. They consist of two functional units, the guanine nucleotide-binding Gα subunit and the Gβγ dimer (Hamm 1998). In the inactive state, GDP is bound in the α subunit of the heterotrimer. Agonist-activated GPCRs induce the release of GDP upon agonist binding, and subsequently, GTP, which is present in much higher intracellular concentrations than GDP, binds to the empty nucleotide-binding pocket in Gα (for short review, see Wieland and Michel 2005). It is generally believed that the conformational change induced by GTP binding releases the Gβγ dimer from the GTP-liganded Gα. Both can directly interact with specific effector proteins. Note, however, that the concept of heterotrimer dissociation has recently been challenged (Bünemann et al. 2003). After the hydrolysis of GTP to GDP and inorganic phosphate by the intrinsic GTPase activity present in Gα, the G protein returns to its inactive heterotrimeric state. In the early 1980s, the group of N. Kimura (for review, see Kimura 1993) were the first to show a contribution of the plasma membrane-bound fraction of NDPK to the activation of heterotrimeric G proteins by GTP formation. Thereafter, data reported by a variety of groups including the laboratory of Karl H. Jakobs (Wieland and Jakobs 1989; Jakobs and Wieland 1989; Wieland et al. 1991) supported that notion. In this paper, we will mainly focus on recent work that describes the identification of a complex formed by Gβγ and the NDPK B isoform and discuss its role in the activation and stability of heterotrimeric G proteins.

## GTP formation by NDPK: more than simple replenishment of G protein hydrolysed GTP?

One of the major reactions catalysed by NDPK is the phosphate transfer from ATP to GDP. This reaction, especially when catalysed by the membrane-bound fraction of NDPK, can maintain steady levels of GTP despite its constant hydrolysis during the activation of cycle of heterotrimeric G proteins and monomeric GTPases. Numerous in vitro studies, mainly performed in crude membrane preparations, have revealed G protein activation through this enzymatic activity of NDPK (reviewed by Otero 1990, 2000; Kimura 1993; Piacentini and Niroomand 1996). Already in this early days, it was found that locally formed, NDPK-derived GTP or its poorly hydrolysable analogue GTPγS are more potent in activating heterotrimeric G proteins than exogenously added GTP or GTPγS (Jakobs and Wieland 1989; Wieland and Jakobs 1992; Niroomand et al. 1997). Two hypotheses to explain such data have mainly been discussed: (1) substrate channelling, which in this case means local, NDPK-catalysed formation of GTP from ATP and GDP in immediate vicinity of the G protein (Kimura and Shimada 1990; Otero et al. 1988; Jakobs and Wieland 1989; Wieland et al. 1992) or (2) the transfer of the high energy phosphate intermediately present on His118 of the NDPK directly onto the GDP still bound to the Gα subunit of the heterotrimer or monomeric GTPases (Kikkawa et al. 1990; Randazzo et al. 1991).

## NDPK catalysed GTP formation on G protein bound GDP: is it possible?

Today, it appears clear that the second hypothesis is wrong, and experimental data believed to support it were based on artefacts. Structural data obtained from crystals of purified G protein α subunits (Lambright et al. 1994; Mixon et al. 1995) and of the heterotrimeric G protein (Wall et al. 1995) show that the bound GDP is deeply buried in the G protein α subunit and even less accessible in the heterotrimer (see Fig. 1). Also, the phosphohistidine in the NDPK monomer is hidden in a small cleft within the structure (Janin et al. 2000; Lascu and Gonin 2000), and thus, a transfer of the phosphate onto GDP bound to Gα is not possible without large alterations in the three-dimensional structures. A transfer to GDP bound to monomeric GTPases is at least not likely (Scheffzek et al. 1995). The experimental artefacts mentioned above were due to a spontaneous release of GDP from G proteins. This GDP served as a substrate for phosphotransfer by the NDPK, and the formed GTP then bound back to the G protein (Randazzo et al. 1991, 1992a,b; Lutz et al. 2002). Even an approach where GDP has been cross-linked to the monomeric GTPase Rad (Zhu et al. 1999) is questionable by its experimental design (Otero 2000), as NDPK is able to transfer phosphate also onto denatured proteins and thus also on the covalently linked GDP (Engel et al. 1995).

## NDPK-mediated nucleotide channelling: a possible mechanism?

The channelling of GTP into the G protein would only be possible when a close association, i.e. a complex formation of at least a monomeric NDPK molecule with the heterotrimeric G protein or one of its subunits occurs. Indeed, experimental data have been reported already in the late 1980s, which support such a complex of membranous NDPK with the stimulatory G protein of adenylyl cyclase ($G_s$; Kimura and Shimada 1988a,b, 1990) or the retinal G protein transducin ($G_t$; Orlov et al. 1996; Klinker and Seifert 1999). Nevertheless, the contribution of local GTP formation to effector regulation was disputed or not detected in other systems (Xu et al. 1996; Sorota et al.

