Mol Cell Biochem (2009) 329:51–62
DOI 10.1007/s11010-009-0120-7

The mammalian Nm23/NDPK family: from metastasis control to cilia movement

Mathieu Boissan · Sandrine Dabernat · Evelyne Peuchant · Uwe Schlattner · Ioan Lascu · Marie-Lise Lacombe

Received: 7 January 2009 / Accepted: 2 April 2009 / Published online: 22 April 2009

Abstract Nucleoside diphosphate kinases (NDPK) are encoded by the NME genes, also called NM23. They catalyze the transfer of γ-phosphate from nucleoside triphosphates to nucleoside diphosphates by a ping-pong mechanism involving the formation of a high energy phospho-histidine intermediate [1, 2]. Besides their known functions in the control of intracellular nucleotide homeostasis, they are involved in multiple physiological and pathological cellular processes such as differentiation, development, metastastic dissemination or cilia functions. Over the past 15 years, ten human genes have been discovered encoding partial, full length, and/or tandemly repeated Nm23/NDPK domains, with or without N-or C-terminal extensions and/or additional domains. These genes encode proteins exhibiting different functions at various tissular and subcellular localizations. Most of these genes appear late in evolution with the emergence of the vertebrate lineage. This review summarizes the present knowledge on these multitalented proteins.

M. Boissan · M.-L. Lacombe (✉)
INSERM UMRS_938, UMPC Université Paris 06,
75012 Paris, France
e-mail: Marie-Lise.Lacombe@inserm.fr

S. Dabernat · E. Peuchant
INSERM U876, Université de Bordeaux-2,
33076 Bordeaux, France

U. Schlattner
INSERM U884, Université Joseph Fourier,
38041 Grenoble, France

I. Lascu
Institut de Biochimie et Génétique Cellulaires, CNRS UMR 5095, Université de Bordeaux-2, 33077 Bordeaux, France

Keywords Nucleoside diphosphate kinase · Signaling · Cancer · Mitochondria · Phospholipid

Introduction

In human 10 genes have been identified to be a part of the Nm23/NDPK family (Fig. 1). In this review, the genes will be named NME and proteins Nm23-H1 to -H9 (H stands for the human isoforms). NDPK A to D will be used as synonyms for Nm23-H1 to H4, for which NDPK activity has been unequivocally demonstrated. The tenth gene, RP2 encoding a truncated Nm23/NDPK named XRP2, is not included. NME genes encode one (NME1 to NME6 and NME9) or several (NME7 and NME8), conserved NDPK domains, either full length or truncated. The NDPK domain occurs individually or associated with extra-domains (NME5, NME7 to NME9, and RP2). In addition, a pseudogene encoding a Nm23-H2 homologue was located on chromosome 12q24.31 [3]. All the rodent orthologues have been found in mouse (except for NME9) and rat (except for NME5) at synthenic positions. A phylogenetic tree with the Nm23/NDPK family members divides into two distinct groups [4] (Fig. 2). Group I includes four genes encoding proteins sharing 58 to 88% identity and endowed with NDPK activity (NDPK A to D). Group II includes genes encoding more divergent proteins sharing only 22% to 44% identity with group I enzymes and between each other (Table 1). Most of group II genes are essentially expressed in ciliated structures such as primary cilia and sperm flagella. The only exception is NME6, which is also the only group II member whose gene product has NDPK activity [5]. The Nm23/NDPKs have been shown to be involved in major cellular events such as proliferation, differentiation, development, apoptosis, and metastasis dissemination, through mechanisms still largely unknown.

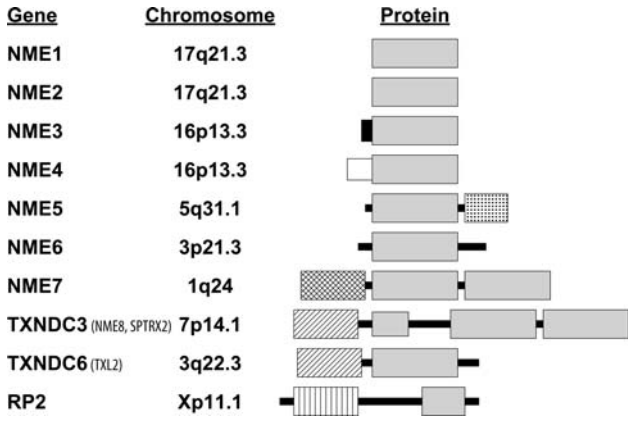

Fig. 1 The Nm23/NDPK family members. The official gene symbols are from the HUGO Gene Nomenclature Committee. The domain organization in the proteins are indicated as follows: ▭: NDPK; ■: hydrophobic peptide; ▭: mitochondrial targeting signal; ▦: dpy-30; ▩: DM10; ▨: thioredoxin and ▥: tubulin-specific chaperone protein co-factor C (TBCC). Alternate gene names are indicated in parentheses. TXNDC6 should be named NME9

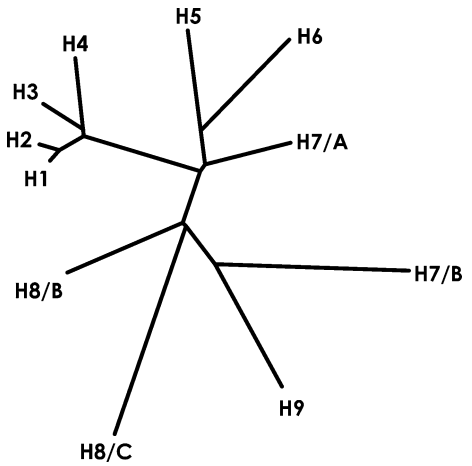

Fig. 2 Phylogenetic tree for the isoforms of the human Nm23/NDP kinase family. It includes NDPK domains of all isoforms except the truncated ones (domain A of the NME8 gene product and XRP2). Information for the tree was obtained from a ClustalW alignment of the common part of the protein sequences. The various isoforms are indicated as H1 to H9

This review will focus on (1) group I family members, essentially the A and B isoforms, their main known partners and the various pathways in which they are involved, (2) the properties specific to the group II family members, (3) data recently released on mitochondrial NDPK, (4) an update on the structural properties of the mammalian NDPKs, and (5) the mouse transgenic models.

Materials and methods

The gene symbols conform the HUGO nomenclature (http://www.genenames.org). The ID numbers of the human

Table 1 Human Nm23/NDP kinases (% identity)

Group I				Group II							
	H2	H3	H4	H5	H6	H7A	H7B	H8A	H8B	H8C	H9
H1	**88**	**67**	**58**	*32*	*31*	*31*	*29*	*NS*	*22*	*28*	*30*
H2	**100**	**65**	**59**	*34*	*33*	*30*	*29*	*NS*	*22*	*28*	*29*
H3		**100**	**61**	*30*	*37*	*29*	*29*	*28*	*25*	*26*	*30*
H4			**100**	*32*	*31*	*29*	*28*	*NS*	*25*	*24*	*26*
H5				*100*	*40*	*44*	*27*	*31*	*35*	*34*	*38*
H6					*100*	*38*	*29*	*31*	*32*	*32*	*35*
H7/A						*100*	*29*	*33*	*36*	*31*	*34*
H7/B							*100*	*NS*	*22*	*33*	*34*
H8/A								*100*	*NS*	*22*	*38*
H8/B									*100*	*26*	*33*
H8/C										*100*	*31*

Group I and II Nm23/NDPKs are represented in bold and italic values, respectively.Values represent the % identity between the NDPK domains of each protein. XRP2 protein, which possesses one partial NDPK domain missing the catalytic histidine and presenting 25 and 21% identity with Nm23-H2 and Nm23-H6, respectively, and no significant homology with the other isoforms, was not included in the table. The various isoforms are indicated as H1 to H10. NS: non significant

NME gene products are from the SwissProt/TrEMBL data base (http://expasy.org/sprot): for NME1: P15531; NME2: P22392; NME3: Q13232; NME4: O0746; NME5: P56597; NME6: O75414; NME7: Q9Y5B8; NME8 (=TXCDN3): Q8N427; TXCDN6 (=TXCL2=NME9): Q86XW9 and for RP2: O75695. The Nm23/NDPK sequences of other organisms can be found within the same website or at http://www.ncbi.nlm.nih.gov. Alignments were performed using the ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and the percentages of identity were obtained using BLAST programs of the ncbi and expasy sites. The tree was drawn according to a program available at: http://www.phylogeny.fr [6]. Expression data can be obtained at: http://symatlas.gnf.org/SymAtlas.

