REVIEW

Nme family of proteins—clues from simple animals

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Abstract Nucleoside-diphosphate kinases (Nme/Nm23/
NDPK) are evolutionarily conserved enzymes involved in
many biological processes in vertebrates. The biochemical
mechanisms of these processes are still largely unknown. The
Nme family consists of ten members in humans of which
Nme1/2 have been extensively studied in the context of carci-
nogenesis, especially metastasis formation. Lately, it has been
proven that the majority of genes linked to human diseases were
already present in species distantly related to humans. Most of
cancer-related protein domains appeared during the two main
evolutionary transitions—the emergence of unicellular eukary-
otes and the transition to multicellular metazoans. In spite of
these recent insights, current knowledge about cancer and status
of cancer-related genes in simple animals is limited. One possi-
ble way of studying human diseases relies on analyzing genes/
proteins that cause a certain disease by using model organism
that represent the evolutionary level at which these genes have
emerged. Therefore, basal metazoans are ideal model organisms
for gaining a clearer picture how characteristics and functions of
Nme genes changed in the transition to multicellularity and
increasing complexity in animals, giving us exciting new evi-
dence of their possible functions in potential pathological con-
ditions in humans.

Keywords Non-bilaterian metazoans · Porifera · NDPK ·
Nm23 · Nme

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Introduction

The Nme gene/protein family, initially called nucleoside di-
phosphate kinase (Nm23/NDPK), consists of evolutionarily
conserved proteins present in all three domains of life:
Bacteria, Archaea and Eukarya (Bilitou et al. 2009). This
family of proteins was originally named after the first identi-
fied member, Nm23-H1/Nme1, which is responsible for me-
tastasis suppression of at least some tumour types (Steeg et al.
1988). The Nme1/NDPKA and Nme2/NDPKB proteins rep-
resent two subunits of a well-known, housekeeping enzyme—
nucleoside-diphosphate kinase (NDPK). The NDPKs form
NTPs and dNTPs by transferring the terminal phosphate from
(d)NTPs and are therefore responsible for the maintenance of
the cellular nucleotide pool. These subunits can assemble into
the enzymatically active hexamer in all possible combinations
(A6, A5B……. AB5, B6) (Gilles et al. 1991). Beside this
basic function, the Nme genes/proteins have several other
biochemical roles: They function as transcription regulators
(Postel 2003), protein kinases (Hartsough et al. 2002) and
DNases (Fan et al. 2003). Involvement of Nme1 in metastasis
suppression opened a new field in molecular oncology and
lead to the discovery of other family members in numerous
species. Nme gene/protein family today consists of ten mem-
bers in humans. These proteins possess diverse biological
roles in the cells and are involved in proliferation, develop-
ment, differentiation, ciliary functions, vesicle transport and
apoptosis (Boissan et al. 2009). In spite of extensive scientific
research in this field for the last two decades, the mechanisms
by which the members of this family execute their biological
functions are still under investigation.

Nme proteins in vertebrates have been separated into two
groups on the basis of phylogenetic analysis, the presence of
protein domains and exon/intron structure. Human Nme1-
Nme4 proteins belong to group I, while Nme5-Nme9 belong
to group II. Proteins of the group I display a single type NDK

domain whereas group II proteins display a single or several NDK domains of different types, associated or not with extra-domains. The Nme10 protein displays a separate evolutionary history since it seems evident that its NDK domain was inserted relatively recently (Desvignes et al. 2009).

Recently, several studies suggest that it would be opportune to study genes/proteins that cause a certain disease by using model organisms that represent the evolutionary level at which these genes have emerged. The majority of disease genes (genes linked to heritable genetic diseases) were already present in the eukaryotic ancestor, and the second largest number has arisen around the time of evolution of multicellularity. The comparative genomics showed that many disease genes can be found in species distantly related to humans (Domazet-Loso and Tautz 2008). Similar has been suggested for cancer genes (genes associated with cancer, responsible for the malfunction of interactions between cells in a multicellular organism) (Domazet-Loso and Tautz 2010). Genome of *Amphimedon queenslandica* and data from other sponges revealed the origin of genes involved in cellular cooperation associated with multicellularity in animals (Harcet et al. 2010; Srivastava et al. 2010). Furthermore, many of the genes previously associated specifically with animals have recently been found in their unicellular relatives (Suga et al. 2013). These genes usually have specialized and specific functions in complex animals such as vertebrates or insects. The presence of genes associated with multicellularity in unicellular organisms as well as the presence of highly specialized genes in simple non-bilaterian animals inevitably raises a question about their functions in these different contexts. In some cases, it is possible that the true basic function of a gene is hidden by myriads of interactions in complex organisms. In other cases, the biochemical properties of a protein may change during evolution, and/or it may be co-opted for a different function. Many of these genes/proteins are involved in cancer formation in more developed species, especially when in their malfunctioning or mutated form. Cancer likely appeared in parallel with multicellularity and the development of true tissues and organs. Most of the cancer-related protein domains appeared during the two major evolutionary transitions—the origin of the first cell and the transition to multicellular metazoans (Domazet-Loso and Tautz 2010). Current knowledge about cancer as well as about the properties and functions of cancer-related genes in simple animals is scarce. Therefore, in most cases, we can only speculate about their original function, whether it has changed or how it might have affected their cancer-causing potential. For instance, a lot of metastasis suppressors as well as Nme proteins in particular have an effect on cell adhesion (Bago et al. 2009). The adhesion process is essential for establishment of multicellularity. Precise regulation of adhesion is linked to the increasing complexity in animals; the appearance of extracellular matrix, different cell types and tissues elaborate organs and organ systems. Therefore, it would be important to understand how characteristics and functions of these genes changed in the transition to multicellularity and with increasing complexity in animals. In this work, we review the current knowledge of Nme genes/proteins in basal animals as well as the available experimental data about their properties and functions.