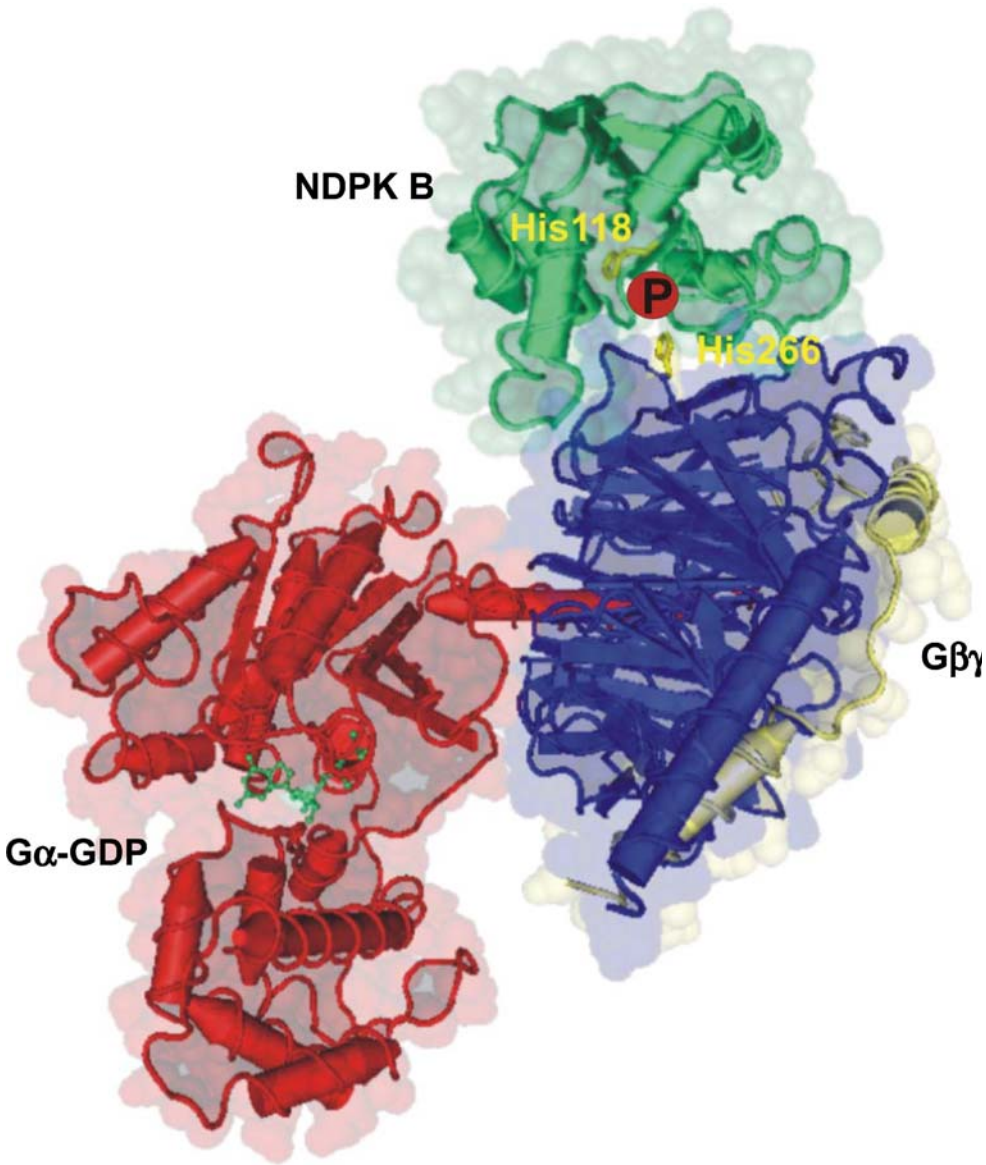


**Fig. 1** Three-dimensional structure of NDPK B complexed with a heterotrimeric G protein. Ribbon diagrams of a trimeric G protein ($G\alpha_s$-GDP [*red*], $G\beta_1$ [*blue*] and $G\gamma_2$ [*yellow*]) were superimposed with NDPK B (*green*). The possible structural vicinity of the histidine residues of NDPK B (His118, *yellow*) and Gβ (His266, *yellow*) is illustrated. The phosphate is shown in *red*. The GDP molecule in the nucleotide binding site in Gα is depicted in *green*