The Nm23/NDPKs family members (group I)

Within group I, all the proteins are enzymatically active (Fig. 3) and hexameric [4]. NDPK A and B, which are 88% identical at the protein level, are the most abundant and, by far, the most studied, especially because NDPK A is a metastasis suppressor [7, 8]. Both are mainly cytoplasmic enzymes, but they can be also found, at least transiently, associated to membranes and in nuclei (Herak-Bosnar, this issue). Transcripts encoding a longer form of Nm23-H1 (Nm23-H1B; [9]) and a read-through transcript encoding part of NDPK A and the complete NDPK B [10] have been reported but with no specific localization or function of the

Nm23 / NDPK	Expression	Subcellar localization	NDPK activity
H1	**ubiquitous**	**Cytoplasm/nuclei**	**+**
H2	**ubiquitous**	**Cytoplasm/nuclei**	**+**
H3	**ubiquitous**	**Cytoplasm/mito?**	**+**
H4	**ubiquitous**	**Mitochondria**	**+**
H5	flagella, cilia	Microtubule assoc.	–
H6	ubiquitous	Cytoplasm / Mito.	+/–
H7	flagella, cilia	?	ND
H8	flagella, cilia	?	–
H9	flagella, cilia	Microtubule assoc.	ND
H10	ubiquitous	Cell membrane	ND

Fig. 3 Expression, subcellular localization and enzymatic activity of the various human Nm23/NDPKs. Group I and II Nm23/NDPKs are in black and gray letters, respectively. (−) indicates that the purified recombinant protein was devoid of catalytic activity. ND: not determined

corresponding proteins. NDPK C (also named Dr-Nm23) is involved in differentiation and apoptosis of myeloid and neuroblastoma cells [11, 12]. It was reported to be, at least partly, located to mitochondria [13] but only NDPK D possesses the canonical mitochondria targeting sequence and is exclusively located in this organelle [14, 15] (details further below). Besides their role in nucleoside triphosphate synthesis, a variety of other functions have been attributed to Nm23/NDPKs. These include DNA binding, transcription modulation, and DNA cleavage [16–18]. It has also been reported that the high-energy phosphate of the intermediate in the NDPK reaction could be transferred to other proteins suggesting a role as a "histidine kinase" [19–22]. Identifying partners of the Nm23/NDPK proteins should provide new insight into the cellular functions of these multi-faceted proteins.

GTP-binding proteins-dependent pathways

Among the multiple proteins interacting with the NDPK/Nm23 family members, many are related directly or indirectly to the small and heterotrimeric G-proteins, to their exchange factors or to their regulatory factors. Because group I Nm23 proteins are NDP kinases responsible for GTP synthesis, it was hypothesized that these proteins might activate G-protein [23]. A mechanism involving the direct phosphorylation of the GDP bound to the G-protein to form G-protein-GTP by the NDPKs has been proposed but was never proven and was sterically unlikely [24]. Another mechanism would be the GTP channeling by NDPKs in close vicinity of the G-proteins and their activation through the GDP–GTP exchange reaction. An effective channeling would require an interaction between NDPK and G-proteins. However, the different reports in the literature are paradoxically more related to an inhibitory role of Nm23 on small G-protein activity, due to a sequestration of the G-protein itself or of its exchange or regulatory factors, for example Rad [25, 26], Tiam1 (Rac1) [27], KSR (Ras) [28], Lbc (RhoA) [29], Dbl-1 (cdc42) [30]. Dynamin, a GTPase playing a critical role in endocytic coated vesicle formation, has been shown to directly interact with Nm23 ([31]; see below).

Nm23-H1/H2 were proposed to play a nucleotide-charging role for tubulin during the process of microtubule assembly. Indeed, several studies reported the Nm23 co-purification with microtubules. However, no direct interaction with Nm23-H1/H2 could be observed [32–34]. Interestingly, the group II members, Nm23-H8 [35] and Nm23-H9 [36], were shown to directly bind microtubules.

Considering heterotrimeric G-proteins, in addition to the receptor-induced GDP/GTP exchange, G protein alpha subunit can be activated by phosphate transfer via a plasma membrane-associated complex of NDPK B and G protein beta–gamma dimers. NDPK B acts as a histidine kinase phosphorylating Gβ at a histidine residue [21]. It should be mentioned that it is a NDPK dimer, which transfer its phosphate to a surface-located histidine on the G$\beta\gamma$. The phosphate can then be transferred to GDP forming GTP subsequently activating Gαs and Gαi (for review: [22]). A phosphohistidine phosphatase, which is capable to specifically dephosphorylate the G$\beta\gamma$ dimer, is also a potential regulator of this pathway. The heterotrimeric G-protein activation by NDPK B, independent of the classical G protein coupled receptor (GPCR) induced GDP/GTP exchange, contributes to the regulation of basal cAMP level in the cell [37]. Interestingly, the observed increase in NDPK content of plasma membranes in hearts from patients suffering of congestive heart failure [38, 39] could be of pathophysiological importance. Concerning an other group I NDPK isoform, a yeast two-hybrid screen of a human brain cDNA library identified Nm23-H3 as an interacting partner of the $(5\text{-HT})_{2A}$ serotonin receptor, a GPCR [40].

Regulation of vesicular trafficking and cell adhesion

Several data point to a role of Nm23 in the secretory and endocytic pathways to regulate vesicle trafficking. Nm23-H2 interacts with COPII, the cytosolic coat protein complex II that mediates vesicle formation from the endoplasmic reticulum (ER) and which is essential for ER to Golgi trafficking [41]. Accordingly, the involvement of NDPK in vesicle movement was evidenced in lower organisms. Indeed, a proteomic approach with the *Dictyostelium discoideum* amoeba identified a NDPK associated to secretory vesicles typical of the prespore differentiation stage [42, 43]. Genetic studies have shown that the drosophila homolog of Nm23, Awd, regulates dynamin-dependent internalization processes such as synaptic vesicle endocytosis [44] and vesicle transport-mediated

turnover of homologs of FGF and PDGF/VEGF receptor [45, 46]. Facilitation by Nm23-H1 of dynamin-based endocytosis, in particular of E-cadherin, was also reported in epithelial mammalian cells during the disassembly of adherens junctions [47]. This result is not in accordance with the metastasis suppressor role of Nm23-H1 since E-cadherin internalization is expected to disrupt cell–cell contacts. Possibly, maintenance of E-cadherin at the cell surface is a dynamic process where Nm23 regulates the endocytosis/recycling balance. A role of Nm23 in endocytosis may explain Nm23 functions in metastasis suppression through the downregulation of cell surface receptors involved in cell motility and angiogenesis and the arrest in signaling pathways. Recently, the lysophosphatidic acid receptor EDG2 was found to be overexpressed in metastatic cancer cells expressing low levels of Nm23-H1 [48]. It would be interesting to determine whether a similar endocytic mechanism by Nm23 is applicable to EDG2 regulation.

Nm23-H2 was found to bind to ICAP1-α, a negative regulator of cell adhesion mediated by β1 integrin supporting cell migration [49]. Thus, Nm23 could exert its metastatic suppressor activity by sequestering ICAP1-α and antagonizing its function.

Other interactions

Nm23-H1 and -H2 were identified as DNA binding factors involved in the control of transcription [16] and possessing DNAse activity ([50]; Kaetzel, this issue). These Nm23 isoforms were also reported to interact with nuclear receptors such as estrogen receptors and members of the ROR/RZR nuclear orphan receptor family [51–53]. Moreover, the interaction between viral antigens of the Epstein-Barr virus such as EBNA-3C and EBNA1 with Nm23-H1 suppresses the ability of Nm23-H1 to inhibit tumor cell migration, therefore promoting metastasis [54, 55]. Both viral antigens overcome the anti-metastatic activity of Nm23-H1 in nude mice [56]. Similarly, the E7 oncoprotein of human papilloma virus binds Nm23-H1 and regulates tumor cell motility [57]. A link with apoptosis has been established by the study of Fan et al. [17], who demonstrated that Nm23-H1 is a granzyme A-activated DNase during T lymphocyte-mediated apoptosis and that the nucleosome assembly protein SET binds to and inhibits Nm23-H1.

