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The Human Nm23/Nucleoside Diphosphate Kinases

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Biochemical experiments over the past 40 years have shown that nucleoside diphosphate (NDP) kinase activity, which catalyzes phosphoryl transfer from a nucleoside triphosphate to a nucleoside diphosphate, is ubiquitously found in organisms from bacteria to human. Over the past 10 years, eight human genes of the nm23/NDP kinase family have been discovered that can be separated into two groups based on analysis of their sequences. In addition to catalysis, which may not be exhibited by all isoforms, evidence for regulatory roles has come recently from the discovery of the genes *nm23* and *awd*, which encode NDP kinases and are involved in tumor metastasis and *Drosophila* development, respectively. Current work shows that the human NDP kinase genes are differentially expressed in tissues and that their products are targeted to different subcellular locations. This suggests that Nm23/NDP kinases possess different, but specific, functions within the cell, depending on their localization. The roles of NDP kinases in metabolic pathways and nucleic acid synthesis are discussed.

KEY WORDS: Nm23; NDP kinase; mitochondria; testis; dynein; metastasis.

INTRODUCTION

Nucleoside diphosphate (NDP) kinase was first discovered in yeast (Berg and Joklik, 1953) and in pigeon breast muscle (Krebs and Hems, 1953). Studies generally show that the enzyme is rather nonspecific with the levels of activity varying, depending on the tissue and the subcellular localization. Although activity is observed mainly in cytosol in rat (Cheng *et al.*, 1973), it is also found in nuclei (Prem veer Reddy and Pardee, 1980), in mitochondria (Herbert *et al.*, 1955; Jacobus and Evans, 1977) and associated with plasma membranes (Kimura and Shimada, 1988). In human erythrocytes as well as other mammalian tissues, electrophoretic profiles were complex, suggesting that several isozymes were present (Cheng *et al.*, 1971, 1973).

The first primary structures for NDP kinases were reported in 1990 for *Myxoccus xanthus* (Munoz-Dorado *et al.*, 1990), *Dictyostelium discoideum* (Lacombe *et al.*, 1990) and rat (Kimura *et al.*, 1990). This led to the discovery (Wallet *et al.*, 1990) that the products of two independently isolated regulatory genes are NDP kinases. The two genes are *nm23*, involved in mammalian tumor malignancy (Steeg *et al.*, 1988; Rosengard *et al.*, 1989), and *awd*, crucial for *Drosophila* development (Dearolf *et al.*, 1988; Biggs *et al.*, 1988). The observation that NDP kinases were involved in complex regulatory processes was unexpected and has sparked considerable interest in these proteins.

Thus far, more than seventy cDNA sequences for NDP kinases have been obtained in a wide variety of organisms, showing a strong conservation throughout the phylogenetic kingdom. In human, eight genes (*nm23*-H1 to *nm23*-H8) have been documented. The first human *nm23* cDNA was isolated by screening a human fibroblast cDNA library (Rosengard *et al.*,

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1989) with the murine *nm23* cDNA previously isolated on the basis of its reduced expression in highly metastatic murine melanoma cell lines (Steeg *et al.*, 1988), thus the name nm23 for "nonmetastatic clone 23." A second human gene, *nm23*-H2 was identified by its high homology to *nm23*-H1 (Stahl *et al.*, 1991). The *nm23*-H3 gene (also known as DR-*nm23*) was identified by differential screening of a cDNA library obtained from chronic myelogenous leukemia cells (Venturelli *et al.*, 1995).

The remaining five members of the *nm23* gene family were identified by searching for homologous sequences in the expressed sequence tags database (dbEST) (Milon *et al.*, 1997; Munier *et al.*, 1998; Mehus *et al.*, 1999). These genes have been named *nm23*-H4 to *nm23*-H8 on the basis of their homology to the earlier *nm23* genes although, as far as we know, they do not affect metastatic potential. The genes *nm23*-H5 and *nm23*-H6 were independently discovered by functional complementation of p53-induced apoptosis (Tsuiki *et al.*, 1999).

The alignment of sequences (Fig. 1) and the phylogenetic tree (Fig. 2) show that the *nm23*/NDP kinase gene family divides into two distinct groups. The group I genes encode proteins that generally have highly homologous counterparts in other vertebrate species and possess the classic enzymic activity of a NDP kinase. This group includes NDP kinases A–D (Nm23-H1 to -H4), which share 58 to 88% identity with each other with no gap or insertion. The protein products of the group II genes (Nm23-H5 to -H8) are more divergent as the sequences share only 25 to 45% identity with the first group proteins and between each other. Members of group II present a high level of identity to the three tandemly repeated NDP kinase domains found in the intermediate chain (IC1) of the outer arm dynein of sea urchin sperm axoneme (Ogawa *et al.*, 1996) and, with the exception of Nm23-H6, are expressed predominantly in human testis. Only one product of group II genes (Nm23-H6) has been demonstrated to catalyze the NDP kinase reaction. Activity was not found for the Nm23-H5 protein and the proteins are not yet available for *nm23*-H7 and -H8.

This review will focus mainly on the more recently described members of the family, *nm23*-H3 to -H8, since NDP kinases A and B (Nm23-H1 and Nm23-H2) are the subject of other reviews in this issue. For the purpose of this review and in accordance with precedence in the literature, we will use letters to designate NDP kinases (in theory NDP kinase A to H) and *nm23*-H1 to *nm23*-H8 to designate the genes. There is also precedence for designating the genes as *NME1*, etc. When no catalytic activity has been demonstrated, the gene product is named only according to the gene. The characteristics and properties of the various isoforms will be detailed below and are summarized in Table I.