1998). The major drawback in all those studies was that the enzymatic activity of NDPK was used as a major read-out for detection of the presence of the enzyme. Due to its extremely high enzymatic activity, contaminations with small amounts of NDPK, which are still present in G protein-enriched fractions, might have led to misinterpretations, and therefore, a more specific role of NDPK than the simple replenishment of GTP from ATP and GDP has been thoroughly questioned (Otero 2000). Recent data, however, indicate that NDPK-mediated nucleotide channelling do occur by complex formation with other nucleotide consuming proteins (Crawford et al. 2005, 2006a,b). The NDPK isoform NDPK A forms a complex with the AMP-activated protein kinase α1 isoform (AMPK). In this complex, AMPK phosphorylates NDPK A on a serine residue and thereby regulates "substrate channelling", which in this case is shielding of NDPK-generated ATP from the consumption in the surroundings. Taking these data and the recently discovered complex formation of the NDPK B isoform with Gβγ dimers (Cuello et al. 2003, see below) into account, channelling of GTP at heterotrimeric G proteins is still the most likely hypothesis for NDPK-mediated G protein activation.

## GTP formation by intermediately phosphorylated G protein β subunits: an alternative phosphate transfer reaction?

The group of Karl H. Jakobs was the first to report that the β subunit of G proteins can carry a high energetic phosphate (Wieland et al. 1991). This phosphate was

characterised to be a phosphoamidate on a histidine residue, which was transferable onto GDP thus leading to GTP formation (Wieland et al. 1993), G protein activation and regulation of effector activity (Wieland et al. 1992). GDP was the only cellular NDP substrate for that phosphate transfer reaction. In contrast to the NDPK, the phosphohistidine intermediate is not formed by incubation of the protein with the required NTP but required a histidine kinase activity which phosphorylates Gβ within the Gβγ dimer. Intermediately occurring histidine phosphorylation of Gβ has been detected in a variety of mammalian tissues (Nürnberg et al. 1996), including human platelets (Hohenegger et al. 1996) and rat pancreatic β cells (Kowluru et al. 1996). The identity of the proposed membrane-bound histidine kinase to catalyse this reaction remained, however, elusive (Nürnberg et al. 1996, Kowluru 2002). Also, in this phosphotransfer reaction, the phosphorylation of GDP bound to Gα was a matter of debate (Wieland et al. 1993; Kaldenberg-Stasch et al. 1994; Hohenegger et al. 1996; Kowluru et al. 1996; Niroomand et al. 1997). The same experimental constraints, which cannot exclude the spontaneous release of the Gα-bound GDP and re-binding of formed GTP, apply here, and some experimental data are clearly arguing against a direct transfer on Gα-bound GDP (Hohenegger et al. 1996).

## NDPK B, the Gβ histidine kinase within the NDPK B/Gβγ dimer complex?

The first, evidence that the histidine kinase, which phosphorylates Gβ, is a NDPK was presented by Cuello et al. (2003). The Gβ phosphorylating activity was enriched from $G_t$ preparations out of bovine rod outer segments membranes and preparations of the inhibitory G proteins of adenylyl cyclase ($G_{i/o}$) out of bovine brain membranes. Fractions in which histidine phosphorylation of Gβ was enriched have been obtained by different purification protocols out of the respective source. Most interestingly, out of both tissues, these fractions contained a small amount of Gβγ dimers, which has been separated from the vast majority of the heterotrimeric G protein, and NDPK, which could be detected by its enzymatic activity, autophosphorylation at His118 and specific antibodies. Although several NDPK isoforms are abundantly expressed in the source tissues, isoform-specific antibodies revealed a selective enrichment of NDPK B. By size exclusion chromatography, the molecular mass of the kinase activity was estimated to be 60–70 kDa. Kowluru et al. (Kowluru 2002; Kowluru et al. 2002) reported a Gβ phosphorylating "histidine kinase" activity of rat pancreatic β cells with a similar size. This kinase activity could be stimulated by the known NDPK activator mastoparan. In addition, membranes of these cells contained only NDPK B but not NDPK A. The estimated molecular mass of the "histidine kinase" would match with a complex formed of Gβγ and monomeric NDPK B (Gβ 35–36 kDa, Gγ 5–12 kDa, NDPK 17–21 kDa). Indeed, a direct interaction of Gβγ with the NDPK could be demonstrated by co-immunoprecipitation experiments (Cuello et al. 2003). A Gβ as well as a NDPK B-specific antiserum precipitated both phosphorylated NDPK B plus phosphorylated Gβ from the retinal preparations.