Nm23-H1 interacts with STRAP, a TGF-β receptor-interacting protein, and inhibits TGF-β signaling [58]. Nm23-H1, when unbound to STRAP under DNA damage or other stress conditions, interacts with p53 and positively regulates its function, including p53-induced apoptosis and cell cycle arrest. p53 activation by Nm23-H1 is mediated by decreasing the association between p53 and mdm2, a negative regulator of p53 [59]. In addition, a very recent report describes a direct interaction between Nm23-H1 and the macrophage migration inhibitory factor (MIF). This interaction is critical for alleviation of MIF-mediated suppression of p53 activity by promoting the dissociation of MIF from MIF–p53 complex [60]. The interaction between Nm23-H2 and Diva, a member of the Bcl-2 family, suggests another mechanism by which Nm23 could regulate apoptosis [61]. An additional partner of Nm23 is h-prune, which belongs to the superfamily of phosphoesterases [62]. The Zollo group (see this issue) has shown that Nm23-H1 forms a complex with h-prune. This interaction results in a decrease in the level of free Nm23-H1 and promotes metastasis [63].

In addition, the Skolnik group demonstrates a new role of NDPK in the control of the Ca^{++} activated K^{+} channel KCa3.1 activity by direct histidine phosphorylation of the channel alpha subunit and its dephosphorylation by a histidine phosphatase [64, 65]. This is another example, in addition to the heterotrimeric G proteins, of histidine phosphorylation/dephosphorylation influencing a biological process in mammals.

In conclusion, the complexity of the described interactions identifies Nm23 proteins as multifunctional proteins. Moreover, these interactions modulate biological functions of either Nm23 isoforms or their binding partners. This hints at the multiple ways by which Nm23 could affect cell signaling and cell functions. In particular, interactions with binding partners are thought to sequester bioavailable Nm23, thereby antagonizing biological properties of Nm23 such as its metastasis suppressive function.

The Nm23/NDPKs family members (group II)

In group II, NDPK enzymatic activity has been demonstrated only for the product of NME6 [5] (Fig. 3). Two other members can now join this group. TXNDC6 (thioredoxin domain containing 6) is also named TXL2 (thioredoxin-like 2) [36]. This gene is not yet mentioned as NME9 in data banks, but according to the nomenclature agreed in the NM23 scientific community, it should be named NME9. Like Nm23-H8, it possesses a N-terminal thioredoxin domain (46% identical), but is followed by one, instead of three, NDPK domain containing the catalytic histidine. The 10th family member is the RP2 gene, which encodes the XRP2 protein involved in X-linked retinitis pigmentosa, an inherited affection of the retina due to photoreceptor degeneration [66].

No additional information on NME6 and NME7 gene products has been provided since our previous review [4]. Despite their thioredoxin domain, no thioredoxin activity could be detected in Nm23-H8 and TXCDN6. Importantly,

all the members of group II, except for NME6, encode proteins mainly located in cilia and/or spermatozoa flagella. Nm23-H5 was located in the flagella of spermatids and spermatozoa, adjacent to the central pair and outer doublets of axonemal microtubules [67]. The Nm23-H5 NDPK domain is fused at its C terminus to a Dpy-30 domain, shown to be a member of the histone H3 methylation complex in yeast and to be involved in the regulation of X chromosome dosage compensation in *C. elegans* [68]. Nm23-H7 possesses a DM10 domain followed by two tandemly repeated full-length NDPK domains [4]. The DM10 domain might act as a flagellar NDPK regulatory module or as a unit involved specifically in axonemal targeting or assembly [69]. NME8 was first reported to be exclusively expressed in testis, mainly in primary spermatocytes and the protein was reported to be an integral component of the fibrous sheet of spermatozoa [70]. The organization into three NDPK domains found in Nm23-H8 was also found in dynein intermediate chains of sea urchin sperm and *Ciona intestinalis* flagellum axoneme [71, 72]. Since no orthologs have been found in higher organisms, it was suggested that during evolution, the NME8 ancestor shifted from an axonemal to a periaxonemal localization [73]. Recently, NME8 mRNA was also detected in human trachea and nasal cells suggesting a role in other ciliated structures [35]. Indeed, a mutation in the gene was found in primary ciliary dyskinesia, a genetic disease characterized by failure in cilia movement resulting in chronic respiratory tract infection, left–right asymmetry randomization and male infertility. This finding discloses a key role of NME8 in ciliary functions [35]. Curiously, single-nucleotide polymorphisms (SNP) in the 5′ region of the NME8 gene were associated with osteoarthritis [74]. NME9 is highly expressed in testis but also in lung and other ciliated cell containing tissues. The protein is associated with microtubular structures such as lung airway epithelium cilia and the manchette and axoneme of spermatids. Both NME8 and NME9 encode proteins possessing an N-terminal thioredoxin domain with the conserved residues of the active site (CGPC) but thioredoxin activity has not been demonstrated and their function is unknown in flagella [70].

XRP2 contains two major domains. The N-terminal domain shares amino acid sequence similarity with the tubulin-specific chaperone protein co-factor C (TBCC). This domain acts as a GTPase-activating protein (GAP) for beta-tubulin [75] and is also an efficient GAP for the small G protein Arf-like 3 [76]. The C-terminal domain of XRP2 is about 25% identical with the N terminal half of NDPK B and is lacking the catalytic histidine. Most of the reported disease linked RP2 mutations are expected to result into truncated proteins with loss of the C-terminal domain. Interestingly, XRP2, like Nm23-H1 [50] and Nm23-H5, -H7, and -H8 [77], exhibits DNA exonuclease activity [78].

Remarkably, among group I protein, NDPK A and B are differently expressed and located in postmeiotic maturating germinal cells with a transient nuclear localization of NDPK A in round spermatids. Both proteins are present in mature sperm flagella [67] and in primary cilia [79]. Therefore, members of both NDPK groups could play a role in the flagellar motility and in the ciliary functions. They could also be involved in intraflagellar transport (IFT), the motor-dependant movement of particles along the axoneme [80].

The mitochondrial NDPKs

A NDPK activity specific to mitochondria was described in 1955 [81], two years after the discovery of the enzyme. The activity was reported in both the matrix and the extra-matrix compartments, and also associated with the contact sites connecting the inner and outer membranes [82]. The submitochondrial localization was greatly variable, depending on the species and the tissue examined. In mammals, a prominent matrix activity was found in the heart while in the liver the activity was mainly associated with an extra-matrix compartment [83]. The membrane-bound or soluble state of the enzyme remained often undefined.

Many functions have been proposed for mitochondrial NDPK. In the matrix, the enzyme can provide NTP for protein and nucleic acid synthesis or can be associated to the Krebs cycle to synthesise ATP at the expense of GTP provided through succinylthiokinase [84]. A matrix NDPK was also proposed to be involved in short chain fatty acid catabolism, and very recently to play a role in iron homeostasis by furnishing GTP [85]. In the intermembrane/cristae space, NDPK was proposed to supply cellular NTP at the expense of NDP, freely diffusible from cytoplasm through the outer membrane, and of ATP, synthesized by oxidative phosphorylation [86].

In mammals, the NME4 gene encodes an exclusive mitochondrial isoform, NDPK D, possessing a specific organelle targeting sequence [14, 87]. Two other human genes, NME3 (encoding NDPK C) and NME6, have been reported to encode proteins that are, at least partly, located to mitochondria [5, 13]. However, no specific mitochondrial targeting sequence could be detected in these proteins and no submitochondrial localization was reported, although NDPK C possesses a 17 aa N-terminal hydrophobic peptide which could anchor the protein to membranes [88]. NDPK D is bound to the inner membrane through an electrostatic interaction between a basic motif (Arg89-Arg90-Lys91), unique to NDPK D and located at the surface of the hexamer,

and cardiolipin, a phospholipid specific of the mitochondrial inner membrane. Mutation of the central arginine (R90D) strongly reduces the phospholipid interaction in model liposomes containing anionic phospholipids or with mitochondrial membranes in HeLa cells in vivo. In mitochondria, NDPK D exhibits a preferred orientation toward the intermembrane space. However, a subpopulation of proteins is only accessible to substrates or antibodies if the inner membrane is lysed by detergent, indicating that the enzyme can also face the matrix or may be hidden within complexes or cristae structures. In HeLa cells, with naturally almost undetectable NDPK-D, stable expression of wild type but not of R90D mutant led to membrane-bound enzyme in vivo. Respiration was significantly stimulated by the NDPK substrate TDP in mitochondria containing wild-type NDPK-D, but not in those expressing R90D mutant yet catalytically equally active. This indicates local ADP regeneration in the mitochondrial intermembrane space and a tight functional coupling of NDPK-D with oxidative phosphorylation, which depends on its membrane-bound state [15]. Corroborating such a model, plant mitochondrial NDPK was found associated with the adenine nucleotide translocator (ANT) [89].