Non-bilaterian metazoans—simple (early) animals

According to the current taxonomy, there are four phyla of non-bilaterian metazoans: Porifera (sponges), Placozoa, Cnidaria and Ctenophora (comb jellies). Genomes of five non-bilaterian species are available to this date: sponge *A. queenslandica*, ctenophore *Mnemiopsis leidyi*, placozoan *Trichoplax adhaerens* and cnidarians *Hydra vulgaris* and *Nematostella vectensis*. Non-bilaterians are often considered to have branched off at the base of the animal tree of life before the origin of Bilateria and are thus referred to as "basal metazoans" (Fig. 1). Major characteristics of all four basal metazoan groups are radically different developmental programs (compared to other animals) and adult body plans with no bilateral symmetry. Sponges can be radially symmetrical although most are asymmetrical, placozoans lack symmetry and cnidarians are radially symmetrical. Most ctenophoran species have modified radial (biradial) symmetry. All other

Fig. 1 Previously proposed relationships of the five deep clades of animals (modified from Ryan et al. 2013). *A* Coelenterata hypothesis. *B* Ctenophora as sister to Bilateria. *C* Porifera as sister group to the rest of Metazoa. *D* Ctenophora as sister group to the rest of Metazoa. *E* Placozoa as sister group to the rest of Metazoa. *F* Diploblastica hypothesis
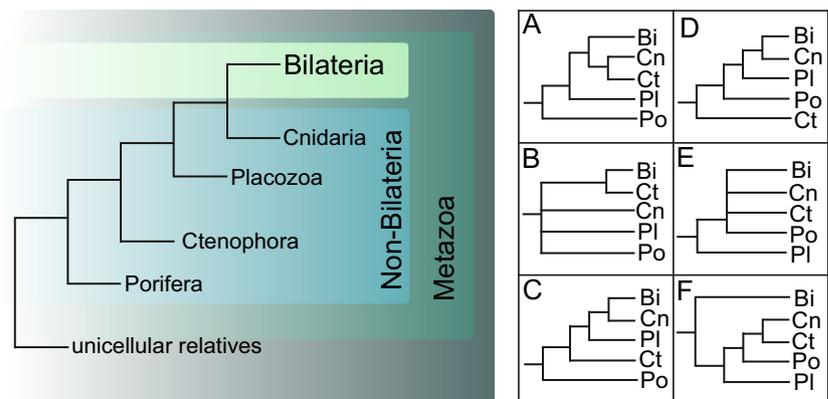
animals belong to the group Bilateria. Almost all Bilateria are triploblastic and bilaterally symmetrical (except echinoderms which are radially symmetrical as adults but have bilaterally symmetrical larvae). Cnidarians and ctenophores are diploblasts—they have only two primary germ layers: the ectoderm and the endoderm. Status of sponges is unclear with some authors claiming that they have no germ layers. Development of placozoans is largely unknown because no viable embryos have been recovered (Martindale 2005; Eitel et al. 2011).