The phosphorylation of Gβ could be reconstituted by combining purified Gβγ dimers from bovine brain or $G_t$βγ with the Gβ phosphorylating activity from bovine brain (Cuello et al. 2003) Therefore, radioactive labelling and subsequent proteolytic analysis of $G_t$βγ, which consists of the isoforms $Gβ_1$ and $Gγ_1$, as well as of less-defined bovine brain Gβγ dimers, were used to identify the histidine residue 266 (His266) as the phosphorylated intermediate in $Gβ_1$. A homologous histidine residue is conserved in the mammalian $Gβ_1$–$Gβ_4$ isoforms but not in $Gβ_5$, and only the imidazolyl side chain of this His is exposed on the surface of the G protein (see Fig. 1). All seven additional imidazolyl side chains are deeply buried in the seven-blade propeller structure of Gβ (Wall et al. 1995) and are therefore not likely accessible to kinases. In contrast, by superimposing the three-dimensional structure of a NDPK B monomer (Webb et al. 1995) and a heterotrimeric G protein (Fig. 1), it appears structurally feasible that His118 of the NDPK B and His266 of $Gβ_1$ can come close enough to allow a phosphorelay from one histidine residue to the other. Such a phosphate transfer is well known in bacteria and used to mediate recognition of chemotactic stimuli (for review, see Falke et al. 1997).

Most important, the biochemical and structural data, which point to NDPK B acting as histidine kinase in a NDPK B/Gβγ complex, could be substantiated by functional data obtained from stable cell clones of immortalized neonatal rat cardiac myocytes (H10 cells; Hippe et al. 2003). In membranes of NDPK B-overexpressing H10 cells, an increase in the content and activity of NDPK B as well as the formation of NDPK B/Gβγ complexes was detected. When Gβ phosphorylation was analysed in membranes of H10 cells stably overexpressing NDPK A, NDPK B or its catalytically inactive mutant NDPK B-H118N (H118), an increase in Phospho-Gβ was observed in the NDPK B but not in the NDPK A or H118-overexpressing cells. Thus, an increase in functional NDPK B/Gβγ complexes apparently increases the amount of intermediately phosphorylated Gβ subunits. Furthermore, adenovirus-mediated overexpression of $Gβ_1γ_2$ dimers in the NDPK B-overexpressing H10 cells further increased Gβ phosphorylation by about twofold. This increase was absent when an adenovirus encoding $Gβ_1γ_2$ dimers

in which His266 of $G\beta_1$ was mutated to leucine ($G\beta_1H266L\gamma_2$) was used (Hippe et al. 2005).