NDPK-D was also able to cross-link anionic phospholipid-containing liposomes in vitro, owing to its symmetrical hexameric structure exposing three R90 on two opposite faces, suggesting that NDPK-D could promote intermembrane contacts. This may be the structural basis for another new and unexpected property of NDPK D, which is facilitating intermembrane lipid transfer [90]. Using different in vitro liposome model systems, lipid transfer between two bilayers occured as soon as cardiolipin was present in the membranes, i.e., NDPK was in its membrane-bound state. These data shed a new light on yet undescribed functions of a mitochondrial NDPK.

Structural basis of Nm23 regulatory mechanisms

Since the last review on the Nm23 structure [91], little progress has been made. All group I mammalian NDPKs solved in crystal were found to be hexameric and no structure of group II proteins have been reported. The crystal structure of NDPK A/Nm23-H1 has been obtained by three groups (PDB code 1JXV, [92]; PDB code 1UCN, complexed with ADP, Ca2+ and Pi, [93]; PDB code 2HVD complexed with ADP, [94]). In addition, structures of Nm23-H3 (PDB code 1ZS6; no publication) and that of the Nm23-H1 S120G mutant (PDB code 2HVE) found in neuroblastoma [95] have also been solved [94]. The Nm23-H3 structure has been anticipated in a model published by Erent et al. [96] based on the crystal structure of the Nm23-H2 [97]. From these data, it can be concluded that all group I NDPKs possess almost identical 3D structures. Nm23-H3 contains a hydrophobic 17 aa N-terminal extension (as compared to Nm23-H1 and -H2) which could not be modeled and is, therefore, not present in the PDB files. Structure and function of this unique N-terminal extension, possibly responsible for targeting Nm23-H3 to membranes has not been addressed yet.

The crystal structures of the wild type and S120G mutant of Nm23-H1 are identical, except for the absence of the serine side-chain in close vicinity of the catalytic histidine (His118). No water molecule is present in the mutant protein in the position of the serine γ-oxygen of wild type protein. The absence of a sizeable structural effect of the mutation in the native state would explain their similar catalytic parameters. The differential cellular effects could be due to the presence of the mutated protein in a partially unfolded form. As it has been shown earlier through denaturation–renaturation experiments in vitro, the mutant protein accumulated as a "molten globule" folding intermediate rather than native monomer [98]. We can assume that this form accumulated within the cell and induced deleterious effects. However, it crystallized as an hexamer with correctly folded monomer, due to the stabilizing effect of the quaternary structure [99]. Recently, Zhou et al. [100] studied the effect of the double mutant P96S/S120G of Nm23-H1. This mutant combines the folding defect of S120G mutation with the Killer-of-prune mutation, which destabilizes the hexamer [101]. The authors showed that the protein was present in cells upon transfection of the mutant cDNA, but was enzymatically inactive, had lost the migration inhibitory effect observed with the wild type enzyme and was therefore non-native. This interesting double mutant might be useful for studying the effects of the protein in a non-native state (probably a folding intermediate) in cells. It also demonstrates the importance of the hexameric structure to maintain the S120G mutant protein in its native state.

Finally, it was puzzling to observe that the phosphorylation of the active site of the S120G mutant stabilizes the native hexamer [102]. A detailed analysis of the quaternary structure of Nm23 and of its mutants in vivo is still missing.

Transgenic mouse models

The human and murine NDPKs are highly homologous [103]; human NDPK A and B share 94 and 98% identity with their mouse counterparts, respectively. In the mouse, NDPK B protein displays high levels of expression in most tissues while NDPK A protein expression is more restricted to tissues originating from the ectoderm such as the

nervous system and the epithelia of tissues developing through mesenchymal/epithelial interactions followed by branching morphogenesis such as the lungs, the kidneys, and the mammary gland [103]. The mouse NME1 gene invalidation was carried out to provide a powerful model for studying the functions of NDPK A [104]. Absence of NDPK A did not affect the overall embryonic development and the animals reached adulthood viable and fertile. A modest negative impact on body weight was observed and NDP kinase activity was decreased accordingly to NME1 gene expression in several tested tissues, suggesting that the loss of enzymatic activity was not compensated by other isoforms. Interestingly, NME1$^{-/-}$ females were not able to feed their litters since the pups died of dehydration within the first 3 days of life. This trait is dependent on the genetic background since the severity of postnatal death was higher in congenic strains as compared to that observed in litters from mixed genetic backgrounds. Further analysis showed that NME1 expression was detected in the early anlagen of the mammary gland epithelium and was maintained throughout the maturation of the gland. Macroscopic analysis of whole mount glands showed delayed ductal branching and elongation in the mutant glands, which became normal upon gestation and parturition, suggesting that the lack of NDPK A slows down the growth of the mammary gland. Despite a normal milk production, and apparent secretion of the milk in the lactiferous ducts, the absence of NDPK A leads to deficiency in the final ejection at the level of the nipples (SAD, EP, unpublished data), through unknown mechanisms. This observation is under current analysis.

The NME1-deficient mouse strain allowed to study in vivo the impact of the absence of NDPKA protein on metastatic dissemination. Double transgenic animals were produced by crossing NME1$^{-/-}$ mice with the ASV strain, displaying SV40 large T antigen expression in the liver and prone to hepatocellular carcinomas (HCC) [105]. In parallel, HCC in WT or mutant mice were induced by diethylnitrosamine injection. In both models of HCC, the lack of NDPK A had no effect on the number or amplitude of growth of preneoplastic and neoplastic lesions despite an increase in NME1 and/or NME2 gene expression during hepatocellular carcinogenesis. Very interestingly, in the ASV HCC model, the metastasis incidence to the lungs was increased in mutant mice and was associated with a loss of NME1 expression in the WT. This study was proving for the first time in vivo the anti-metastatic activity of the NME1 gene [105]. However, the mechanisms involved are still largely unsolved.

Interestingly, genes identified by differential transcriptomic analyses of carcinoma cell lines, overexpressing or not NME1, were also shown to be deregulated in the tumoral liver of NME1$^{-/-}$ mice (see reviews of the group of P. Steeg in the same issue). The invalidation of NME2 gene has not been reported. However, the double knock out of NME1 and NME2 genes was obtained, facilitated by the localization of both genes on the same chromosome about 5 kbp apart [106]. This strain is currently analyzed by the group of Edith Postel. The double NME1/NME2 knock-out mice are severely hypotrophic and die at birth probably due to severe anemia resulting of major defects in erythropoeisis (Postel et al., personnal communication and this issue).

Concluding remarks

The multiplicity of the Nm23/NDPK family members suggests that Nm23/NDPK proteins possess different and specific functions within the cell, depending on their tissular and subcellular localization. Over the past 15 years, ten human genes of the Nm23/NDPK family have been discovered that can be separated into two groups based on the analysis of their sequences. Members of group I are ubiquitous. It is fascinating to observe how they regulate complex cell signaling pathways from development to metastasis dissemination, requiring or not the nucleoside diphosphate kinase activity. It was shown that, in networks with other nucleotide metabolizing enzymes such as adenylate kinases, creatine kinases and glycolytic enzymes, NDPKs participate in high energy phosphoryl transfer and signal communication in the cell [107].

The abundant list of Nm23 binding proteins illustrates the multifunctionality of group I members, being much more than « house keeping enzymes » . All the group I recombinant isoforms can form heterohexamers (I. L., M.L. L., unpublished data). If this is also the case in vivo, formation of such heteropolymers could introduce a supplementary level of regulation within the cell. It is noteworthy to mention here that the heterohexameric state as well as the intracellular oligomeric state (monomer, dimer, hexamer,…) of NDPKs are still open issues. Interestingly, a GTP-dependent organization of NDPK B into ordered filaments in vitro was reported very recently [108]. The physiological relevance of this polymer formation deserves further investigation. In addition, many examples in the literature suggest that Nm23-H1 and Nm23-H2 may be present but functionally inactivated by interacting proteins. This is particularly relevant for the anti-metastatic activity of the Nm23-H1 isoform that is lost, even in the presence of this isoform as soon as it is sequestered by different binding partners. To date, only two mouse gene knock-out models are available, invalidating either NME1 or both, NME1 and NME2. Transgenic murine models for the other NME genes would be of great interest to decipher their functions.

