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EDITORIAL

Progress on *Nme* (NDP kinase/Nm23/Awd) gene family-related functions derived from animal model systems: studies on development, cardiovascular disease, and cancer metastasis exemplified

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The enzyme nucleoside diphosphate kinase (NDP kinase or NDPK) was discovered in the 1950s as biochemical activity that removes the terminal phosphate from a nucleoside triphosphate (NTP) and adds it to a nucleoside diphosphate (NDP). Thus, the correct biochemical name for the enzyme is NTP/NDP transphosphorylase, and it is generally regarded as a housekeeping enzyme required for nucleotide homeostasis. At least four of the ten *Nme* gene family products (*Nme1–Nme4*), also called group I *Nme* proteins, carry that enzymatic activity. A far more complex story was started in the 1990s when it became evident that enhanced cancer metastasis was linked to reduced expression of a gene named *nm23*, which turned out to be identical to human *Nme1* = NDPK A. To date, the function of *Nme1* underlying its metastasis suppressor activity has not been clarified. It is hoped that fundamental cellular and molecular insights into the role of NDP kinase/

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Nm23/awd proteins in development may explain its pathophysiological roles. Based on the reports made on the 9th International Congress of the NDP Kinase/Nm23/awd Gene Family in August 2013 in Boston, USA, experts in the field have summarized their ideas and views in review articles or contributed novel original research to a special issue of *Naunyn-Schmiedeberg's Archives of Pharmacology*. Within this editorial article, we propose a novel, potentially unifying hypothesis, which developed from studies from model organisms, but may help to understand the role of *Nme* proteins in cardiovascular diseases as well as in cancer.

Developmental studies in model systems

Studies of normal physiological functions of *Nme* genes using model organisms provide an excellent opportunity to unravel the underlying mechanism of *Nme* action. Indeed, studies using model systems have suggested a basic, unifying principle of *Nme* actions: *Nme* (or at least the group I members *Nme1–4*) is critical for vesicle and macromolecular transport, and *Nme* proteins perform this cellular function by acting as a scaffold.

Drosophila melanogaster

The only ortholog of the *Nme* genes in *Drosophila*, *a*bnormal *w*ing *d*iscs (*awd*), was discovered soon after the human *Nme1* (also known as *Nm23H1* or *NDPK A*) metastasis suppressor activity was identified (Dearolf et al. 1988; Nallamothu et al. 2009; Steeg et al. 1988). Early studies in *Drosophila* showed that *awd* mutant larval brain exhibited mitotic defects correlated with defective microtubule polymerization (Biggs et al. 1990). Subsequently, *awd* has been implicated in the process of endocytosis in multiple tissues, including neurotransmitter uptake at the neuromuscular junctions (Krishnan et al. 2001), surface receptor internalization that modulates the chemotaxis response in tracheal cell and border cell migration (Dammai et al. 2003; Nallamothu et al. 2008), recycling of adherens junction components (Woolworth et al. 2009), and promotion of early-to-late endosome transition that is critical for Notch signaling in follicle cells and in imaginal discs (Ignesti et al. 2014). These endocytic functions were associated with the functions of dynamin and Rab5, suggesting a GTP-supplier function for these monomeric GTPases.

Interestingly, *awd* protein activity is negatively regulated by the kinase action of 5′ adenosine monophosphate-activated protein kinase (*AMPK*) (Onyenwoke et al. 2012). *AMPK* is a key regulator of cellular energy homeostasis (Hardie et al. 2003). It is activated during starvation. Previous studies have shown that *AMPKα* mutation could cause epithelial disruption and proliferation in the follicle cells but only under energy stress (Mirouse et al. 2007). Since loss of *awd* results in delocalization of adherens junction and breakdown of epithelial characteristics (Woolworth et al. 2009), the *AMPK-awd* axis may the key component in the regulation of nutrient-dependent epithelial integrity.