Sponges

The sponges (phylum Porifera, pore-bearing) are among the simplest and probably the most ancient group of animals estimated to have separated from other metazoans more than 800 million years ago (Love et al. 2009). They are sessile as adults and lack true tissues and organs as well as any recognizable sensory or nervous structures. Body of a sponge is made of three layers, polygonal cells called pinacocytes cover the outside of the sponge. The inside layer is characterized by choanocytes—cells with a flagellum surrounded by a collar. The middle layer called mesohyl is a matrix of glycoproteins with several types of motile cells and skeletal elements (calcareous or siliceous spicules and/or protein spongin). More complex and bigger sponges usually have additional structural features, such as specialized choanocyte chambers and a network of water channels, but the basic structure is always conserved. Sponge bodies are covered by numerous small openings (pores) called ostia and have at least one big opening—osculum. Due to the coordinated action of choanocytes, water carrying food and oxygen enters the sponge body through ostia, moves through the channels and chambers and exits the sponge through the osculum. Besides producing the water current, the choanocytes filter food particles, which are subsequently digested by the cells in the mesohyl. Currently, there are some 8,500 valid sponge species inhabiting diverse marine and freshwater habitats divided into four classes: Demospongia, Hexactinellinda, Calcarea and Homnoscleromorpha. In contrast to their simple morphology, sponges have complex genomes (Srivastava et al. 2010). Many of their genes are strikingly similar to vertebrate homologues (Harcet et al. 2010). In addition, vertebrate orthologues of numerous sponge genes participate in highly specific cellular processes whose malfunction leads to initiation and/or progression of various diseases (Cetkovic et al. 2004).

Placozoans

Placozoans are a small group of very simple animals. The placozoan body consists of two layers of epithelial cells and an additional layer of loosely arranged fibre cells between them. The lower layer, which is in contact with the substrate, has ciliated and gland cells that are used for moving, adherence and feeding. No specialized muscle or nervous structures are present. Placozoans are normally less than 2 mm in diameter, asymmetrical and without any kind of body polarity. For a long time, only a single placozoan species was known—*Trichoplax adherens*. Recent studies show that the phylum is much more diverse (Voigt et al. 2004; Pearse and Voigt 2007). *Trichoplax* genome is compact but with many genes involved in complex cellular processes in "higher" animals (Srivastava et al. 2008). Placozoans and sponges do not possess true tissues and are thus sometimes grouped together into Parazoa.

Cnidarians

The cnidarians keep the basic three-layer body structure with an outside and an inside epithelium and a gelatinous mesoglea between them. However, they possess important evolutionary innovations: basement membrane, muscle cells, simple nervous system and sensory organs. Distinguishing feature of cnidarians are cnidocytes—explosive cells with harpoon-like structures and toxic content. Cnidocytes are used to capture prey and as a defense from predators. Cnidarians appear in two quite different forms: The medusa (jellyfish) is a free-swimming form, while the polyp is attached to the substrate, sometimes connected with other polyps into a colony. Both forms are radially symmetrical with tentacles surrounding the mouth. There are over 10,000 described species of cnidarians that belong to one of the four classes: Hydrozoa, Scyphozoa, Cubozoa and Anthozoa. (Collins 2002).

Ctenophores

The ctenophores or comb jellies have the same basic body structure as the cnidarians—an outside and an inside epithelium with mesoglea between them. Due to the similarity, the two groups were historically classified in the same phylum—Coelenterata. Still, there are some important differences. Ctenophores do not have cnidocytes, their epithelia have two cell layers and they possess multicilliated cells. These cells form usually eight rows (combs) along the body, which are used for swimming, thus making ctenophores the largest organisms that use cilia for locomotion. Like cnidarians, ctenophores have muscle cells as well as simple nervous system and sensory organs. Ctenophores, cnidarians, and Bilateria have true tissues and thus belong to the supergroup Eumetazoa. There are around 150 ctenophoran species divided into two classes: Nuda and Tentaculata. The latter have long tentacles with glue-producing cells that are used to capture prey.

Phylogenetic relationship among basal Metazoa

The phylogenetic relationships among non-bilaterian phyla and their relationship with the Bilateria are still not well

understood and are a subject of much debate. Different hypothesis about the relationships of the five deep clades of animals are shown in Fig. 1. Traditional view is that the sponges are the earliest branching lineage (Nielsen et al. 1996). Phylogenomic studies based on the genomic sequences of a sponge (*A. queenslandica*), a placozoan (*T. adherens*) and a cnidarian (*N. vectensis*) are in concordance with the traditional view—sponges are basal and placozoans are a sister group to Eumetazoa (Putnam et al. 2007; Srivastava et al. 2008, 2010). Other studies using large datasets produced somewhat different results, e.g. placing placozoans as the most basal group (Schierwater et al. 2009). However, there are also high-impact studies that place ctenophores at the base of the animal tree of life (Dunn et al. 2008). This work has received some strong criticism with critics interpreting the results as an artifact of the phylogenetic methods used (Philippe et al. 2009; Pick et al. 2010). A recently published ctenophore genome paper (Ryan et al. 2013) again places ctenophores as the earliest branching animals. If true, this finding would have a huge impact on our understanding of early animal evolution. It would mean that the last common ancestor of Metazoa was relatively complex and had true tissues, muscle cells, nervous and sensory systems, while sponges became simpler due to their highly specialized lifestyle of sessile water filterers.