## GPCR-independent G protein activation by NDPK B/Gβγ complexes: a mechanism to regulate basal activity?

Most of the data reporting on phosphate transfer reactions by NDPK or by Gβ and the resulting G protein activation were based on studies in cell membranes or partially purified protein fractions. Therefore, they have been suspected to be the result of these artificial conditions, and their relevance for signal transduction in living cells has consequently been questioned (Hohenegger et al. 1996; Clapham and Neer 1997; Otero 2000). The cell clones of stable transfected H10 cells, which overexpress NDPK B at different levels, as well as H118 or NDPK A enabled Hippe et al. (2003) to address the question of NDPK B-mediated G protein activation in an intact cell model. The expressions of $G\alpha_s$, $G\alpha_i$ and $G\beta\gamma$ were not different between the individual cell clones, and the cellular GTP and ATP levels as well as basal cAMP formation were similar. Increasing adenoviral overexpression of the adenylyl cyclase stimulatory Gα subunit $G\alpha_s$ induced, however, huge differences in basal, GPCR-independent cAMP accumulation. Whereas cAMP accumulation increased linearly with amount of overexpressed $G\alpha_s$ in all cell clones, the slope of this linear increase, however, correlated with the amount of overexpressed NDPK B in the respective cell clone. For example, when compared to control H10 cells, the increase in cAMP levels in response to $G\alpha_s$ was about fourfold higher at each level of $G\alpha_s$ expression in the cell clone with a threefold higher NDPK activity. The cell clone threefold, expressing the inactive NDPK B mutant H118, displayed a reduced cAMP accumulation (by about 33%) in response to $G\alpha_s$ compared to control H10 cells. Overexpression of NDPK A was without effect on cAMP synthesis, which data showed that the activation of $G\alpha_s$ was strictly dependent on the catalytic activity of the NDPK B isoform. The combined stimulatory effect of NDPK B and $G\alpha_s$ overexpression on cAMP formation was confirmed by measurements of adenylyl cyclase activity in membranes of the H10 cell clones, linking the data in living cells to the previously disputed biochemical analyses in cell membranes (Hippe et al. 2003). Notably, like already shown in a variety of membrane systems in the early 1990s, in the H10 membrane in vitro assay, the NDPK B and $G\alpha_s$-dependent stimulation of adenylyl cyclase was largely suppressed when the phosphate acceptor substrate GDP was replaced by its analogue, guanosine 5′-O-(2-thio)diphosphate (GDPβS). GDPβS binds to Gα subunits with similar affinity than GDP but is a poor substrate for NDPK (Niroomand et al. 1997) and Phospho-Gβ (Wieland et al. 1993). Most interestingly, an even stronger reduction in $G\alpha_s$-dependent cAMP accumulation then with H118 mutant was obtained when $G\beta_1H266L\gamma_2$ was adenovirally overexpressed in H10 cells. Compared to the overexpression of wild-type $G\beta_1\gamma_2$, cells overexpressing comparable levels of $G\alpha_s$ and $G\beta_1H266L\gamma_2$ exhibited about 55% lower cAMP levels (Hippe et al. 2005). Similar data were obtained by comparison of adenoviral overexpression of wild-type $G\beta_1\gamma_2$ and $G\beta_1H266L\gamma_2$ in cultured neonatal rat cardiac myocytes. When an identical approach was used in isolated adult rat ventricular cardiomyocytes and single cell fractional shortening was measured, overexpression of $G\beta_1H266L\gamma_2$ but not of wild-type $G\beta_1\gamma_2$ significantly reduced myocyte contractility. In contrast, overexpression of either $G\beta_1H266L\gamma_2$ or wild-type $G\beta_1\gamma_2$ similarly supported β-adrenoceptor-induced cAMP synthesis and increase in contractility in H10 cells, neonatal and adult rat ventricular myocytes. Therefore, all this functional data provide evidence for a physiological relevance of NDPK-mediated G protein activation in GPCR-independent, basal activation of G proteins in living cells (Fig. 2). Like a variety of proteins, e.g. dynamin I, phocein (Baillat et al. 2002), ICAPα (Fournier et al. 2002), KSR (Hartsough et al. 2002) and AMPK (Crawford et al. 2005, 2006b), Gβγ dimers form a complex with a specific isoform of NDPK, which in case of heterotrimeric G proteins is NDPK B. At least one additional, so far unidentified, protein appears to be required and might function as scaffold for this complex (Cuello et al. 2003). This subpopulation of G protein complexed with NDPK B can be activated by a phosphate transfer: In the living cell, most likely, ATP binds to the NDPK B as NTP substrate in that complex and its γ-phosphate is transferred onto His118 in the NDPK B. Acting as protein histidine kinase, this high energetic phosphate is transferred onto His266 in Gβ. Out of that phosphoamidate bond, the phosphate is transferred specifically onto GDP, and the formed GTP leads to receptor-independent G protein activation. At present, the experimental data support such a model for $G_s$, $G_t$ and other members of the $G_i$ family. A contribution of NDPK-mediated G protein activation for members of the $G_q$ or $G_{12}$ families of G proteins has not been reported yet. As already discussed above, experimental evidence as well as the three-dimensional structure of a heterotrimeric G Protein (Fig. 1) does not support a direct transfer onto Gα-bound GDP. The distance between His266 and the bound GDP molecule within the Gα subunit is far too large to allow such a transfer. Nevertheless, it appears feasible that the GDP bound in Gα dissociates with a certain frequency from the binding pocket and thus comes in close proximity to Phospho-His266 in Gβ. The phosphate is transferred onto that GDP, and the newly formed GTP can bind with