Caenorhabditis elegans

Like *Drosophila*, *C. elegans* has a single *Nme* gene named *ndk-1*. Knockout of *ndk-1* displayed sterility and a protruding vulva (Masoudi et al. 2013). Vulva development in *C. elegans* is largely determined by the canonical MAP kinase signaling. Epistasis study demonstrated that *ndk-1* acts downstream of, or in parallel to, *lin-45*, the ortholog of the proto-oncogene serine/threonine-protein kinase *Raf*, and upstream of *mek-2* and *mpk-1*, the orthologs of the mitogen-activated kinases *MEK* and mitogen-activated protein kinase (*MAPK*), respectively. This placed the *ndk-1* function at the same level as the kinase suppressors of *ras* (*Ksr*). *Ksr* encodes a scaffold protein that coordinates the MAPK relay system. Interestingly, it has been shown that mammalian *Nme1* protein binds Ksr and can function as a protein kinase for this scaffold (Hartsough et al. 2002). However, in mammalian cells, the phosphorylation is inhibitory, while in *C. elegans*, the interaction between *ndk-1* and *Ksr* promotes MAPK signaling. Such difference may reflect different tissue context, although whether the worm *ndk-1* protein can phosphorylate *Ksr* has yet to be determined.

In subsequent studies (Fancsalszky et al. 2014), the Takács-Vellai group also discovered that *ndk-1* is additionally involved in the migration of distal tip cell and engulfment and clearance of apoptotic corpses by gonadal sheath cells. These functions were placed downstream of *ced-10*, the ortholog of the monomeric GTPase Rac1, and showed a genetic interaction with *dyn*, the ortholog of the monomeric GTPase dynamin.

Danio rerio

The zebrafish is an excellent developmental model for organogenesis, because of the transparent body of the animal. Taking advantage of this model, Wieland et al. have shown that association of *Nme2* (*NDPK B*) with the G protein βγ dimer (Gβγ) is required for G protein function in vivo (Hippe et al. 2009). This function in turn is critical for cardiac contractility, as *Nme2*, but not *Nme1*, knockdown resulted in cardiac phenotype characterized by a severely impaired cardiac contractility of both chambers of the heart, pericardial edema, and bradycardia (Hippe et al. 2011b). Such phenotype is linked with the failure of heterotrimeric G protein subunits and their resident caveolae to be transported to the cell surface. Indeed, *Nme2* was found to associate with caveolae in normal cells (Hippe et al. 2011a; Hippe et al. 2011b).

More recently, the same laboratory also demonstrated that zebrafish larvae depleted of *Nme2* displayed severe

malformations specifically in vessels formed by angiogenesis. Importantly, a similar phenotype was observed in *Nme2*-deficient mice when the animals were subjected to oxygen-induced retinopathy. In this model, the number of preretinal neovascularizations in *Nme2* (−/−) mice was strongly reduced in comparison with wild-type littermates (Feng et al. 2014). This defect may be correlated with the observation that *Nme2* depletion impaired vascular endothelial growth factor (VEGF)-induced sprouting and hampered the VEGF-induced spatial redistributions of the VEGF receptor type 2 (VEGFR2) and VE-cadherin at the plasma membrane.

Mus musculus

Somewhat surprising, the mice deficient in the *Nme1* or the *Nme2* gene have not yielded profound developmental defects, which is likely due to *Nme* isoform redundancy and/or compensatory mechanisms. *Nme1* (−/−) females cannot feed their pups as a result of growth retardation of the mammary glands and defects in the final step of mammary duct maturation of the nipple leading to duct obstruction (Deplagne et al. 2011).

Nme2 (−/−) mice are phenotypically normal at birth with a normal life span. They exhibit, however, a mild impairment of heart contractility at the age of 6 months or older. Although T and B cell development is normal in *Nme2* (−/−) mice, $K_{Ca}3.1$ channel activity and cytokine production are markedly defective in T helper 1 (Th1) and Th2 cells (Di et al. 2010). This phenotype is consistent with the previous finding that the *Nme2* protein activates the potassium channel $K_{Ca}3.1$ via histidine phosphorylation of the C terminus of the channel. This activation is required for T cell receptor-stimulated Ca^{2+} influx and proliferation of activated naive human CD4 T cells (Srivastava et al. 2006a).