Within basal metazoan phyla, there are also many unresolved relationships. Sponges are notoriously difficult to classify. There is, for example, an ongoing debate regarding monophyly of the phylum and the position of the calcareous sponges (class Calcarea) (Worheide et al. 2012). The position of Myxozoa, a group of simple parasitic animals, is also uncertain. They are currently considered to be highly specialized cnidarians (Jimenez-Guri et al. 2007), although some authors claim that they are more closely related to Bilateria (Zrzavy and Hypsa 2003). It is unlikely that we will soon have definite answers for many of the questions regarding the phylogeny of the non-bilaterian animals. In order to get a clearer picture of early animal evolution, we will need to broaden the phylogenomic sampling, improve the phylogenomic tree construction methods and integrate the molecular methods with classical biology (Philippe et al. 2011).

Nme genes/proteins

Group I

In the last 20 years, the Nme group I has been thoroughly investigated. Therefore, a lot has been discovered about the biochemical and genetic properties of its members, at least in vertebrate representatives. Group I protein members are generally highly homologous among themselves and compared to

orthologues in different species and their corresponding genes have similar exon-intron structures (Desvignes et al. 2009; Perina et al. 2011a). These proteins are active as hexamers and all possess the nine residues essential for the stability and kinase activity (Morera et al. 1995; Min et al. 2000; Perina et al. 2011a). Group I encompasses four human Nme genes/proteins, Nme1-Nme4 and their homologues (Fig. 2). It has been suggested that the group I *Nme1/2* and *Nme3/4* genes arose from an ancestor gene common to all chordates by the first round of whole genome duplication (1R) which occurred early in the vertebrate lineage (Desvignes et al. 2009). *Nme3* and *Nme4* arose from a *cis*-duplication of *Nme3/4* gene that occurred probably before or around teleost radiation, while *Nme1* and *Nme2* separated through *cis*-duplication after the emergence of amphibians. Duplications of group I *Nme* genes are not exclusive for vertebrates. It has been observed that they occurred independently more than once within the same lineages in non-bilaterian metazoans (cnidarians and calcareous sponges) (Desvignes et al. 2009; Perina et al. 2011a). Group I *Nme* genes were analyzed in two demosponges *A. queenslandica* and *Suberites domuncula* (Perina et al. 2011a). Only one gene coding for group I Nme enzyme was found in the genome of the sponge *A. queenslandica* (Srivastava et al. 2010) and *S. domuncula* cDNAs (Harcet et al. 2010). Searches of NCBI's dbEST revealed the presence of cDNAs that encode two group I Nme proteins in the calcareous sponges *Sycon raphanus* and *Leucetta chagosensis*. Phylogenetic analyses indicated their independent duplications (Perina et al. 2011a). Independent duplications are also observed in cnidarians *H. vulgaris* and in *N. vectensis*, as well as in ctenophore *M. leidyi* (Fig. 3). All of these Nme proteins are similar in primary structure and possess conserved residues essential for NDPK activity. Biochemical and functional characterizations of non-bilaterian Nme proteins from group I are still not well documented. Perina and collaborators (Perina et al. 2011a) presume that the group I Nme gene/protein of the metazoan last common ancestor was structurally and functionally similar to the multifunctional enzyme it is today. It has been demonstrated that sponge genes coding for group I Nme protein are intron rich and these introns are relatively short. Analyses of introns show that the ancestral metazoan group I *Nme* gene was also intron rich and probably had all four introns that are still present in most extant basal metazoan homologues. Furthermore, analysis of sponge *NmeGpI* promoters revealed that some of the motifs crucial for human promoter activity are also present in sponges. Interestingly, under the same search parameters, these motifs have not been found in the corresponding choanoflagellate promoter which indicates a possible change in *Nme1* regulation in the metazoan lineage. Analysis of protein activity showed that the sponge NmeGp1Sd protein possesses a hexameric form and has a similar level of kinase activity as the human homologues.