THE HUMAN NM23/NUCLEOSIDE DIPHOSPHATE KINASE GENE/PROTEIN FAMILY

The Human NDP Kinases (Group I)

X-ray crystallography and gel permeation analyses indicate that the group I proteins (NDP kinases A–D) are hexamers of identically folded subunits (Webb *et al.*, 1995; Moréra *et al.*, 1995, Min *et al.*, 2000; Erent *et al.*, 1999; Milon *et al.*, 2000). These four isoforms possess the NDP kinase active site motif (NXXHG/ASD) and are catalytically active with similar kinetic parameters (Gonin *et al.*, 1999). They all possess the nine residues that are most essential for catalysis and stability of a prototypical NDP kinase as defined by X-ray crystallography and site-directed mutagenesis. Their genes present similar intron–exon organization (Dooley *et al.*, 1994; J.Y. Daniel and M.L. Lacombe, unpublished observation, 2000).

All four recombinant proteins of the first group can be reconstituted into heterohexamers *in vitro* (I. Lascu, personal communication, 2000). Formation of such heteropolymers *in vivo* could introduce another level of regulation of the subcellular localization and/or functions of NDP kinases within the cell.

NDP Kinases A and B (Nm23-H1 and -H2)

Gilles *et al.* (1991) demonstrated that NDP kinase A and B, purified from human erythrocytes and sequenced chemically, are identical to the *nm23*-H1 and *nm23*-H2 gene products with no post-translational modifications (Gilles *et al.*, 1991). They can form *in vitro* and *in vivo* homohexamers as well as heterohexamers possessing different ratios of the respective subunits (Gilles *et al.*, 1991; Urano *et al.*, 1992), thus explaining the complex electrophoretic profiles observed earlier. It is tempting to speculate that these various isoforms could possess specific cellular functions. Indeed, the control of metastatic potential involve mainly NDP kinase A and the role as the PuF