Group II includes proteins that are, except Nm23-H6, mainly related to ciliary functions. Importantly, a mutation in the NME8 gene was found responsible of a primary ciliary

dyskinesia [35] demonstrating that a NME gene mutation could be responsible for a genetic disease. Interestingly, only proteins of the group II are associated with extra-domains such as Dpy-30, DM10, and thioredoxin. These domains could regulate Nm23 localization and/or functions possibly by modulating their interaction with various partners.

Another remark of great interest is the recent identification of a charged motif at the surface the hexameric NDPK [15, 109]. This motif is composed of three basic residues (R-R-K) in NDPK D and is responsible for the NDPK D binding to mitochondria inner membrane phospholipids, especially cardiolipin. In NDPK A to C, this motif is composed of one acidic residue surrounded by two basic residues (K/R-D/E-R) and was shown, very recently, to be responsible for NDPK B binding to phosphatidyl inositols and phosphatidic acid of model liposomes and ER [109]. This interaction could scaffold extended membrane networks, and suggest a possible role for NDPK B, as well as phosphoinositides and/or acidic phospholipids, in modulating ER membrane network morphogenesis. NDPK A possesses the same motif (K-D-R), which could be involved in its nucleotide dependent binding to membrane phospholipids, as very recently reported [110]. This finding could provide new research fields for the future.

In conclusion, over the past couple of decades, extensive analysis has been done to decipher the roles of NME genes in the physiopathological aspects of the cell. Data have been accumulated showing how complex and versatile this family of genes was. We started from one enzymatic activity to 10 different isoforms with specific features. The roles of these different isoforms undoubtedly require specific targeting or location within the cell. Major advances have been made since P. Steeg's pioneering first assumption [7] on the anti-metastatic potential of the NME1 gene. However, the precise mechanisms are still under question and more work is needed to obtain a clear model of the role of Nm23 proteins in cell physiology.

Acknowledgments The authors are greatly indebted to Pr. J. Y. Daniel, who created the first NM23 transgenic mice, for his continued interest in these works. We thank Yves Chrétien for assistance in figure preparation and C. Mailleau for technical assistance. This work was supported by the Germaine de Stael program for Franco-Swiss collaboration (U.S.; M.L.L.), the Agence Nationale de la Recherche (chaire d'excellence to U.S.), the Institut National de la Santé et de la Recherche Médicale (INSERM) and grants (to M.L.L.) from the Groupement des Entreprises Françaises contre le Cancer (GEFLUC) and from the Association pour la Recherche contre le Cancer (ARC).

References

1. Cleland WW (1963) The kinetics of enzyme-catalyzed reactions with two or more substrates or products. III. Prediction of initial velocity and inhibition patterns by inspection. Biochim Biophys Acta 67:188–196. doi:10.1016/0006-3002(63)91816-X
2. Norman AW, Wedding RT, Black MK (1965) Detection of phosphohistidine in nucleoside diphosphokinase isolated from Jerusalem artichoke mitochondria. Biochem Biophys Res Commun 20:703–709. doi:10.1016/0006-291X(65)90073-2
3. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chevre JC, Froguel P, Bell GI (1996) Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature 384:455–458. doi:10.1038/384455a0
4. Lacombe ML, Milon L, Munier A, Mehus JG, Lambeth DO (2000) The human Nm23/nucleoside diphosphate kinases. J Bioenerg Biomembr 32:247–258. doi:10.1023/A:1005584929050
5. Tsuiki H, Nitta M, Furuya A, Hanai N, Fujiwara T, Inagaki M, Kochi M, Ushio Y, Saya H, Nakamura H (1999) A novel human nucleoside diphosphate (NDP) kinase, Nm23-H6, localizes in mitochondria and affects cytokinesis. J Cell Biochem 76:254–269. doi:10.1002/(SICI)1097-4644(20000201)76:2<254::AID-JCB9>3.0.CO;2-G
6. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:W465–W469. doi:10.1093/nar/gkn180
7. Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, Sobel ME (1988) Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 80:200–204. doi:10.1093/jnci/80.3.200
8. Steeg PS (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895–904. doi:10.1038/nm1469
9. Ni X, Gu S, Dai J, Cheng H, Guo L, Li L, Ji C, Xie Y, Ying K, Mao Y (2003) Isolation and characterization of a novel human NM23-H1B gene, a different transcript of NM23-H1. J Hum Genet 48:96–100. doi:10.1007/s100380300014
10. Valentijn LJ, Koster J, Versteeg R (2006) Read-through transcript from NM23-H1 into the neighboring NM23-H2 gene encodes a novel protein, NM23-LV. Genomics 87:483–489. doi:10.1016/j.ygeno.2005.11.004
11. Venturelli D, Martinez R, Melotti P, Casella I, Peschle C, Cucco C, Spampinato G, Darzynkiewicz Z, Calabretta B (1995) Overexpression of DR-nm23, a protein encoded by a member of the nm23 gene family, inhibits granulocyte differentiation and induces apoptosis in 32Dc13 myeloid cells. Proc Natl Acad Sci USA 92:7435–7439. doi:10.1073/pnas.92.16.7435
12. Amendola R, Martinez R, Negroni A, Venturelli D, Tanno B, Calabretta B, Raschella G (2001) DR-nm23 expression affects neuroblastoma cell differentiation, integrin expression, and adhesion characteristics. Med Pediatr Oncol 36:93–96. doi:10.1002/1096-911X(20010101)36:1<93::AID-MPO1021>3.0.CO;2-3
13. Negroni A, Venturelli D, Tanno B, Amendola R, Ransac S, Cesi V, Calabretta B, Raschella G (2000) Neuroblastoma specific effects of DR-nm23 and its mutant forms on differentiation and apoptosis. Cell Death Differ 7:843–850. doi:10.1038/sj.cdd.4400720
14. Milon L, Meyer P, Chiadmi M, Munier A, Johansson M, Karlsson A, Lascu I, Capeau J, Janin J, Lacombe ML (2000) The human nm23-H4 gene product is a mitochondrial nucleoside diphosphate kinase. J Biol Chem 275:14264–14272. doi:10.1074/jbc.275.19.14264
15. Tokarska-Schlattner M, Boissan M, Munier A, Borot C, Mailleau C, Speer O, Schlattner U, Lacombe ML (2008) The nucleoside diphosphate kinase D (NM23-H4) binds the inner