Interestingly, *Nme1*(−/−); *Nme2*(−/−) double knockout mice are undersized, die perinatally, and exhibit a spectrum of hematological phenotypes including severe anemia, impaired maturation of erythrocytes, and abnormal hematopoiesis in the liver and bone marrow (Postel et al. 2009). The underlying molecular and cellular defects of this phenotype are not yet clear, but it is reasonable to speculate that defective chemotactic signaling regulated by receptor trafficking may be involved.

Functions of the *Nme* family gene products related to cardiovascular disease

Especially, *Nme2* has been associated with functions in the cardiovascular system. Hippe et al. 2007 reported that *Nme2* forms a complex with the β subunit of the heterotrimeric G_s protein in cardiomyocytes thereby regulating cardiac contractility. In a subsequent study, it was found that two distinct functions of *Nme2* are involved in that regulation (Hippe et al. 2011a). One requires the histidine kinase activity which phosphorylates His266 in Gβ (Cuello et al. 2003) and feeds into a phosphorelay fueling GTP for G protein activation. The second is apparently a scaffold function regulating the formation of caveolae which does not require enzymatic activity but complex formation with caveolins (Hippe et al. 2011a).

Nme2 as protein histidine kinase

As outlined in the review by Attwood and Wieland (2014), phosphorylation and dephosphorylation of protein histidine residues is quite common in signal transduction pathways in prokaryotes and lower eukaryotes, like yeast fungi and plants. In recent years, increasing evidence has been presented that *Nme2* can act as protein histidine kinase in mammals. The so far described few substrates of the *Nme2*, the channels $K_{Ca}3.1$ and TRPV5 (Cai et al. 2014; Srivastava et al. 2006b), the G protein β subunit (Cuello et al. 2003), and annexin 1 (Muimo et al. 2000) fall into at least two subgroups. The histidine phosphorylation of the channels follows the classical paradigm in which phosphorylation of a protein alters its confirmation and/or activity. Both channels exhibit higher open probability upon histidine phosphorylation, and this can be reversed upon dephosphorylation by the phosphohistidine-specific phosphatase PHP. As mentioned above, the importance of the regulation of these channels has been shown in mouse models (Cai et al. 2014; Di et al. 2010). Although also a substrate for PHP (Maurer et al. 2005), histidine-phosphorylated Gβ does not show altered activity and thus does not belong to the first category. His266 in Gβ apparently serves as a storage site for a high energetic phosphate group which can be retransferred onto GDP. The newly formed GTP is subsequently used to activate the heterotrimeric G protein (Hippe et al. 2007).

Nme2/caveolin interaction

The association of caveolin-1 and caveolin-3 with *Nme2* has first been detected in the zebrafish but later confirmed also in mammalian cells (Hippe et al. 2009). Accordingly, *Nme2* as well as caveolin-3 (the prominent caveolin isoform in striated muscle) depleted zebrafish exhibit an impaired cardiac contractility (Hippe et al. 2011b; Hippe et al. 2009). Interestingly, the *Nme2* knockdown in the zebrafish also impaired vessel formation by angiogenesis, a phenotype also seen under pathological conditions in *Nme2* (−/−) mice (Feng et al. 2014). Apparently, the VEGF-induced spatial redistribution of the VEGFR2 is attenuated in *Nme2*-deficient endothelial cells. As the VEGFR2 resides in caveolae and the absence of caveolin-1 causes endothelial dysfunction as well as angiogenesis defects (Lin et al. 2007; Sonveaux et al. 2004), it is very likely that these defects are also related to the disturbed

Nme2/caveolin interaction. Caveolin oligomers are already formed in the endoplasmic reticulum (Hayer et al. 2010). Thus, the reported facilitation of coat protein complex II (COPII)-dependent vesicular transport from ER exit sites by *Nme2* (Kapetanovich et al. 2005) highlights a localization where protein complexes of *Nme2* with caveolins might assemble.