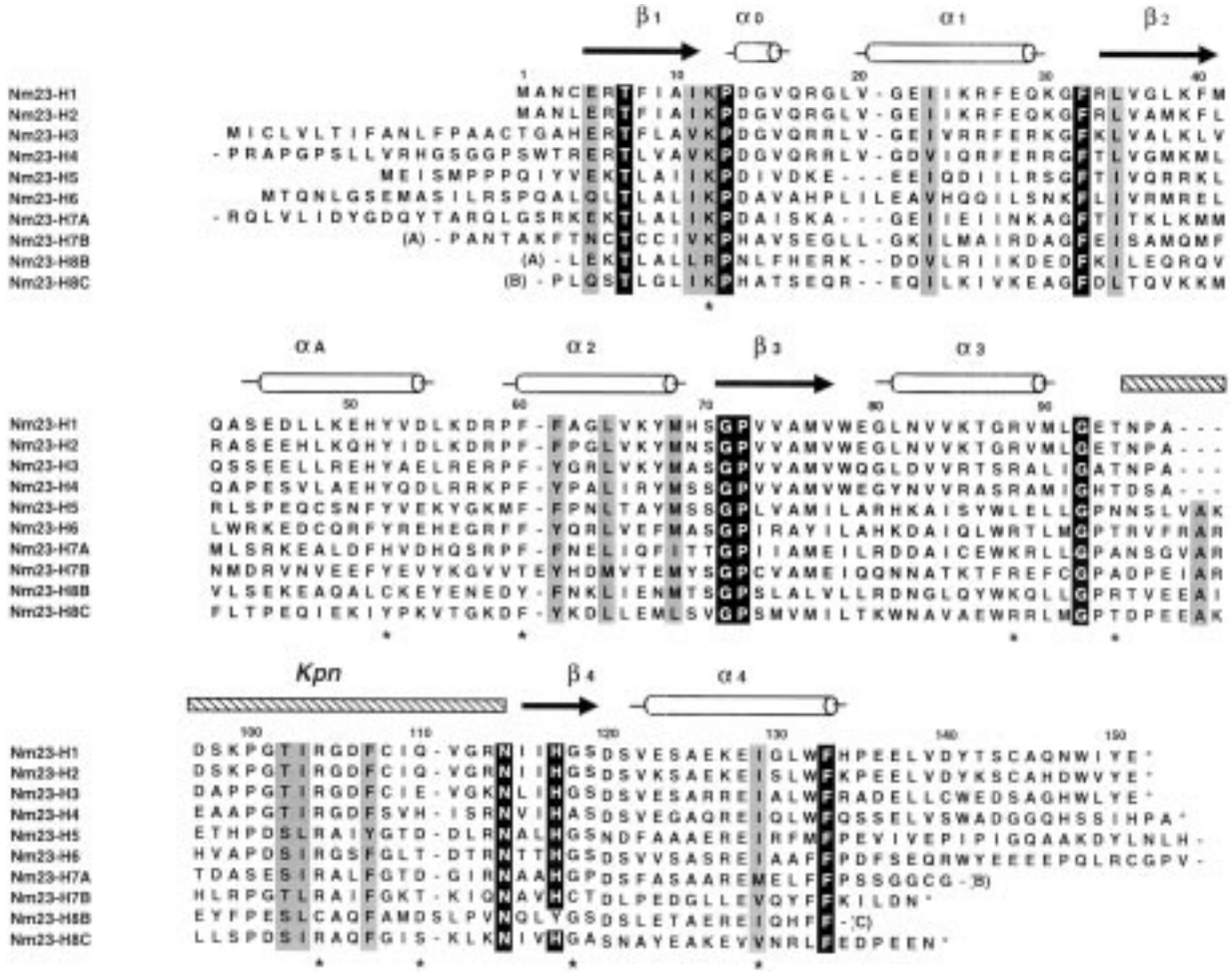

Fig. 1. Primary sequence comparison between the NDP kinase domains of the human Nm23/NDP kinase proteins. The above numbering refers to the Nm23-H1 sequence. Gaps are indicated by dashes and the COOH terminal residue by (°). The consensus sequence represents identical (black boxes) and conserved (grey boxes, according to Hanks *et al.* 1988) residues within all sequences. (*) Indicates the residues involved in catalysis and stability. Residue 96 is the site of the Kpn mutation found in *Drosophila*. Secondary structural elements, defined in Moréra *et al.* (1995) are also indicated.

transcription factor is restricted to NDP kinase B (see other reviews in this issue).

In normal physiological conditions, NDP kinases A and B are involved in proliferation, development, and differentiation (for review: Lombardi *et al.*, 2000). During mouse embryogenesis, increased levels of NDP kinases are observed during organogenesis coincident with differentiation (Lakso *et al.*, 1992). In various cell models, it was shown that, depending on the cell type, NDP kinase expression is positively or negatively associated with differentiation. Enforced expression of *nm23*-H1 in the breast-derived metastatic cell line MDA-MB435 induces growth arrest and formation of organized glandular structures (Howlett *et al.*, 1994). Transfection of *nm23*-H1 cDNA enhances NGF-induced neural cell differentiation (Gervasi *et al.*, 1996; Ishijima *et al.*, 1999).

In contrast, differentiation of human colon-derived Caco2 cells to enterocytelike structures, as well as myeloid and lymphoid differentiation of hematopoietic progenitors, are accompanied by decreased levels of NDP kinase A and B (Lacombe, unpublished observation, 2000; Willems *et al.*, 1998). Similarly, *nm23*-H1 expression decreases during differentiation of megakaryoblastic MEG-01 and promyelocytic HL60 leukemia cell lines (Yamashiro *et al.*, 1994). NDP kinase B also acts as a differentiation-inhibiting factor in mouse myeloid leukemia cells (Okabe-Kado

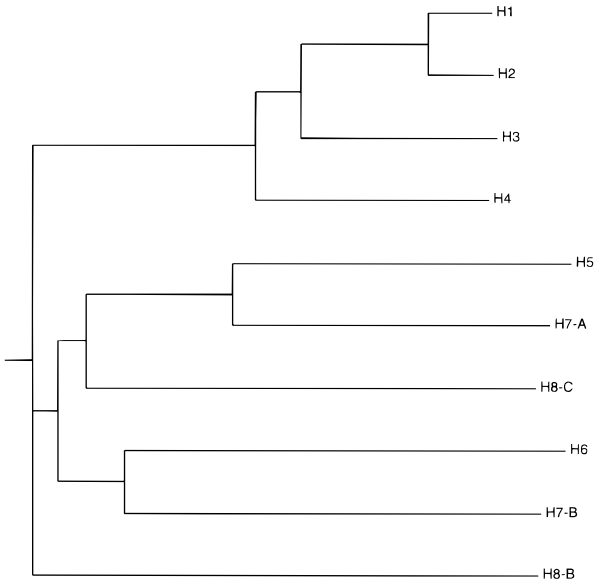

Fig. 2. Phylogenetic tree for the isoforms of the human *nm23*/NDP kinase gene family. The tree was drawn using the Phylodendron program. Information for the tree was obtained from a ClustalW alignment of sequences in Fig. 1 by using the default values and the Phylip output format option.

et al., 1995). These data suggest that NDP kinases A and B participate in cellular proliferation/differentiation by yet undefined molecular mechanisms.

Very few analyses of the differential subcellular localization of A and B NDP kinases have been reported. In breast cancer-derived cell lines, both proteins are primarily in the cytosol, particularly abundant at the periphery of the nuclei, and are partly associated with the microtubular network of interphasic cells (Pinon *et al.*, 1999). The B isoform also appears in nuclei, in accord with its role as a transcription factor (Kraeft *et al.*, 1996; Pinon *et al.*, 1999). Interestingly, NDP kinases location is greatly modified in dividing cells. They form aggregates and are not associated with the mitotic spindle microtubules (Pinon *et al.*, 1999).

The rodent equivalents of NDP kinases A and B are 95 to 98% identical to their human counterparts. This indicates that the duplication leading to the two homologous genes occurred before rodent and human speciation. This duplication seems restricted to vertebrates since equivalents of A and B NDP kinases have been found in *Xenopus* (Ouatas *et al.*, 1997) and zebrafish (Mehus and Lambeth, 1999), but not in *Drosophila* or other lower eukaryotic species.