Fig. 2 Schematic architecture of domains present in Nme representatives. *Numbers* indicate amino acids. Proteins are represented in a scale 1:10 (1 mm=10 amino acids). Protein domains have been indicated with *coloured boxes*, and each protein has been searched against SMART/Pfam databases. Abbreviations of domain names are retrieved from SMART/Pfam databases and indicated in the figure. Shortened names include the following: transmembrane domain (TM) and mitochondrial localization signal domain (MLS)
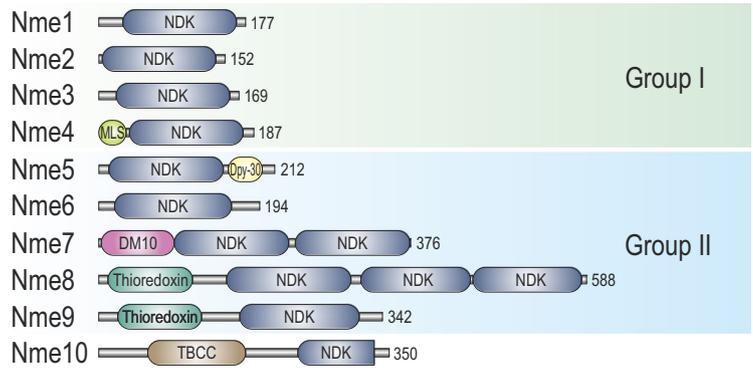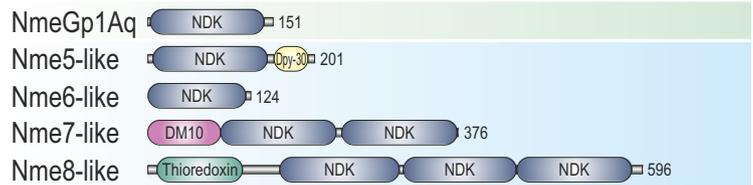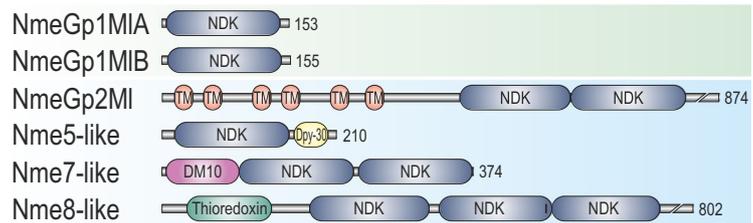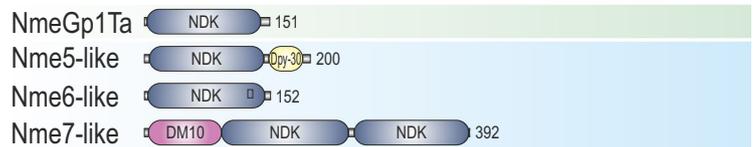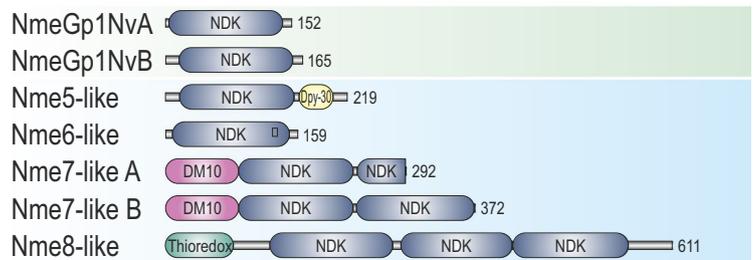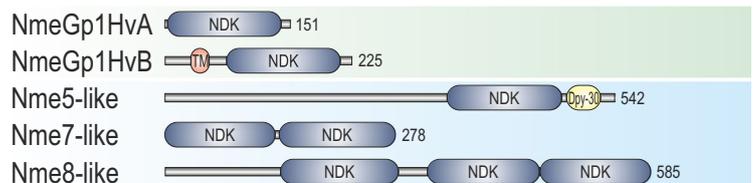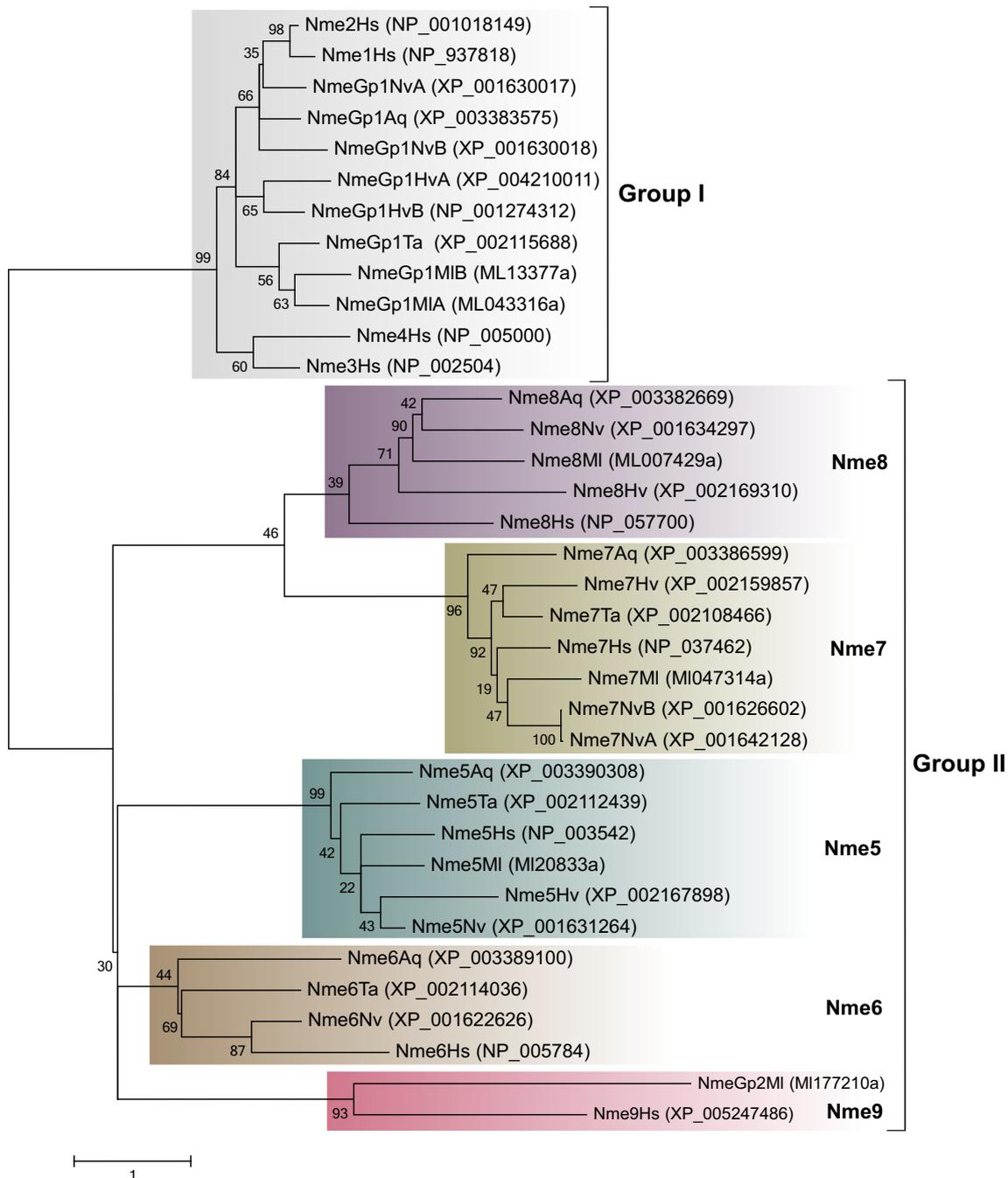
Fig. 3 Maximum likelihood phylogenetic tree of Nme proteins from representative species. Bootstrap values ML (>60 %) are given above nodes and MCMC (>0.6) (below nodes). Accession numbers of sequences used are given after species names. The *scale bar* indicates the genetic distance of the branch lengths. The collected Nme proteins were aligned with the PROMALS multiple alignment tool, using default settings (Pei and Grishin 2007). The multiple alignment was subjected to a maximum likelihood (ML) analysis using MEGA6 (Tamura et al. 2013). The model for ML analysis was selected with ProtTest 2.4 (Abascal et al. 2005) and the Akaike information criterion (AIC) (Posada and Crandall 1998), which indicated the Le_Gascuel_2008 model (LG + I + G) (Le and Gascuel 2008). Bayesian MCMC analysis was performed in MrBayes v. 3.1.2. (Ronquist and Huelsenbeck 2003). Bootstrap tests were performed with 1,000 replicates