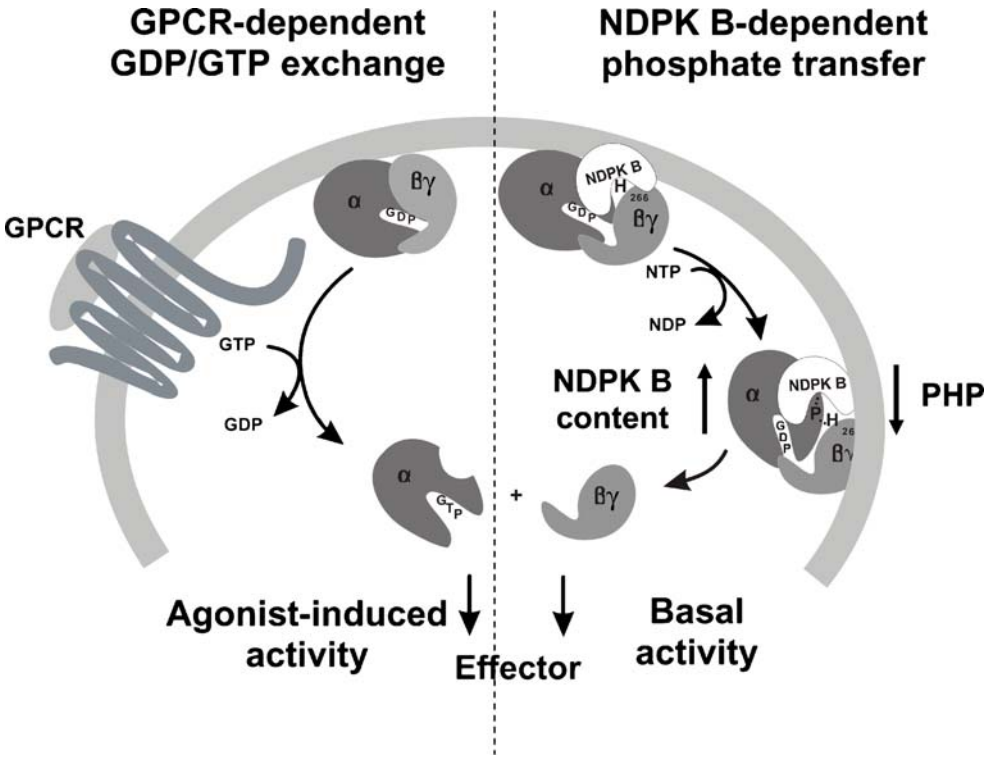


**Fig. 2** GPCR-dependent and alternative NDPK B-dependent G protein activation. Agonist binding to a GPCR triggers GDP/GTP exchange on Gα and release of Gβγ, leading to effector responses (*left side*). In the subpopulation of heterotrimeric G proteins complexed with NDPK B, a phosphotransfer from NTP, preferably ATP, to His118 in NDPK B and subsequently onto His266 of Gβ, results in a high energetic phosphate, which promotes the formation of GTP, leading to GPCR-independent G protein activation and thus regulation of basal effector activities. Two possible mechanisms modulating this pathway are shown. Increased plasma membrane content of NDPK B, as detected in CHF will increase basal G protein activity. Phosphohistidine phosphatase (*PHP*), which can specifically dephosphorylate Gβ, is a potential counter regulator of NDKP B-dependent G protein activation

high affinity to the still empty binding pocket. Thus, the G protein will be activated. This model would explain many of the data obtained in membranes during the 1980s and 1990s and as discussed above, allow for the "old" hypothesis of GTP channelling in which the NDPK-formed GTP is shielded from the surroundings and thus has an advantage.

In a recent report (Mäurer et al. 2005), it was described that the first identified mammalian phosphohistidine phosphatase (PHP; Ek et al. 2002; Klumpp et al. 2002) is able to dephosphorylate Gβ, but not NDPK B, in reconstituted systems and in H10 cells membranes. Its stable overexpression in living H10 cells also interferes with Gβ phosphorylation. Therefore, PHP might be an endogenous regulator of basal NDPK-dependent G protein activation. Apparently, it targets the formation of Phospho-His266 in Gβ (see Fig. 2), the importance of which has been proven by mutation analysis.

## Stimulation of phosphorelay by agonist-liganded GPCRs: more than an interesting hypothesis?

From the purification of the NDPK B/Gβγ complexes (Cuello et al. 2003), it was, however, concluded that the majority of G proteins is not complexed with NDPK B. These G proteins still can be activated by the classical pathway of GPCR-induced GDP/GTP exchange. Nevertheless, a variety of data accumulated by different laboratories in the early days of investigations on NDPK and G protein activation (reviewed in Otero 1990; Piacentini and Niroomand 1996) as well as some data on G protein activation by phosphorylated Gβ subunits (Kaldenberg-Stasch et al. 1994) argued for a role of phosphate transfer in GPCR-induced G protein activation. These speculations culminated in a hypothesis in which the GPCR/G protein complex functions as GTP synthase and a phosphorelay would be the essential mechanism of G protein activation (Nederkoorn et al. 1998). Besides the fact that these data were only based on theoretical calculations, the formation of a phosphohistidine on His183 of Gβ was crucial to this hypothesis. The identification of His266 as the phosphorylated His in Gβ and the structural impossibility to relay this phosphate to His183 are arguing against this phosphorelay hypothesis. Also, the experimental data so far accumulated in studying the influence of $G\beta_1H266L\gamma_2$ on β-adrenoceptor-induced cAMP synthesis and contractility (Hippe et al. 2005) do not support a role of NDPK B/Gβγ complexes in GPCR-induced G protein activation In addition, measurement of isoproterenol-stimulated GTPγS binding in membranes of Sf9 insect cells (Kühn et al. 2002) in which the human $\beta_2$-adrenoceptor was reconstituted with $G\alpha_s$ and either wild-type $G\beta_1\gamma_2$ or with $G\beta_1H266L\gamma_2$ by baculovirus infection revealed no difference between wild-type and mutant Gβγ. Both equally supported agonist-stimulated GTPγS binding. Therefore, a direct stimulation of NDPK B/Gβγ-mediated phosphate transfer by agonist-liganded GPCRs is not very likely.