NDP Kinase C (Nm23-H3 or DR-Nm23)

nm23-H3 cDNA was identified through differential screening of a blast-crisis chronic myelogenous leukemia (CML) cDNA library (Venturelli *et al.*, 1995). The mRNA was preferentially expressed in the blast crisis transition of CML, characterized by growth advantage and differentiation arrest of CML blast cells. The protein possesses an N-terminal extension of 17 amino acids as compared to NDP kinases A and B. The *nm23*-H3 gene expression seems to be inversely correlated with differentiation since a marked decline in mRNA was observed when human CD34+ peripheral blood cells were induced to differentiate toward the myeloid pathway upon addition of IL3 and granulocyte colony-stimulating factor. Moreover, overexpression of *nm23*-H3(DR) by transfection of 32Dc13 cells, derived from mouse bone marrow, inhibited differentiation in granulocytes and caused apoptosis. Likewise, a high expression of *nm23*-H3 was observed in several solid tumor derived cell lines (Martinez *et al.*, 1997). However, this relationship between gene expression and cell differentiation seems to vary, depending on the cell line studied, which has also been observed with *nm23*-H1. This phenomenon is demonstrated by *nm23*-H3 transfection experiments in neuroblastoma cells that show a positive correlation between differentiation and expression of *nm23*-H3 (Amendola *et al.*, 1997). Considering normal tissues, the expression of *nm23*-H3 is ubiquitous, but is particularly expressed in some structures of the brain, such as, cerebellum and pituitary gland. However, the overall expression of *nm23*-H3 was much lower than that of *nm23*-H1 and -H2 (M. L. Lacombe, unpublished observations, 2000).

Studies of the expression of NDP kinase C fused to the NH2 terminus of the green fluorescent protein (GFP) showed a cytoplasmic-punctated staining for the fused protein (Martinez *et al.*, 1997), but no exact location in the cytoplasm was reported. The murine equivalent of NDP kinase C was observed to be localized to the membranes of the Golgi apparatus and endoplasmic reticulum (Barraud *et al.*, 1999). The full-length recombinant protein could not be efficiently expressed in *E. coli*. However, the recombinant protein truncated to the length of NDP kinases A and B was obtained in very high yield and easily purified (Erent *et al.*, 1999). NDP kinase C is remarkably stable to heat treatment (T_m, 82°C as compared to 50–60°C for NDP kinase A and B and 40°C for NDP kinase D),

Table 1. The Human Nm23/NDP Kinase Family

Isoform	Size (aa)	Mass (Da)	p*I*	Locus	Tissular expression[b]	Subcellular localization[c]	NDPK activity	Comments
Nm23-H1	152	17,149	5.83	17q21.3	Ubiquitous[1] (kidney, liver, intestine, brain)	Cytoplasmic	Yes	Overexpressed in tumors; inverse correlation with metastatic potential
Nm23-H2	152	17,298	8.52	17q21.3	Ubiquitous[2] (heart, liver, pancreas, kidney)	Cytoplasmic, nuclear	Yes	Overexpressed in tumors; transcription factor (PuF) for c-*myc* proto-oncogene
Nm23-H3	168	18,903	6.91	16q13[a]	Ubiquitous[1] (pituitary gland, cerebellum, adrenal gland)	Cytoplasmic	Yes	Overexpression suppresses granulocyte differentiation and induces apoptosis of myeloid cells. N-terminus of 17 aa
Nm23-H4	187	20,659	10.3	16p13.3	Ubiquitous[2] (prostate, liver, heart)	Mitochondrial	Yes	Associated with mitochondrial membranes (contact sites?); N-terminus of 33 aa
Nm23-H5	212	24,236	5.89	5q21.3	Testis (traces in brain and kidney)[2]	Nd[d]	Not found	Expressed in male germinal cells C-extension of 51 aa
Nm23-H6	186	21,142	8.51	3p21.3	Ubiquitous (skeletal muscle, placenta)[2] (kidney, heart, spleen)[3]	Mitochondrial, Cytoplasmic	Yes	A role in regulation of cell growth and cell cycle progression?
Nm23-H7	376	42,492	6.03	1q24	Mainly in testis,[3] (also in liver, heart, brain, ovary, small intestine, and spleen)	Nd[d]	Nd	Duplicated NDP kinase domain. N-terminus of 85 aa
Nm23-H8	588	67,270	4.9	7	Mainly in testis[3]	Nd[d]	Nd	N-terminal thioredoxin domain; triplicated NDP kinase domain; human dynein IC1 equivalent?

[a] Another chromosomal localization was reported in 16p13.3 (GenBank acc. no: AL031718).
[b] The tissular expression was analyzed by hybridization of specific probes to the Human RNA Master blot (1) and to Northern blot (MTN blot) (2), both available from Clontech, and by RT-PCR (3). Tissues which present the highest expression are indicated in parenthesis.
[c] Chromosomal localizations of *nm23*-H1 to -H8 genes were reported by Varesco *et al.* (1992), Backer *et al.* (1993), Martinez *et al.* (1997), Milon *et al.* (1997), Munier *et al.* (1998), Mehus *et al.* (1999), and Tsuiki *et al.* (1999), Mehus and Lambeth (1999), and Mehus and Lambeth, unpublished, based on high throughput genomic DNA sequence from chromosome 7.
[d] nd, not determined.

due to tighter subunit interactions, as shown by the modeling of the three-dimensional structure of NDP kinase C onto the NDP kinase B structure (Erent *et al.*, 1999). To date, genes homologous to human *nm23*-H3 have been identified in rodent and zebrafish.