NmeGp1Sd shows nonspecific DNA-binding with single-stranded circular DNA, like the human Nme2, but does not possess the ability of human Nme2 to cleave negatively supercoiled DNA in the NHE sequence of *c-myc* promoter. NmeGp1Sd interacts with its human orthologue/homologue in human cultured cells and shows the same subcellular localization pattern as Nme1. Furthermore, if expressed in human tumour cells, the NmeGp1Sd significantly diminishes its migration potential which leads to the conclusion that the sponge Nme protein can replace its human homologue/orthologue in

at least some of its biological functions which are usually associated with "higher" metazoans (Perina et al. 2011a). Migration of cells in complex metazoan phyla is limited to specific and tightly controlled processes in the embryonic development, wound healing or immune response. The anchorage and migration of cells depends on a large number of molecules that enable the movement through the extracellular matrix composed of various protein elements such as fibronectins and collagens. Sponges do not possess true tissues or organs but simple varieties of these molecules as well as the mesohyl—a space between cell layers that resembles a primitive extracellular matrix. Cell migration in sponges is present in several different processes, one being the movement of special variety of cells, the amoeboid cells, through the mesohyl. It has been established for quite some time that sponges possess a large variety of highly sophisticated genes usually linked to processes known to be engaged exclusively in higher metazoan species (the components of the extracellular matrix, tyrosine kinases, neuronal like receptors, etc. (Mueller 2001)). The possible activity and function of these genes/proteins in simple animals as sponges are not yet understood, but it is possible that they participate in ancient precursor processes which are actually the foundation for the complex signalling networks of higher animals. Therefore, finding the function of the ancient variants of proteins that are still present in extant simple metazoans is important for understanding the functions of their homologues in intricate processes of complex metazoan organisms (such as mammals). In accordance with this view, we can presume that the migration of amoeboid cells through the mesohyl and the movement of cells through the ECM may originate from the same ancient process present in the last common ancestor of all metazoans.