Cancer-related functions of the *Nme* gene family

Nme1 as metastasis suppressor

Metastasis is the movement of tumor cells from their initial site of origin to distant sites of the body and their progressive colonization of those sites. Metastases, by direct organ compromise or by complications of their treatment, are the major cause of cancer mortality. Differential expression of the *Nme* gene family was first identified in experimental melanoma metastasis. *Nme1* was more highly expressed in poorly metastatic sublines of the K-1735 murine melanoma than in related, highly metastatic sublines (Steeg et al. 1988). Transfection of *Nme1* into a highly metastatic melanoma line had no effect on primary tumor size but significantly reduced metastasis (Leone et al. 1991), the founding definition of a metastasis suppressor gene. Multiple confirmatory studies have been published in several cancer histologies (reviewed in Marino et al. (2012)), excluding leukemias, lymphomas, and neuroblastoma. Upon developing chemically induced liver cancer, *Nme1*-deficient mice formed significantly greater lung metastases than wild-type mice (Boissan et al. 2005), confirming a metastasis suppressor activity. Recent articles have expanded the breadth of metastasis suppression to *Nme1* in UV radiation-induced melanoma (Jarrett et al. 2013) and lung cancer (Fan et al. 2013) and to *Nme3* (also named *Nm23-DR*) in colorectal cancer (Qu et al. 2013). The function of *Nme1* has also expanded to some aspects of tumorigenesis (Jarrett et al. 2011). The potential role of *Nme2* in tumorigenesis and metastasis is discussed in the reviews by Li et al. (2014) and Chowdhury (2014), respectively, in this issue of *Naunyn-Schmiedeberg's Archives of Pharmacology*.

Multiple aspects of metastasis have been implicated in *Nme1* suppression. Chiefly studied is tumor cell motility and invasion, the latter being traversal of an extracellular matrix. Invasion involves reversible changes in cell adhesion, motility, and proteolysis, often fueled by gene expression changes in the epithelial-mesenchymal transition (EMT). Surprisingly, no single set of pathways has been shown to be *Nme1*-dependent. Rather, a bewildering host of adhesion molecules (Boissan et al. 2010; Fournier et al. 2002; Kaetzel et al. 2014), motility factors, signaling pathways (Hartsough et al. 2002; Masoudi et al. 2013; Murakami et al. 2008; Otero 1997; Otsuki et al. 2001; Roymans et al. 2000; Seong et al. 2007; Tanaka et al. 2012; You et al. 2014), proteolytic events (Khan et al. 2001), EMT hallmarks (Zhao et al. 2013), and other transcriptional programs (Horak et al. 2007) have been functionally linked to *Nme1*. A new, important finding showed that lysosomal cysteine cathepsins degraded *Nme1* proteins under the direction of C-Abl and Arg, limiting invasiveness (Fiore et al. 2014). That invasion and motility may be fundamental to *Nme1*'s biological effects is supported by reports that its orthologs regulate these processes in *Drosophila* and *C. elegans* (Fancsalszky et al. 2014; Nallamothu et al. 2008).

Colonization of a distant site is also a fundamental part of cancer metastasis and may be the most amenable to translational development. For many cancer patients, evidence of initial spread is apparent at the time of initial diagnosis and surgery, such as involved lymph nodes. Thus, development of a motility or invasion inhibitor may be unusable since it has already occurred. Colonization of a distant site, evidenced by imaging, may have started but remains incomplete in many cancer patients. Experimental metastasis assays, in which tumor cells are inoculated into the circulation and bypass initial invasion steps, confirmed a metastasis suppressor activity for *Nme1*. Intravidal microscopy showed that control and *Nme1* transfectants of a breast cancer cell line reached the lung at comparable rates but that Nme1-overexpressing tumor cells survived in a distant organ more poorly (Horak et al. 2007).