NDP Kinase D (Nm23-H4)

NDP kinase D possesses a N-terminal extension of 33 amino acids as compared to NDP kinases A and B, with characteristics of a mitochondrial-targeting signal (Milon *et al.*, 1997). When expressed in *E. coli*, the full length protein was retained in inclusion bodies and could not be reactivated by renaturation, while a truncated protein, missing the N-terminus, is expressed as a soluble protein, which is catalytically active. NDP kinase D is highly labile toward heat or urea treatment, since it was 50% inactivated upon preincubation at 40°C or in 3 *M* urea. Interestingly, NDP kinase D naturally possesses, at position 129, a serine residue equivalent to the Kpn mutation of *Drosophila*. This point mutation in Awd NDP kinase causes lethality in the genetic context of the *prune* eye color mutation (Biggs *et al.*, 1988) by a still unknown mechanism. The Kpn mutation is located in a loop, thus named

the Kpn loop, characterized by three-dimensional structure, which plays a crucial role in the stability of the hexamers (Lascu *et al.*, 1992; Chiadmi *et al.*, 1993). Site-directed mutagenesis shows that this serine is responsible for the marked lability of NDP kinase D, since the S129P mutant is greatly stabilized, as shown by a shift in T_m of 25°C (from 40 to 65°C).

The mitochondrial localization of NDP kinase D was first demonstrated by confocal microscopy examination of HEK 293 cells transfected with a GFP fusion protein (Milon *et al.*, 2000) showing a green fluorescent punctiform pattern superimposable with the labeling of a mitochondria specific dye. That NDP kinase D is specifically and uniquely located to mitochondria of these cells was also demonstrated by Western blot transfer using specific polyclonal antibodies raised against the recombinant enzyme. There is no evidence for an association of either NDP kinase A or NDP kinase B to mitochondria of HEK 293 cells. Import into mitochondria is accompanied by cleavage of the N-terminus. A protein of the size of the full-length protein could not be detected in the HEK 293 cell homogenate, indicating that there is no accumulation of the preprotein in cytoplasm and that a close coupling between translation and mitochondrial import may exist for this enzyme.

NDP kinase D was shown to be associated with the outer and inner mitochondrial membranes and was not found in the soluble fractions. NDP kinase D and porin distribute similarly among the fractions, thus strongly suggesting that NDP kinase D is associated, like porin, with contact sites between the outer and the inner membrane. NDP kinase is a peripheral membrane protein, but the mechanism of association is unknown. Further information regarding its orientation toward the matrix side or the intermembrane space will be required to more precisely define its role in nucleotide synthesis and other functions of mitochondria (Milon *et al.*, 2000).

In contrast to NDP kinase D, mitochondrial NDP kinases found in other species have been reported to be soluble, either in the matrix or intermembrane space. A pigeon NDP kinase was located to the matrix by biochemical criteria (Lambeth *et al.*, 1997). Interestingly, it also possesses the equivalent of the Kpn mutation. Intermembrane space NDP kinases have been identified in *Dictyostelium discoideum* (Troll *et al.*, 1993) and pea (Escobar Galvis *et al.*, 1999). To date, genes homologous to human *nm23*-H4 have been found in rodent, pigeon, and chicken.

The Group II Nm23/NDP Kinases: Nm23-H5 to Nm23-H8

The products of group II genes are highly divergent among themselves (28 to 45% identity) and as compared to group I proteins (25 to 34% identity). As shown in Fig. 1, the group II sequences are characterized by a three amino acid insertion (X-A-R/K) in the Kpn loop with additional alterations (insertions/deletions) near residue 21 (sequence numbering for NDP kinases A/B). These sequence alterations are also found in the NDP kinase repeats of the intermediate chain (IC1) of sea urchin axonemal dynein. The NDP kinase active site motif is not strictly conserved in the second group. Only Nm23-H6 and domain C of Nm23-H8 have all of the residues previously deemed essential for nucleotide binding and catalysis (see Fig. 1). Group II proteins also differ from group I by considerable variation in the lengths of their N- and C-terminals.

Interestingly, group II proteins show a higher identity than group I NDP kinases with the NDP kinase repeats in the axonemal dynein intermediate chain of sea urchin, the most closely related protein that is presently found in the databases. Interestingly, both Nm23-H7 and Nm23-H8 possess tandemly repeated NDP kinase domains as does IC1.

Nm23-H5

The *nm23*-H5 cDNA sequence was reported in 1998 by Munier *et al.* (1998). Identical sequences were obtained in the laboratories of Lambeth (GenBank acc. no: AF067724) and Nakamura (GenBank acc. no: U90450). In comparison to NDP kinases A and B, the Nm23-H5 sequence has eight additional residues at the N-terminal, 51 additional residues at the C-terminal, a two-residue deletion near residue 21 of NDP kinase A, and a three residue insertion in the Kpn loop. The Nm23-H5 protein has only seven out of the nine residues deemed crucial for enzyme structure and activity and the recombinant protein was inactive. The *nm23*-H5 gene is abundantly and almost exclusively expressed in testis with only trace amounts of mRNA detected in brain and kidney (Munier *et al.*, 1998). The cellular localization of *nm23*-H5 mRNA in human testis was determined by *in situ* hybridization to be along the basement membrane of spermatogonia as well as early spermatocytes, distant from the boundary of the tubules. These results suggest that *nm23*-H5 could be specifically involved in early stages of sper-

matogenesis. However, the localization of the Nm23-H5 protein is not yet known and it is possible that it will be found in mature spermatocytes. Indeed, delayed expression of proteins as compared to mRNA has been observed during germinal cell maturation (Yelick *et al.*, 1989). There are no genetic disorders associated with the gene locus to date. The EST database contains evidence of a homologous gene in rodents.