### NDPK B/Gβγ complexes: are they required for G protein stability in vivo?

The data so far summarised did not address the question whether the formation of NDPK B/Gβγ complexes are of physiological relevance for G protein-mediated signal transduction in a living, multicellular organism. A recent attempt used the morpholino phosphorodiamidate oligonucleotide-induced gene depletion in zebrafish embryos (Sumanas and Larson 2002, Iversen and Newbry 2005) to study this issue (Mehringer et al. 2006). A selective knockdown of the zebrafish orthologues of the NDPK isoforms A and B as well as $G\beta_1$ subunits was made by injection of specific morpholino antisense oligos compared to control embryos injected with scrambled oligos. Depletion of NDPK B, but not NDPK A, led to severely compromised atrial and ventricular cardiac function associated with pericardial edema and insufficient blood flow. Histology and immunofluorescence staining revealed intact development and no structural defects in the knockdown hearts. The knockdown of the two zebrafish $G\beta_1$ orthologues, $G\beta_1$ and $G\beta_{1like}$, individually or in combination caused a very similar phenotype with an identical cardiac dysfunction. As a consequence of the NDPK B depletion, a nearly complete loss of $G\beta_1$ and $\gamma_2$ proteins was detected. Vice versa, the depletion of $G\beta_{1/1like}$ was associated with the specific loss of the NDKP B orthologue. Most interestingly, in the NDPK B as well as in the $G\beta_{1/1like}$ knockdown animals, the expression of the Gα subunits of all four G protein subfamilies, $G_s$, $G_i$, $G_q$ and $G_{12}$, was almost completely suppressed. In contrast, the expression of several other marker proteins was unaffected, and only the content of the mRNA encoding the targeted protein was strongly reduced. A similar loss of all Gα subunits was recently reported for small interfering RNA-induced silencing of $G\beta_1$ and $G\beta_2$ in J774A.1 mouse macrophages (Hwang et al. 2005). In summary, the data point to an enhanced posttranslational degradation of heterotrimeric G proteins or their remaining components when either the NDKP B or $G\beta_1$ as important contributors in the protein complex are missing. For the first time, they provide in vivo evidence for an essential role of the interaction of NDPK B with Gβγ dimers to maintain G protein expression and thus normal cardiac contractility.

### NDPK–G protein interaction: are there alterations in disease?

Besides the rather huge amount of literature dealing with the tumour suppressor function of NDPK (for review, see Steeg et al. 2003), very little information about alterations in other diseases is available. A contribution of the phosphate transfer by NDPK B/Gβγ complexes was so far implicated in diabetes and congestive heart failure (CHF).

The notion that G protein activation via NDPK B/Gβγ complexes might be altered in diabetes are based on findings in Goto–Kakizaki rats, a model for human type 2 diabetes, which exhibit impaired insulin secretion in response to glucose. These animals display a reduction of about 50% in NDPK activity (Metz et al. 1999), NDPK autophosphorylation and histidine kinase activity compared to control rats (Kowluru 2003). In addition, it was recently shown that some polyunsaturated fatty acids, which are well-known stimulators of insulin secretion, increase NDPK activity, NDPK autophosphorylation and Gβ phosphorylation in rat pancreatic islet cells (Kowluru 2004).