Nme1 protein may interrupt the metastasis process by binding metastasis-promoting proteins in a scavenger like function. The number of validated protein/protein interactions (PPI) of the *Nme* protein family is large and growing. It includes viral proteins capable of causing invasion and metastasis (Banerjee et al. 2014; Murakami et al. 2005; Qin et al. 2011; Subramanian et al. 2001), oncogenes (Fishbach and Settleman 2003; Ignesti et al. 2014; Iwashita et al. 2004; Jarrett et al. 2013; Murakami et al. 2008), and other factors promoting aggressiveness and metastasis (D'Angelo et al. 2004; Horak et al. 2007; Reymond et al. 1999). Some of these interactions were disputed as they relied exclusively on the use of antibodies whose specificity was suspect. Alternatively, interactions of *Nme1* protein and other proteins may be indirect, part of a signaling complex. But even when reliable antibodies are available, the fact that two proteins can be co-precipitated from cell lysates does not ensure that they exist in a complex in a given intact cells. Cell lysis, for example, may disrupt subcellular compartmentalization and thus allow for PPIs actually not present in the native environment. For these reasons, independent lines of evidence should be presented to allow for the conclusion that two proteins are part of a larger protein complex and actually interact. An example for many of such independent lines of evidence would be the binding of h-prune to Nme1 and Nme2 in mammalian tumorigenic cells. Starting from a genetic interaction model based on homology

to *prune* and *Drosophila awd* (Reymond et al. 1999), it was found that the phosphorylation of *Nme1* serine residues 122 and 125 by casein kinase I was regulating this PPI (Garzia et al. 2008), thereby enhancing WNT signaling and vesicular trafficking. The resulting increase in cell motility finally propagates cell escape from the primary tumor site and metastases (Carotenuto et al. 2014; Carotenuto et al. 2013; Garzia et al. 2008). In a review in this issue of *Naunyn-Schmiedeberg's Archives of Pharmacology* (Vlatkovic et al. 2014), the authors raised a pivotal question to all PPI studies, "the issue of false or incorrect syllogisms in PPI functional studies." Based on the arguments raised by Vlatkovic et al., the manifold of proposed interactions of *Nme* proteins with other proteins have to be carefully reevaluated for mis- or overinterpretation of the actual data. Often, the proposed direct interaction is more a "tip of an iceberg" situation where one partner is sitting on the top; the others, although detected by the used functional assays, are located somewhere else. Such results therefore give only very limited insights in the complex situation "below the surface of the water" (Galasso and Zollo 2009). Therefore, systems biology analyses based on in vivo observations may be an appropriate approach to validate the hypotheses regarding the multiple *Nme* protein family interaction partners and the related functions.

Another potential contribution of the *Nme* family to cancer progression lies in its interaction with DNA causing genomic instability (Kaetzel et al. 2014). The disruption of an Nme ortholog in *E. coli* was reported to produce a mutator phenotype and thus was opening research in that field (Lu et al. 1995). Due to artifactual binding of Nme to DNA and DNA-binding proteins, this line of research has been fraught with missteps but, recently, some consistent patterns have emerged. Interactions of *Nme* with dynamin have been reported in multiple species and may extend to other aspects of the endocytosis, e.g., Rab5-dependent processes (Baillat et al. 2002; Dammai et al. 2003; Ignesti et al. 2014). The knockdown of *Nme1* in vitro caused abnormal chromosomal ploidy resulting from a failure in cytokinesis (Conery et al. 2010), which can be attributed to the provision of GTP (Boissan et al. 2014). As discussed by Kaetzel et al. 2014, *Nme1* apparently increases genetic stability in melanoma, possibly by a 3′–5′ exonuclease activity as well as NTP fueling to DNA polymerases. In line with this interpretation, *Nme1* promoted the repair of UV-induced DNA damage to limit melanoma formation (Jarrett et al. 2011; Yang et al. 2009; Zhang et al. 2011).