Nm23-H6

The *nm23*-H6 cDNA sequence was independently reported by the Lambeth and Nakamura laboratories (Mehus *et al.*, 1999; Tsuiki *et al.*, 1999). In comparison with NDP kinases A and B, the inferred protein has seven additional residues at the N-terminal and 22 extra residues at the C-terminal. Homologous sequences have been identified in pig, bovine, rat, mouse, zebrafish, and *Drosophila* (Mehus and Lambeth, unpublished data, 2000). The Nm23-H6 protein has one extra residue near residue 21 of NDP kinase A and three extra residues in the Kpn loop. The Nm23-H6 protein has all nine residues deemed crucial for enzyme structure and activity and the characteristic sequence around the active site histidine. A fusion protein with glutathione *S*-transferase (GST) showed activity, although it may be lower than that of NDP kinase A (Tsuiki *et al.*, 1999). Nm23-H6 could, therefore, be named NDP kinase F. Unlike *nm23*-H5, H7, and H8, RT-PCR studies show that *nm23*-H6 is expressed in most human tissues studied, although at a rather low level (Mehus *et al.*, 1999). Immunocytochemical and Western blot analyses of U2OS cells showed that Nm23-H6 partially colocalizes with mitochondria (Tsuiki *et al.*, 1999). Overexpression of *nm23*-H6 in SAOS2 cells results in growth suppression and generation of multinucleated cells. Thus, the protein may play a role in cell growth and cell cycle progression (Tsuiki *et al.*, 1999). The *nm23*-H6 gene locus has been implicated in a variety of malignant tumors (Fearon, 1997).

Nm23-H7

Work on the *nm23*-H7 cDNA (unpublished, GenBank acc. no: AF153191) and its homologs in rat, mouse, *Drosophila*, and zebrafish has been carried out by Mehus and Lambeth. The Nm23-H7 protein contains two NDP kinaselike regions. In comparison with NDP kinases A and B, the H7 protein has an 85-residue extension at its N-terminal, but a C-terminal truncation of 13 residues. Each NDP kinase repeat has three additional residues in the Kpn loop and is lacking three of the residues deemed crucial for catalysis. RT-PCR analysis of several human tissues indicates that the *nm23*-H7 gene, like *nm23*-H5 and *nm23*-H8, is predominantly expressed in testes. However, appreciable amounts of message is also found in liver, heart, brain, ovary, small intestine, and spleen. It is not known if the native Nm23-H7 is an axonemal protein, but its duplication of the NDP kinase domain is reminiscent of the sea urchin IC1 structure. The *nm23*-H7 gene is composed of 12 exons spanning over 180 kb. The genes for *nm23*-H7 and the beta subunit of Na^+/K^+-ATPase are on opposite strands and overlap in such a way that the 3′ UTRs of the two gene transcripts are complementary and capable of hybridization. The *nm23*-H7 gene is located in or near the hereditary prostate cancer susceptibility locus.

Nm23-H8

The *nm23*-H8 gene was identified in 1999 by Mehus and Lambeth (GenBank acc. no: AF202051). Analysis of high-throughput genomic DNA suggests that the *nm23*-H8 gene is comprised of 17 exons spanning over 50 kb. The full-length cDNA predicts a protein that includes a partial (domain A, not shown) and two full repeats (domains B and C, Fig. 1) of the NDP kinase core sequence, thus resembling the sea urchin dynein IC1. The first repeat is homologous to NDP kinase only over the first 60–75 residues. The second and third repeats are 40–45% identical to the equivalent repeats in IC1. Only domain C possesses the catalytic site signature (NXXH) found in NDP kinases and the three domains of IC1. The catalytic site histidine of domain B is replaced by a tyrosine, which suggests interesting features for the catalyzed reaction, should it occur. Like the sea urchin IC1 protein, Nm23-H8 has an N-terminal sequence that is homologous to thioredoxin. This thioredoxin sequence is also found in the 14 and 16 kDa light chains in the dynein of the flagellar outer arm in *Chlamydomonas reinhardtii*. A tissue survey by RT-PCR indicates that the *nm23*-H8 gene is primarily expressed in testes.

METABOLIC ROLES OF MAMMALIAN NDP KINASES

For nearly 40 years, NDP kinases were known only through their catalytic activity. Until 1990, the

widely accepted roles of NDP kinases were: (1) to use ATP as a donor to synthesize the nonadenylic NTPs needed for nucleic acid synthesis and several important metabolic intermediates including UDP-glucose and a few CDP-lipid derivatives; (2) to catalyze transphosphorylation between GTP produced in the Krebs cycle and ADP; and (3) to provide GTP for protein synthesis, G-protein signaling, and tubulin polymerization. Although recent research has focused on NDP kinase functions that do not require catalysis, the catalytic efficiency of NDP kinases A, B, C, and D (Gonin *et al.*, 1999) suggest that their catalytic activity has been perfected by evolution and is not a secondary property of the protein.