Group II

In contrast to group I members, homologues of almost all human group II members are present in non-bilaterian metazoans (Fig. 2). Desvignes and coworkers (Desvignes et al. 2010) found that numerous metazoan species display a full set of the group II *Nme* genes with the exception of Placozoa. Their data demonstrated that Nme5-Nme8 were already present in the genome of the common ancestor of choanoflagellates and metazoans and emerged around eukaryote radiation. Most group II genes/proteins are present in early-branching eukaryotic lineages. Two exceptions are Nme8, which is probably a choanoflagellate/metazoan innovation, and Nme9, which originated from an incompletely translocated duplication of Nme8 after separation of eutherians and metatherians. Human group II members are highly divergent among themselves. Multimeric form has not been demonstrated for any of the group II members. The available data suggest that only Nme6 displays NDPK activity,

although this finding is still debatable (Tsuiki et al. 1999; Yoon et al. 2005; Perina et al. 2011b). The Nme5 protein has only seven out of nine residues crucial for the NDPK activity, and therefore, it is highly improbable that it is enzymatically active. Nme5 is expressed in human testes, in the flagella of spermatids and spermatozoa, in association with axoneme microtubules, where it is probably required for sperm motility (Munier et al. 1998, 2003). Almost all metazoan Nme5 possess an additional C-terminal Dpy-30 domain (Fig. 2). The role of Dpy-30 is still unclarified. However, it is known for its involvement in histone methylation, trans-Golgi trafficking, assembly or functioning of cilia and carcinogenesis (Xu et al. 2009; Gopal et al. 2012). In mammals, Dpy-30 is crucial in cell fate specification of embryonic stem cells (Jiang et al. 2011). All of the non-bilaterian metazoans with sequenced genomes possess Nme5 with both characteristic domains (Fig. 2) which may indicate its importance in animal evolution. However, its functions are probably not related with the NDPK activity.

Compared to the human Nme1, the human Nme6 protein has 22 additional residues at the C-terminus. Furthermore, three additional residues are present in the Kpn loop, just like in the human Nme5. In non-bilaterian Nme6 homologues, the C-terminus extension is usually not present, but three additional residues in the Kpn-loop are. The only known non-bilaterian Nme6 with a C-terminal extension is the one from the sponge *S. domuncula* (Perina et al. 2011b). Nme6 homologues are not present in cnidarian *H. vulgaris* and ctenophore *M. leidyi*. Tsuiki and coworkers (Tsuiki et al. 1999) demonstrated that the human Nme6 protein has an NDPK activity and that it localizes, at least partly, in mitochondria. Overexpression of Nme6 in SAOS2 cells resulted in growth suppression and formation of multinucleated cells, which suggests that Nme6 has a role in cell growth and cell cycle progression. Conversely, additional NDPK activity measurements of all human Nme proteins revealed that none of the group II members display measurable NDPK activities (Yoon et al. 2005). Recent findings of our group suggest that the sponge Nme6 does not possess measurable NDPK activity and does not localize in mitochondria, at least not in human cells, although it has a putative mitochondrial signal sequence. It lacks two recent introns that comprise miRNAs and has different transcriptional binding sites in the promoter region (Perina et al. 2011b). Therefore, we concluded that the function of Nme6 gene has changed during metazoan evolution possibly in correlation with the structure of the protein. Recent findings demonstrated that Nme6 and Nme7 are important for the regulation of Oct4, Nanog, Klf4, c-Myc, telomerase, Dnmt3B, Sox2 and ERas expression. Knockdown of either Nme6 or Nme7 reduces the formation of embryoid body and teratoma which implies the importance of Nme6 and Nme7 in embryonic stem cell renewal (Wang et al. 2012). This may indicate possible functions in non-bilaterian metazoans that
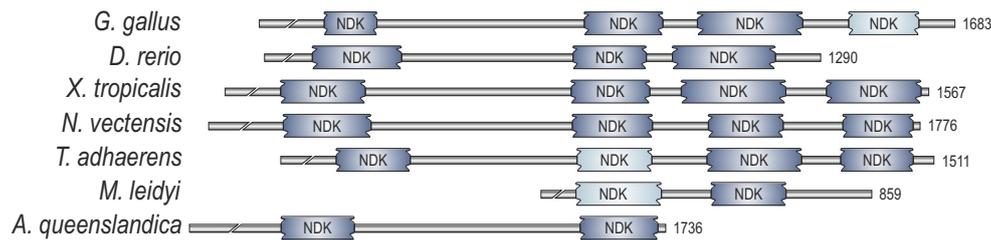
Fig. 4 Schematic representation of truncated inactive NDK domains found in Nme proteins from non-bilaterian metazoans and vertebrate representatives. The names of the species are given on the *left*. *Numbers* indicate amino acids. NDK-truncated domains are illustrated by *coloured boxes* and were defined according to SUPERFAMILY 1.75 (Gough et al. 2001). Brighter NDK domains had *E* value greater than 1.0e-05. Protein database accession numbers for the illustrated proteins are the following: XP_003642206 (*G. gallus*), XP_005164296 (*D. rerio*), XP_002932503 (*X. tropicalis*), XP_001622810 (*N. vectensis*), XP_002108458 (*T. adhaerens*), ML05694a (*M. leidyi*) and XP_003387107 (*A. queenslandica*)