The notion that NDPK/Gβγ-mediated G protein activation is altered in CHF, is based on findings demonstrating an at least threefold higher content in sarcolemmal membranes purified from human myocardium of failing hearts compared to non-failing controls (Lutz et al. 2001; Zhou and Artman 2001). These changes were only seen in the small pool of membrane-associated NDPK and were not observed in either homogenate, cytosol or other remaining fractions. These data, therefore, suggested a translocation from the cytosol to the plasma membrane in cardiac myocytes. An up-regulation of all three major isoforms (NDPK A, B and C) was detectable (Lutz et al. 2004), which is likely due to the formation of heterohexamers by the majority of the NDPK isoforms (Gilles et al. 1991). Furthermore, a substantial GPCR-independent inhibition of adenylyl cyclase activity up to 50% in membranes of failing hearts was demonstrated. These data, therefore, support the above-outlined role of NDPK B/Gβγ complex as modulator of basal G protein activity. Interestingly, the net effect of the NDPK B-mediated regulation of adenylyl cyclase activity in failing hearts was an inhibition, which obviously correlates to the relative prevalence of $G\alpha_i$ in CHF (Neumann et al. 1988). In contrast, in sarcolemmal membranes of canine hearts, NDPK stimulates adenylyl cyclase via $G\alpha_s$ (Niroomand et al. 1997). Thus, it is tempting to speculate that at $G\alpha_s$ and $G\alpha_i$ subunits non-selectively form heterotrimers with NDPK B/Gβγ complexes, and thereby, any alteration in the prevalence of the one or the other Gα subunit decides whether the net effect is receptor-independent stimulation ($G\alpha_s$ prevalence) or inhibition ($G\alpha_i$ prevalence) of adenylyl cyclase activity. Thereby, this mechanism might aggravate the consequence of a rather small (30–50%) increase in $G\alpha_i$ in CHF and contribute to the well-known diminished cAMP signalling observed in failing hearts.

The use of β-adrenoceptor antagonists is meanwhile part of the accepted standard therapy in CHF (Lohse et al. 2003). Interestingly, the elevation of the membrane-associ-

ated NDPK was partially prevented in patients with CHF, which have been treated with β-adrenoceptor antagonists. On the other hand, the progression of cardiac hypertrophy induced by chronic β-adrenoceptor stimulation was paralleled by the increase in membrane-associated NDPK (Lutz et al. 2003). In contrast, an increase in NDPK content was not observed when hypertrophy was induced with thyroid hormone. Consistent with recent reports showing NDPK translocation by stimulation of GPCRs (Gallagher et al. 2003; Rochdi et al. 2004), these data suggest that sustained stimulation of β-adrenergic receptors increases association of NDPK with the plasma membrane. Taking the recent findings in the zebrafish into account, the primary regulation might occur at the level of mRNA encoding the interacting G protein α subunit (Müller et al. 1994). By a formation of stable heterotrimers of the translated protein with NDPK B/Gβγ complexes, this might cause the enhanced association of NDPK with the plasma membrane without transcriptional regulation of the NDPK B itself.

## Conclusion

Over the last 25 years, a large body of work has been accumulated, which now gives evidence for an important role of the interaction of NDPK with heterotrimeric G proteins. The primary observations, which have been disputed for a long time due to a methodology prone to artefacts, have recently been substantiated by studies in living cells and whole organisms. Although we have not finally solved the question of the physiological and pathophysiological function of NDPK B/G protein complexes yet, we meanwhile obtained a raw picture and certainly, directions in which we have to look further.

Besides the complexation of heterotrimeric G proteins with NDPK B on which this review is focused and the well-documented interactions with GPCRs and effectors, it is meanwhile clear that the additional proteins like regulators of G proteins (for review, see Wieland and Mittmann 2003, Abramow-Newerly et al. 2006), scaffold proteins (Chen et al. 2004; Wang et al. 2005), membrane organising proteins like caveolins (Head et al. 2005) and even proteins, which were, until now, believed not to be interaction partners of heterotrimeric G proteins, e.g. endothelial nitric oxide synthase (Andreeva et al. 2006), a part of cellular signal transduction machineries. Thus, we are far away from understanding the complexity of heterotrimeric G protein-mediated signal transduction pathways on the cellular level. Also, generally accepted mechanisms on how heterotrimeric G proteins principally operate have been questioned lately. A recent report raised the question whether G proteins have to be in the GTP-bound form to be active (Ugur et al. 2005; Wieland and Michel 2005), and there is increasing evidence that not all heterotrimeric G proteins dissociate into Gα and Gβγ upon activation (Bünemann et al. 2003; Frank et al. 2005). Taking further into account that, in in vitro systems, even nucleotide-free Gα subunits are active (Lutz et al. 2002), or heterotrimeric G proteins can be activated by pyrimidine nucleotide triphosphates under conditions excluding high energy phosphate relay (Gille et al. 2005), it is evident that investigations on heterotrimeric G proteins and their interaction partners, like those performed by Karl H. Jakobs since 1970 (Jakobs and Schultz 1970), will provide interesting and relevant topics for several decades of future research.

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