mitochondrial membrane with high affinity to cardiolipin and couples nucleotide transfer with respiration. J Biol Chem 283:26198–26207. doi:10.1074/jbc.M803132200
16. Postel EH (2003) Multiple biochemical activities of NM23/NDP kinase in gene regulation. J Bioenerg Biomembr 35:31–40. doi:10.1023/A:1023485505621
17. Fan Z, Beresford PJ, Oh DY, Zhang D, Lieberman J (2003) Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. Cell 112:659–672. doi:10.1016/S0092-8674(03)00150-8
18. Kaetzel DM, Zhang Q, Yang M, McCorkle JR, Ma D, Craven RJ (2006) Potential roles of 3′-5′ exonuclease activity of NM23-H1 in DNA repair and malignant progression. J Bioenerg Biomembr 38:163–167. doi:10.1007/s10863-006-9040-3
19. Engel M, Veron M, Theisinger B, Lacombe ML, Seib T, Dooley S, Welter C (1995) A novel serine/threonine-specific protein phosphotransferase activity of Nm23/nucleoside-diphosphate kinase. Eur J Biochem 234:200–207. doi:10.1111/j.1432-1033.1995.200_c.x
20. Steeg PS, Palmieri D, Ouatas T, Salerno M (2003) Histidine kinases and histidine phosphorylated proteins in mammalian cell biology, signal transduction and cancer. Cancer Lett 190:1–12. doi:10.1016/S0304-3835(02)00499-8
21. Cuello F, Schulze RA, Heemeyer F, Meyer HE, Lutz S, Jakobs KH, Niroomand F, Wieland T (2003) 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 278:7220–7226. doi:10.1074/jbc.M210304200
22. Wieland T (2007) Interaction of nucleoside diphosphate kinase B with heterotrimeric G protein betagamma dimers: consequences on G protein activation and stability. Naunyn Schmiedebergs Arch Pharmacol 374:373–383. doi:10.1007/s00210-006-0126-6
23. Kimura N (1993) Role of nucleoside diphosphate kinase in G-Protein action. In: Dickey BF, Birnbaumer L (eds) Handbook of experimental pharmacology. Springer-Verlag, New York, pp 485–496
24. Otero AS (2000) NM23/nucleoside diphosphate kinase and signal transduction. J Bioenerg Biomembr 32:269–275. doi:10.1023/A:1005589029959
25. Tseng YH, Vicent D, Zhu J, Niu Y, Adeyinka A, Moyers JS, Watson PH, Kahn CR (2001) Regulation of growth and tumorigenicity of breast cancer cells by the low molecular weight GTPase Rad and nm23. Cancer Res 61:2071–2079
26. Zhu J, Tseng YH, Kantor JD, Rhodes CJ, Zetter BR, Moyers JS, Kahn CR (1999) Interaction of the Ras-related protein associated with diabetes rad and the putative tumor metastasis suppressor NM23 provides a novel mechanism of GTPase regulation. Proc Natl Acad Sci USA 96:14911–14918. doi:10.1073/pnas.96.26.14911
27. Otsuki Y, Tanaka M, Yoshii S, Kawazoe N, Nakaya K, Sugimura H (2001) Tumor metastasis suppressor nm23H1 regulates Rac1 GTPase by interaction with Tiam1. Proc Natl Acad Sci USA 98:4385–4390. doi:10.1073/pnas.071411598
28. Hartsough MT, Morrison DK, Salerno M, Palmieri D, Ouatas T, Mair M, Patrick J, Steeg PS (2002) Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. J Biol Chem 277:32389–32399. doi:10.1074/jbc.M203115200
29. Iwashita S, Fujii M, Mukai H, Ono Y, Miyamoto M (2004) Lbc proto-oncogene product binds to and could be negatively regulated by metastasis suppressor nm23-H2. Biochem Biophys Res Commun 320:1063–1068. doi:10.1016/j.bbrc.2004.06.067
30. Murakami M, Meneses PI, Knight JS, Lan K, Kaul R, Verma SC, Robertson ES (2008) Nm23-H1 modulates the activity of the guanine exchange factor Dbl-1. Int J Cancer 123:500–510. doi:10.1002/ijc.23568
31. Baillat G, Gaillard S, Castets F, Monneron A (2002) Interactions of phocein with nucleoside-diphosphate kinase, Eps15, and Dynamin I. J Biol Chem 277:18961–18966. doi:10.1074/jbc.M108818200
32. Gallagher BC, Parrott KA, Szabo G, de Otero S (2003) A receptor activation regulates cortical, but not vesicular localization of NDP kinase. J Cell Sci 116:3239–3250. doi:10.1242/jcs.00630
33. Islam K, Burns RG (1985) Microtubules and nucleoside diphosphate kinase. Nucleoside diphosphate kinase binds to co-purifying contaminants rather than to microtubule proteins. Biochem J 232:651–656
34. Melki R, Lascu I, Carlier MF, Veron M (1992) Nucleoside diphosphate kinase does not directly interact with tubulin nor microtubules. Biochem Biophys Res Commun 187:65–72. doi:10.1016/S0006-291X(05)81459-7
35. Duriez B, Duquesnoy P, Escudier E, Bridoux AM, Escalier D, Rayet I, Marcos E, Vojtek AM, Bercher JF, Amselem S (2007) A common variant in combination with a nonsense mutation in a member of the thioredoxin family causes primary ciliary dyskinesia. Proc Natl Acad Sci USA 104:3336–3341. doi:10.1073/pnas.0611405104
36. Sadek CM, Jimenez A, Damdimopoulos AE, Kieselbach T, Nord M, Gustafsson JA, Spyrou G, Davis EC, Oko R, van der Hoorn FA, Miranda-Vizuete A (2003) Characterization of human thioredoxin-like 2. A novel microtubule-binding thioredoxin expressed predominantly in the cilia of lung airway epithelium and spermatid manchette and axoneme. J Biol Chem 278:13133–13142. doi:10.1074/jbc.M300369200
37. Hippe HJ, Luedde M, Lutz S, Koehler H, Eschenhagen T, Frey N, Katus HA, Wieland T, Niroomand F (2007) Regulation of cardiac cAMP synthesis and contractility by nucleoside diphosphate kinase B/G protein beta gamma dimer complexes. Circ Res 100:1191–1199. doi:10.1161/01.RES.0000264058.28808.cc
38. Lutz S, Mura R, Baltus D, Movsesian M, Kubler W, Niroomand F (2001) Increased activity of membrane-associated nucleoside diphosphate kinase and inhibition of cAMP synthesis in failing human myocardium. Cardiovasc Res 49:48–55. doi:10.1016/S0008-6363(00)00222-4
39. Lutz S, Hippe HJ, Niroomand F, Wieland T (2004) Nucleoside diphosphate kinase-mediated activation of heterotrimeric G proteins. Methods Enzymol 390:403–418. doi:10.1016/S0076-6879(04)90025-0
40. Sheffler DJ, Kroeze WK, Garcia BG, Deutch AY, Hufeisen SJ, Leahy P, Bruning JC, Roth BL (2006) p90 ribosomal S6 kinase 2 exerts a tonic brake on G protein-coupled receptor signaling. Proc Natl Acad Sci USA 103:4717–4722. doi:10.1073/pnas.0600585103
41. Kapetanovich L, Baughman C, Lee TH (2005) Nm23H2 facilitates coat protein complex II assembly and endoplasmic reticulum export in mammalian cells. Mol Biol Cell 16:835–848. doi:10.1091/mbc.E04-09-0785
42. Alexander S, Srinivasan S, Alexander H (2003) Proteomics opens doors to the mechanisms of developmentally regulated secretion. Mol Cell Proteomics 2:1156–1163. doi:10.1074/mcp.R300011-MCP200
43. Wallet V, Mutzel R, Troll H, Barzu O, Wurster B, Veron M, Lacombe ML (1990) Dictyostelium nucleoside diphosphate kinase highly homologous to Nm23 and Awd proteins involved in mammalian tumor metastasis and Drosophila development. J Natl Cancer Inst 82:1199–1202. doi:10.1093/jnci/82.14.1199