need to be tested. Nme7 protein contains two NDK-like domains. Each of the NDK domains has three additional residues in the Kpn loop and is lacking three residues necessary for the enzymatic activity; therefore, it is considered to be NDPK inactive. In mammals, Nme7 activities are associated with ciliary motility. These functions may be ancient and general for all eukaryotes since in *Chlamydomonas*, this protein is tightly associated with the flagellar axoneme (Ikeda 2010). Nme7 homologues possess additional N-terminal DM10 domain whose function is largely unknown. In *Chlamydomonas*, DM10 domain-containing proteins are tightly bound to the flagellar doublet microtubules which may suggests that DM10 domains might act as flagellar NDPK regulatory modules or as units specifically involved in axonemal targeting or assembly (King 2006). Nme7 homologues are present in all analyzed non-bilaterian metazoans with both characteristic domains (Fig. 2). The Nme8 and Nme9 display a similar exon/intron structure but are located on different chromosomes. Therefore, it seems likely that Nme9 originates from an incompletely translocated duplication of Nme8. Both proteins possess thioredoxin domain at the N-terminus. Nme8 is present in all non-bilaterian metazoans except in placozoans (Figs. 2 and 3). In humans, defects in Nme8 cause primary ciliary dyskinesia, indicating its importance in ciliary function (Duriez et al. 2007). Nme8 lacks detectable NDPK activity but has a 3′→5′ exonuclease activity like Nme1/2 from group I, Nme5 and Nme7 from group II and Nme10 (Yoon et al. 2005, 2006). Ancient origin and conservation of Nme8 in animal lineage indicate its importance in metazoan evolution. Beside human Nme5-8 homologues present in non-bilaterian metazoans, a protein named NmeGp2Ml from ctenophoran *M. leidyi* groups together with the human Nme9 (Fig. 3). This grouping probably does not reflect the true relationship between the two proteins but demonstrates that in some lineages, distinct Nme proteins can emerge. The *M. leidyi* NmeGp2Ml protein has as much as six transmembrane regions at the N-terminus followed by two NDK domains (Fig. 2). Additionally, in all non-bilaterian lineages, proteins containing only catalytically inactive truncated NDK domain exist (Fig. 4). These proteins seem to be conserved throughout the metazoan lineage as its homologues are also present in fishes, amphibians and birds (Fig. 4) and they do not display homology to any of the existing members of groups I or II. The phylogenetic tree shows that these proteins group together in a separate clade (data not shown). The truncated NDK domain is most similar to the NDK domain in Nme7; however, on the level of the whole sequence, the similarity is not relevant. Functions of these Nme proteins are yet to be determined.

Conclusion

Currently, available data show that the group I Nme protein in metazoan ancestor already possessed a lot of biochemical properties of human homologues as well as potential to modulate the migratory properties of the cell which seems to be switched on even before the composition of true tissue. There are three groups of unicellular eukaryotes closely related to animals: Choanoflagellata, Filasterea and Ichtyosporea. In all three phyla, there are free-living and colonial species, as well as species whose life cycles include unicellular and colonial or aggregative phases. In order to better understand the function of Nme in cell adhesion, it would be important to study the properties of group I Nme homologues in unicellular relatives of animals. Comparison of homologues from free-living and colonial species may reveal whether the group I Nme has a role in aggregation and colony formation in these organisms. Comparison with animal homologues could demonstrate how these proteins changed during the transition to multicellularity in the ancestor of animals. These analyses might help in understanding the still illusive mechanism of antimetastatic action of Nme and origin of cancer in general. Basic properties of group II members need to be traced much further back in evolutionary history, probably to the ancestor of all eukaryotes. According to the current knowledge, all of the recent group II Nme proteins in non-bilaterian metazoans are NDPK

inactive as are their vertebrate homologues. The biochemical properties of these members are still unclarified but are probably not NDPK activity related.

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