Nonadenylic nucleotides, especially GTP, play a number of key metabolic and regulatory roles in the cell, as mentioned above. In addition, in multicellular eukaryotes, PEP carboxykinase and a GTP-specific succinyl-CoA synthetase, have evolved to use GTP rather than ATP. A fundamental but unresolved question is why these enzymes and processes use GTP rather than the more abundant ATP. Like all NTPs, GTP and ATP have the same standard-state free energies of hydrolysis. However, the logarithm of the ratio of their free concentrations ([NTP]/[NDP]) is used in calculating their free energies within a cellular compartment. The ratios of [GTP]/[GDP] and [ATP]/[ADP] can be maintained differently if they are generated by different metabolic processes and if NDP kinase is prevented from freely equilibrating the guanine and adenine nucleotide pools. As will be discussed further below, certain processes may be coupled to GTP because of the advantage conferred by a GTP/GDP ratio that is higher than the ATP/ADP ratio obtained by oxidative phosphorylation. This raises questions about when the catalytic activity of NDP kinase needs to be absent (or at least tightly regulated) in a given cellular compartment so that there is proper integration and regulation of cellular activities. The degree to which pools of nucleotides are equilibrated is dependent on the amount of NDP kinase, the concentrations of all nucleotides that can serve as substrates, as well as the K_ms of NDP kinase for those nucleotides. These ideas will now be further discussed in the context of mitochondrial bioenergetics.

The Role of NDP Kinase in Mitochondrial Matrix

The discovery of both GTP-specific succinyl-CoA synthetase and NDP kinase in the early 1950s led immediately to the proposal that NDP kinase converts GTP generated by the Krebs cycle to ATP. Ottaway *et al.*, (1981) has suggested that GTP-specific succinyl-CoA synthetase participating in the Krebs cycle could generate a GTP/GDP ratio that is up to 400-fold higher than the ATP/ADP ratio generated by oxidative phosphorylation. Such a high ratio would provide a strong thermodynamic driving force for any process coupled to GTP hydrolysis. Examples of such processes, which vary with tissues and species, may include glucogenesis, heme synthesis, ketone body activation, and processing of matrix AMP (by GTP-AMP phosphotransferase).

Maintenance of a high GTP/GDP ratio in matrix would require that NDP kinase not equilibrate the guanine and adenine nucleotide pools. Indeed, the activity of NDP kinase in matrix in some mammalian tissues seems low in comparison with Krebs cycle activity. Experimental demonstration of significant NDP kinase activity in rat liver mitochondrial matrix has been particularly elusive. In contrast the matrix of pigeon liver mitochondria has an abundance of NDP kinase (Muhonen and Lambeth, 1995). In yeast, where succinyl-CoA synthetase and PEP carboxykinase are specific for ATP, there is no gene encoding a mitochondrial NDP kinase. Models of nucleotide metabolism in matrix are further complicated by the recent discovery that an ATP-specific succinyl-CoA synthetase is also present in higher organisms (Johnson *et al.*, 1998a; b) with its activity exceeding that of the GTP-specific enzyme in some mouse tissues (Johnson and Lambeth, unpublished, 2000).

The preceding discussion emphasizes the need to reassess the role of NDP kinase in energy metabolism in mitochondrial matrix. Yet it is difficult to believe that NDP kinase activity is absent from matrix because of its apparent need for DNA and RNA synthesis. Definitive information on NDP kinase isoforms associated with mitochondria is just beginning to be obtained. NDP kinase D has a presequence that could direct it across the inner membrane. Although experiments show that NDP kinase D is membrane bound (Milon *et al.*, 2000), it is not known whether it faces the intermembrane or matrix space. Nm23-H6 also appears to be associated with mitochondria (Tsuiki *et al.*, 1999). However the cDNA does not predict a canonical matrix-targeting signal (Neupert, 1997).

The Role of NDP Kinases in Supplying NTPs to the Cytosol

Because inner membrane nucleotide transporters are specific for adenine, an NDP kinase providing

nonadenylic NTPS to cytosol must be located outside the mitochondrial inner membrane. Several studies of rat liver mitochondria (reviewed in Muhonen and Lambeth, 1995) indicate that most of the NDP kinase activity is located outside the inner membrane, where it may be associated with contact sites (Adams *et al.*, 1989). These sites are involved in transport of proteins, solutes, and nucleotides (Brdiczka *et al.*, 1998). These sites contain the permeability transition pore, which includes porin, the ATP/ADP carrier, and at least two kinases (Adams *et al.*, 1989). The ADP/ATP carrier and porin are the targets of the pro- and antiapoptotic proteins Bax (Marzo *et al.*, 1998) and Bcl-2 (Shimizu *et al.*, 1999), respectively, that play a major role in the control of apoptosis (Green and Kroemer, 1998) through their control of the permeability transition pores. NDP kinase associated with contact sites could be localized within the intermembrane space, as shown for mitochondrial creatine kinase (Bessman and Carpenter, 1985; Adams *et al.*, 1991). Alternatively, it may be bound to porin within the outer membrane, as determined for hexokinase II (Arora and Pedersen, 1988; Adams *et al.*, 1991) and glycerol kinase (Adams *et al.*, 1991). In either case, NDP kinase may have preferential access to mitochondrial ATP, as it is exported through the ATP/ADP carrier. Nucleotides produced by NDP kinase located outside the mitochondrial inner membrane could be supplied directly to other proteins via protein–protein interactions or released into the cytosol to form a pool that is generally available to other proteins and enzymes. Another possibility involves the shuttling of high-energy phosphate through cytoplasmic NDP kinases to furnish NTP at specific sites via mechanisms similar to those proposed for adenylate kinase and creatine kinase (Dzeja *et al.*, 1998).

Pedersen (1973) carried out extensive studies of a NDP kinase that is released by aging liver mitochondria (Glaze and Wadkins, 1967). The isoform released is NDP kinase B as shown by sequencing of a band excised from a SDS-PAGE gel (Lambeth *et al.*, 1999). This isoform is released under conditions where adenylate kinase, a marker for the intermembrane space, is retained. These results suggest that NDP kinase B can bind to the surface of the outer mitochondrial membrane.