44. Krishnan KS, Rikhy R, Rao S, Shivalkar M, Mosko M, Narayanan R, Etter P, Estes PS, Ramaswami M (2001) Nucleoside diphosphate kinase, a source of GTP, is required for dynamin-dependent synaptic vesicle recycling. Neuron 30:197–210. doi:10.1016/S0896-6273(01)00273-2
45. Dammai V, Adryan B, Lavenburg KR, Hsu T (2003) Drosophila awd, the homolog of human nm23, regulates FGF receptor levels and functions synergistically with shi/dynamin during tracheal development. Genes Dev 17:2812–2824. doi:10.1101/gad.1096903
46. Nallamothu G, Woolworth JA, Dammai V, Hsu T (2008) Awd, the homolog of metastasis suppressor gene Nm23, regulates Drosophila epithelial cell invasion. Mol Cell Biol 28:1964–1973. doi:10.1128/MCB.01743-07
47. Palacios F, Schweitzer JK, Boshans RL, D'Souza-Schorey C (2002) ARF6-GTP recruits Nm23-H1 to facilitate dynamin-mediated endocytosis during adherens junctions disassembly. Nat Cell Biol 4:929–936. doi:10.1038/ncb881
48. Horak CE, Lee JH, Elkahloun AG, Boissan M, Dumont S, Maga TK, Arnaud-Dabernat S, Palmieri D, Stetler-Stevenson WG, Lacombe ML, Meltzer PS, Steeg PS (2007) Nm23-H1 suppresses tumor cell motility by down-regulating the lysophosphatidic acid receptor EDG2. Cancer Res 67:7238–7246. doi:10.1158/0008-5472.CAN-07-0962
49. Fournier HN, Albiges-Rizo C, Block MR (2003) New insights into Nm23 control of cell adhesion and migration. J Bioenerg Biomembr 35:81–87. doi:10.1023/A:1023450008347
50. Ma D, McCorkle JR, Kaetzel DM (2004) The metastasis suppressor NM23-H1 possesses 3′-5′ exonuclease activity. J Biol Chem 279:18073–18084. doi:10.1074/jbc.M400185200
51. Curtis CD, Likhite VS, McLeod IX, Yates JR, Nardulli AM (2007) Interaction of the tumor metastasis suppressor nonmetastatic protein 23 homologue H1 and estrogen receptor alpha alters estrogen-responsive gene expression. Cancer Res 67:10600–10607. doi:10.1158/0008-5472.CAN-07-0055
52. Paravicini G, Steinmayr M, Andre E, Becker-Andre M (1996) The metastasis suppressor candidate nucleotide diphosphate kinase NM23 specifically interacts with members of the ROR/RZR nuclear orphan receptor subfamily. Biochem Biophys Res Commun 227:82–87. doi:10.1006/bbrc.1996.1471
53. Rayner K, Chen YX, Hibbert B, White D, Miller H, Postel EH, O'Brien ER (2008) Discovery of NM23-H2 as an estrogen receptor beta-associated protein: role in estrogen-induced gene transcription and cell migration. J Steroid Biochem Mol Biol 108:72–81. doi:10.1016/j.jsbmb.2007.07.006
54. Subramanian C, Cotter MAII, Robertson ES (2001) Epstein-Barr virus nuclear protein EBNA-3C interacts with the human metastatic suppressor Nm23-H1: a molecular link to cancer metastasis. Nat Med 7:350–355. doi:10.1038/85499
55. Subramanian C, Robertson ES (2002) The metastatic suppressor Nm23-H1 interacts with EBNA3C at sequences located between the glutamine- and proline-rich domains and can cooperate in activation of transcription. J Virol 76:8702–8709. doi:10.1128/JVI.76.17.8702-8709.2002
56. Kaul R, Murakami M, Choudhuri T, Robertson ES (2007) Epstein-Barr virus latent nuclear antigens can induce metastasis in a nude mouse model. J Virol 81:10352–10361. doi:10.1128/JVI.00886-07
57. Mileo AM, Piombino E, Severino A, Tritarelli A, Paggi MG, Lombardi D (2006) Multiple interference of the human papillomavirus-16 E7 oncoprotein with the functional role of the metastasis suppressor Nm23-H1 protein. J Bioenerg Biomembr 38:215–225. doi:10.1007/s10863-006-9037-y
58. Seong HA, Jung H, Ha H (2007) NM23-H1 tumor suppressor physically interacts with serine-threonine kinase receptor-associated protein, a transforming growth factor-beta (TGF-beta) receptor-interacting protein, and negatively regulates TGF-beta signaling. J Biol Chem 282:12075–12096. doi:10.1074/jbc.M609832200
59. Jung H, Seong HA, Ha H (2007) NM23-H1 tumor suppressor and its interacting partner STRAP activate p53 function. J Biol Chem 282:35293–35307. doi:10.1074/jbc.M705181200
60. Jung H, Seong HA, Ha H (2008) Direct interaction between Nm23-H1 and macrophage amigration inhibitory factor (MIF) is critical for alleviation of MIF-mediated suppression of p53 activity. J Biol Chem 283:32669–32679
61. Kang Y, Lee DC, Han J, Yoon S, Won M, Yeom JH, Seong MJ, Ko JJ, Lee KA, Lee K, Bae J (2007) NM23-H2 involves in negative regulation of Diva and Bcl2L10 in apoptosis signaling. Biochem Biophys Res Commun 359:76–82. doi:10.1016/j.bbrc.2007.05.090
62. Reymond A, Volorio S, Merla G, Al-Maghtheh M, Zuffardi O, Bulfone A, Ballabio A, Zollo M (1999) Evidence for interaction between human PRUNE and nm23-H1 NDPKinase. Oncogene 18:7244–7252. doi:10.1038/sj.onc.1203140
63. D'Angelo A, Garzia L, Andre A, Carotenuto P, Aglio V, Guardiola O, Arrigoni G, Cossu A, Palmieri G, Aravind L, Zollo M (2004) Prune cAMP phosphodiesterase binds nm23-H1 and promotes cancer metastasis. Cancer Cell 5:137–149. doi:10.1016/S1535-6108(04)00021-2
64. Srivastava S, Li Z, Ko K, Choudhury P, Albaqumi M, Johnson AK, Yan Y, Backer JM, Unutmaz D, Coetzee WA, Skolnik EY (2006) Histidine phosphorylation of the potassium channel KCa3.1 by nucleoside diphosphate kinase B is required for activation of KCa3.1 and CD4 T cells. Mol Cell 24:665–675. doi:10.1016/j.molcel.2006.11.012
65. Srivastava S, Zhdanova O, Di L, Li Z, Albaqumi M, Wulff H, Skolnik EY (2008) Protein histidine phosphatase 1 negatively regulates CD4 T cells by inhibiting the K+ channel KCa3.1. Proc Natl Acad Sci USA 105:14442–14446. doi:10.1073/pnas.0803678105
66. Schwahn U, Lenzner S, Dong J, Feil S, Hinzmann B, van Duijnhoven G, Kirschner R, Hemberger M, Bergen AA, Rosenberg T, Pinckers AJ, Fundele R, Rosenthal A, Cremers FP, Ropers HH, Berger W (1998) Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat Genet 19:327–332. doi:10.1038/1214
67. Munier A, Serres C, Kann ML, Boissan M, Lesaffre C, Capeau J, Fouquet JP, Lacombe ML (2003) Nm23/NDP kinases in human male germ cells: role in spermiogenesis and sperm motility? Exp Cell Res 289:295–306. doi:10.1016/S0014-4827(03)00268-4
68. Nagy PL, Griesenbeck J, Kornberg RD, Cleary ML (2002) A trithorax-group complex purified from Saccharomyces cerevisiae is required for methylation of histone H3. Proc Natl Acad Sci USA 99:90–94. doi:10.1073/pnas.221596698
69. King SM (2006) Axonemal protofilament ribbons, DM10 domains, and the link to juvenile myoclonic epilepsy. Cell Motil Cytoskeleton 63:245–253. doi:10.1002/cm.20129
70. Miranda-Vizuete A, Sadek CM, Jimenez A, Krause WJ, Sutovsky P, Oko R (2004) The mammalian testis-specific thioredoxin system. Antioxid Redox Signal 6:25–40. doi:10.1089/152308604771978327
71. Ogawa K, Takai H, Ogiwara A, Yokota E, Shimizu T, Inaba K, Mohri H (1996) Is outer arm dynein intermediate chain 1 multifunctional? Mol Biol Cell 7:1895–1907
72. Padma P, Hozumi A, Ogawa K, Inaba K (2001) Molecular cloning and characterization of a thioredoxin/nucleoside diphosphate kinase related dynein intermediate chain from the ascidian, Ciona intestinalis. Gene 275:177–183. doi:10.1016/S0378-1119(01)00661-8
73. Miranda-Vizuete A, Tsang K, Yu Y, Jimenez A, Pelto-Huikko M, Flickinger CJ, Sutovsky P, Oko R (2003) Cloning and

developmental analysis of murid spermatid-specific thioredoxin-2 (SPTRX-2), a novel sperm fibrous sheath protein and autoantigen. J Biol Chem 278:44874–44885. doi:10.1074/jbc.M305475200
74. Mahr S, Burmester GR, Hilke D, Gobel U, Grutzkau A, Haupl T, Hauschild M, Koczan D, Krenn V, Neidel J, Perka C, Radbruch A, Thiesen HJ, Muller B (2006) Cis- and trans-acting gene regulation is associated with osteoarthritis. Am J Hum Genet 78:793–803. doi:10.1086/503849
75. Tian G, Bhamidipati A, Cowan NJ, Lewis SA (1999) Tubulin folding cofactors as GTPase-activating proteins, GTP hydrolysis and the assembly of the alpha/beta-tubulin heterodimer. J Biol Chem 274:24054–24058. doi:10.1074/jbc.274.34.24054
76. Veltel S, Gasper R, Eisenacher E, Wittinghofer A (2008) The retinitis pigmentosa 2 gene product is a GTPase-activating protein for Arf-like 3. Nat Struct Mol Biol 15:373–380. doi:10.1038/nsmb.1396
77. Yoon JH, Singh P, Lee DH, Qiu J, Cai S, O'Connor TR, Chen Y, Shen B, Pfeifer GP (2005) Characterization of the 3′ –> 5′ exonuclease activity found in human nucleoside diphosphate kinase 1 (NDK1) and several of its homologues. Biochemistry 44:15774–15786. doi:10.1021/bi0515974
78. Yoon JH, Qiu J, Cai S, Chen Y, Cheetham ME, Shen B, Pfeifer GP (2006) The retinitis pigmentosa-mutated RP2 protein exhibits exonuclease activity and translocates to the nucleus in response to DNA damage. Exp Cell Res 312:1323–1334. doi:10.1016/j.yexcr.2005.12.026
79. Mitchell KA, Gallagher BC, Szabo G, Otero Ade S (2004) NDP kinase moves into developing primary cilia. Cell Motil Cytoskeleton 59:62–73. doi:10.1002/cm.20025
80. Scholey JM (2008) Intraflagellar transport motors in cilia: moving along the cell's antenna. J Cell Biol 180:23–29. doi:10.1083/jcb.200709133
81. Herbert E, Potter VR, Takagi Y (1955) The phosphorylation of 5′-uridine nucleotides by cell fractions from rat liver. J Biol Chem 213:923–939
82. Brdiczka D, Beutner G, Ruck A, Dolder M, Wallimann T (1998) The molecular structure of mitochondrial contact sites, their role in regulation of energy metabolism and permeability transition. Biofactors 8:235–242. doi:10.1002/biof.5520080311
83. Muhonen WW, Lambeth DO (1995) The compartmentation of nucleoside diphosphate kinase in mitochondria. Comp Biochem Physiol B Biochem Mol Biol 110:211–223. doi:10.1016/0305-0491(94)00123-C
84. Krebs HA, Wiggins D (1978) Phosphorylation of adenosine monophosphate in the mitochondrial matrix. Biochem J 174:297–301
85. Gordon DM, Lyver ER, Lesuisse E, Dancis A, Pain D (2006) GTP in the mitochondrial matrix plays a crucial role in organellar iron homoeostasis. Biochem J 400:163–168. doi:10.1042/BJ20060904
86. Pedersen PL (1973) Coupling of adenosine triphosphate formation in mitochondria to the formation of nucleoside triphosphates. Involvement of nucleoside diphosphokinase. J Biol Chem 248:3956–3962
87. Milon L, Rousseau-Merck MF, Munier A, Erent M, Lascu I, Capeau J, Lacombe ML (1997) nm230-H4, a new member of the family of human nm23/nucleoside diphosphate kinase genes localised on chromosome 16p13. Hum Genet 99:550–557. doi:10.1007/s004390050405
88. Barraud P, Amrein L, Dobremez E, Dabernat S, Masse K, Larou M, Daniel JY, Landry M (2002) Differential expression of nm23 genes in adult mouse dorsal root ganglia. J Comp Neurol 444:306–323. doi:10.1002/cne.10150
89. Knorpp C, Johansson M, Baird AM (2003) Plant mitochondrial nucleoside diphosphate kinase is attached to the membrane through interaction with the adenine nucleotide translocator. FEBS Lett 555:363–366. doi:10.1016/S0014-5793(03)01288-2
90. Epand RF, Tokarska-Schlattner M, Schlattner U, Wallimann T, Epand RM (2007) Cardiolipin clusters and membrane domain formation induced by mitochondrial proteins. J Mol Biol 365:968–980. doi:10.1016/j.jmb.2006.10.028
91. Janin J, Dumas C, Morera S, Xu Y, Meyer P, Chiadmi M, Cherfils J (2000) Three-dimensional structure of nucleoside diphosphate kinase. J Bioenerg Biomembr 32:215–225. doi:10.1023/A:1005528811303
92. Min K, Song HK, Chang C, Kim SY, Lee KJ, Suh SW (2002) Crystal structure of human nucleoside diphosphate kinase A, a metastasis suppressor. Proteins 46:340–342. doi:10.1002/prot.10038
93. Chen Y, Gallois-Montbrun S, Schneider B, Veron M, Morera S, Deville-Bonne D, Janin J (2003) Nucleotide binding to nucleoside diphosphate kinases: X-ray structure of human NDPK-A in complex with ADP and comparison to protein kinases. J Mol Biol 332:915–926. doi:10.1016/j.jmb.2003.07.004
94. Giraud MF, Georgescauld F, Lascu I, Dautant A (2006) Crystal structures of S120G mutant and wild type of human nucleoside diphosphate kinase A in complex with ADP. J Bioenerg Biomembr 38:261–264. doi:10.1007/s10863-006-9043-0
95. Chang CL, Zhu XX, Thoraval DH, Ungar D, Rawwas J, Hora N, Strahler JR, Hanash SM, Radany E (1994) Nm23-H1 mutation in neuroblastoma. Nature 370:335–336. doi:10.1038/370335a0
96. Erent M, Gonin P, Cherfils J, Tissier P, Raschella G, Giartosio A, Agou F, Sarger C, Lacombe ML, Konrad M, Lascu I (2001) Structural and catalytic properties and homology modelling of the human nucleoside diphosphate kinase C, product of the DRnm23 gene. Eur J Biochem 268:1972–1981. doi:10.1046/j.1432-1327.2001.2076.doc.x
97. Morera S, Lacombe ML, Xu Y, LeBras G, Janin J (1995) X-ray structure of human nucleoside diphosphate kinase B complexed with GDP at 2 A resolution. Structure 3:1307–1314. doi:10.1016/S0969-2126(01)00268-4
98. Lascu I, Schaertl S, Wang C, Sarger C, Giartosio A, Briand G, Lacombe ML, Konrad M (1997) A point mutation of human nucleoside diphosphate kinase A found in aggressive neuroblastoma affects protein folding. J Biol Chem 272:15599–15602. doi:10.1074/jbc.272.25.15599
99. Lascu I, Giartosio A, Ransac S, Erent M (2000) Quaternary structure of nucleoside diphosphate kinases. J Bioenerg Biomembr 32:227–236. doi:10.1023/A:1005580828141
100. Zhou Q, Yang X, Zhu D, Ma L, Zhu W, Sun Z, Yang Q (2007) Double mutant P96S/S120G of Nm23-H1 abrogates its NDPK activity and motility-suppressive ability. Biochem Biophys Res Commun 356:348–353. doi:10.1016/j.bbrc.2007.02.066
101. Lascu I, Chaffotte A, Limbourg-Bouchon B, Veron M (1992) A Pro/Ser substitution in nucleoside diphosphate kinase of Drosophila melanogaster (mutation killer of prune) affects stability but not catalytic efficiency of the enzyme. J Biol Chem 267:12775–12781
102. Mocan I, Georgescauld F, Gonin P, Thoraval D, Cervoni L, Giartosio A, Dabernat-Arnaud S, Crouzet M, Lacombe ML, Lascu I (2007) Protein phosphorylation corrects the folding defect of the neuroblastoma (S120G) mutant of human nucleoside diphosphate kinase A/Nm23-H1. Biochem J 403:149–156. doi:10.1042/BJ20061141
103. Dabernat S, Larou M, Masse K, Dobremez E, Landry M, Mathieu C, Daniel JY (1999) Organization and expression of mouse nm23-M1 gene. Comparison with nm23-M2 expression. Gene 236:221–230. doi:10.1016/S0378-1119(99)00288-7
104. Arnaud-Dabernat S, Bourbon PM, Dierich A, Le Meur M, Daniel JY (2003) Knockout mice as model systems for studying nm23/NDP kinase gene functions. Application to the nm23-M1

gene. J Bioenerg Biomembr 35:19–30. doi:10.1023/A:1023561821551
105. Boissan M, Wendum D, Arnaud-Dabernat S, Munier A, Debray M, Lascu I, Daniel JY, Lacombe ML (2005) Increased lung metastasis in transgenic NM23-Null/SV40 mice with hepatocellular carcinoma. J Natl Cancer Inst 97:836–845
106. Masse K, Dabernat S, Bourbon PM, Larou M, Amrein L, Barraud P, Perel Y, Camara M, Landry M, Lacombe ML, Daniel JY (2002) Characterization of the nm23-M2, nm23-M3 and nm23-M4 mouse genes: comparison with their human orthologs. Gene 296:87–97. doi:10.1016/S0378-1119(02)00836-3
107. Dzeja PP, Terzic A (2003) Phosphotransfer networks and cellular energetics. J Exp Biol 206:2039–2047. doi:10.1242/jeb.00426
108. Morin-Leisk J, Lee TH (2008) Nucleotide-dependent self-assembly of nucleoside diphosphate kinase (NDPK) in vitro. Biochim Biophys Acta 1784:2045–2051
109. Baughman C, Morin-Leisk J, Lee T (2008) Nucleoside diphosphate kinase B (NDKB) scaffolds endoplasmic reticulum membranes in vitro. Exp Cell Res 314:2702–2714. doi:10.1016/j.yexcr.2008.06.005
110. Mitchell KA, Szabo G, de S Otero A (2009) Direct binding of cytosolic NDP kinases to membrane lipids is regulated by nucleotides. Biochim Biophys Acta 1793:469–476. doi:10.1016/j.bbamcr.2008.12.009