Numerous reports suggest that NDP kinase activity may provide phosphorylation energy to cytosolic structures, such as the translation apparatus (Sastre-Garau *et al.*, 1992; Sonnemann and Mutzel, 1995), G proteins (Kimura, 1993), microtubules (Nickerson and Wells, 1984; Biggs *et al.*, 1990; Pinon *et al.*, 1999), and chaperones (Leung and Hightower, 1997). However, the evidence for such interactions has generally been controversial and further work is clearly needed.

Role of NDP Kinase in Supplying Nucleotides for Nucleic Acid Synthesis

One of the most important roles for NDP kinase is provision of NTPs for nucleic acid synthesis. Indeed, increased levels of NDP kinases A and B have been observed in cells induced to proliferate (Keim *et al.*, 1992; Lee *et al.*, 1997). How NDP kinases efficiently provide pyrimidine dNTPs is not obvious in view of the intracellular concentrations of dNDPs being about a hundredth that of ADP and GDP (Traut, 1994), while the K_ms of NDP kinase for dNDPs are significantly higher than for ADP and GDP (Schaertl *et al.*, 1999). One way around this problem may be through protein–protein interactions. Indeed, NDP kinase appears to be a part of the DNA synthesis complex in *E. coli* (Ray and Mathews, 1992). Other possibilities include provision of some nucleotides by relatively nonspecific kinases such as pyruvate kinase and phosphoglycerate kinase. Finally, it is of interest to note that a family of nucleotide-specific "nucleoside monophosphate kinases" (e.g., adenylate kinase, uridylate-cytidylate kinase and thymidylate kinase) fulfill the role formerly attributed to a nonspecific kinase. Could similar nucleotide-specific "nucleoside diphosphate kinases" synthesize NTPs? Recent work has shown that the single NDP kinase gene in *E. coli* (Lu *et al.*, 1995), *Saccharomyces cervisiae* (Fukuchi *et al.*, 1993), and *S. pombe* (Izumiya and Yamamoto, 1995) can be disrupted without apparent effect on growth or morphology. However, disruption of the NDP kinase gene in *E. coli* induces a mutator phenotype, probably as a result of imbalance in dNTPs (Lu *et al.*, 1995; Zhang *et al.*, 1996). A few microorganisms (e.g., Mycoplasma species, *Thermotoga maritima*) lack a NDP kinase gene altogether. Such results have challenged the view that NDP kinase is an essential housekeeping enzyme.

CONCLUSION: FURTHER RESEARCH ON NM23/NDP KINASES

We now know that eight isoforms of NDP kinase have evolved in multicellular organisms. It will be interesting to see if additional isoforms are revealed as the human genome is sequenced. In comparison with human, the recently completed *Drosophila* genome

appears to encode only three isoforms of NDP kinase. The multiplicity of isoforms alone suggests that NDP kinases serve much more specialized roles than envisioned before 1990. These roles undoubtedly require targeting or location of specific isoforms to definite sites within the cell. Clearly, some roles are related to regulatory phenomena that may or may not require the nominal catalytic activity of a NDP kinase.

Group I isoforms all possess NDP kinase activity and could be involved in the control of cytoplasmic (NDP kinase A, B, and C), nuclear (NDP kinase B), and mitochondrial (NDP kinase D) nucleotide homeostasis. Interestingly, the three cytoplasmic isoforms play roles in tumor progression, differentiation, and apoptosis. The mechanisms are still ill defined and require future research. These proteins could act as phosphotransferase (Engel *et al.*, 1998; Wagner and Vu, 2000), but trace contamination with unbound nucleotides could at least partly explain the phenomenon (Levit *et al.*, 1999). The same is true for the long-proposed interaction with GTP-binding proteins, which was nevertheless substantiated by very recent data reporting interaction between NDP kinase A and the low molecular weight GTPase Rad (Zhu *et al.*, 1999). The role of NDP kinases interacting with cellular proteins such as glyceraldehyde-3-phosphate dehydrogenase, intermediate filament vimentin (Otero, 1997; Engel *et al.*, 1998; Pinon *et al.*, 1999), and the human equivalent of the *Drosophila prune* gene product (Reymond *et al.*, 1999) has to be further investigated.

The homology of group II isoforms with sea urchin sperm dynein together with the preferential expression of *nm23*-H5, -H7, and -H8 in testis, prompt us to speculate that these proteins may be associated with human sperm axonemes and perhaps other ciliated tissues including trachea, ovarian tubes, or ependymal cells. The movement is possible because the force-generating interaction of the axonemal proteins, dyneins, and microtubules is fueled by ATP and GTP hydrolysis (Shingyoji *et al.*, 1998). It is tempting to propose that at least one of the isoforms predominantly expressed in testis supplies NTP for beating. However, it remains to be demonstrated that any of these three proteins is endowed with catalytic activity. Domain C of Nm23-H8, which is the closest equivalent to IC1 to date, is a promising candidate in that it possesses all the residues required for catalysis. It is also possible that interaction of an inactive isoform with specific axonemal proteins would be necessary to reveal NDP kinase activity. Analysis of the precise localization of these isoforms should help answer this question.

Obviously, there is much work to be done in sorting out the roles, subcellular locations, catalytic properties, and regulation of the eight isoforms presently known in human. Application of techniques involving gene arrays, gene knockouts, immunolocalization, and overexpression/protein characterization will provide much needed information regarding the biological functions of the *nm23*/NDP kinase family.

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