In addition to PPI and DNA interactions, also the protein kinase activity of *Nme1* is discussed to be relevant for its antimetastatic actions. Compared to *Nme2*, there are even fewer known substrates of the kinase activity of *Nme1*. ATP citrate lyase, the first confirmed substrate of *Nme1* acting as protein histidine kinase (Wagner and Vu 1995), is also a substrate to PHP. Its phosphorylation correlates to higher enzymatic activity and to the viability of neuronal cells in culture (Klumpp et al. 2009). Nevertheless, evidence from in vivo models supporting the importance of this regulation is missing. A second substrate for *Nme1* is KSR which is however phosphorylated on an important serine residue (Hartsough et al. 2002). As the phosphorylation of KSR regulates its scaffold function, the finding that in *C. elegans* the interaction of KSR with *Nme1* is relevant for Ras activation (Masoudi et al. 2013) highlights the possibility that *Nme* isoenzymes might also be important kinases on other amino acid side chains. Lapek et al. (2014) describe in this issue of *Naunyn-Schmiedeberg's Archives of Pharmacology* an unbiased screen for so far unidentified phosphorylation on histidine and aspartic acid residue in proteins in a prostatic human epithelial cell model in non-tumorigenic, tumorigenic, and metastatic state. In the latter, the expression of human *Nme1* (and *Nme2*) was reduced. Although it is not clear that the loss of phosphorylated proteins detected in the tumorigenic and metastatic cells is due to the reduced presence of *Nme* isoforms, some of the proteins might turn out to be indeed so far unknown protein substrates for phosphorylation by *Nme*.

Translational advances in cancer therapy based on targeting Nme-dependent processes

Ever since its discovery in 1988, a goal of *Nme* research has been to elevate its expression in micrometastatic tumor cells and prevent their metastatic colonization in cancer patients. An inhibition of the *Nme* enzymatic activity by small molecule inhibitors was initially intended, but the available polyphenols such as ellagic acid (Buxton 2008), although widely discussed to be beneficial in cancer therapy, are unspecific kinase inhibitors and show low potency. Gene therapy approaches were successful preclinically (Li et al. 2006; Li et al. 2009) but remain clinically intractable. A variety of alternative approaches have been pursued. First, elevation of tumor cell expression of *Nme1* protein was demonstrated in vitro and in vivo by high-dose medroxyprogesterone acetate (MPA) (Ouatas et al. 2002; Ouatas et al. 2003), with concomitant inhibition of metastasis (Palmieri et al. 2005). The validity of this approach as therapeutic option was tested in a phase II trial for advanced breast cancer. The experimental regimen could not be validated as patients failed to achieve the necessary plasma levels of MPA (Miller et al. 2014). Other compounds have been reported to elevate tumor cell *Nme1* expression as well (Jiang et al. 1988; Lin et al. 2002; Liu et al. 2000; Natarajan et al. 2002) and thus are additional candidates at least for preclinical testing. A cell permeable *Nme1* construct suppressed development of pulmonary metastases in mice and diminished already established metastases, with a prolongation of overall survival (Lim et al. 2011). A peptide that disrupts the interaction of *Nme1* and *h-Prune* has been identified, and preclinical studies were performed in breast and prostate cancers as well as neuroblastoma (Carotenuto

et al. 2014; Carotenuto et al. 2013; D'Angelo et al. 2004). The results obtained by Carotenuto et al. (2014) (presented in this issue of *Naunyn-Schmiedeberg's Archives of Pharmacology*) which showed the effectiveness and safety of the peptide expression treatment in a mouse prostate cancer model of metastasis support its use in further clinical testing. For example, an in situ delivery of the peptide to patients affected by prostate cancer and undergoing prostatectomy would present an interesting therapeutic option.

Another translational approach is based on the identification of genes which expression patterns were inversely related to that of *Nme1* protein. The lysophosphatidic acid receptor 1 (LPA1, EDG2, LPAR1) was inversely expressed to *Nme1* in multiple cancer cell lines as well as in human breast tumors. The use of LPA1 antagonist was validated in two preclinical models to suppress metastasis in breast cancer without effecting primary tumor formation (Marshall et al. 2012). The analysis of proliferation rates and activation of the MAP kinase pathway in micrometastatic tumor cells in distant organs suggested an induction of metastatic dormancy as underlying mechanism. Orally available forms of this compound are now in advanced preclinical testing. Other inverse expression correlates of *Nme1* have been reported and stand as far as preclinical research leads (Bosnar et al. 2006; Ma et al. 2008; McCorkle et al. 2014; Zhao et al. 2004). The identification of novel metastasis suppressive or metastatic colonization preventive drug candidates will require testing in adjuvant setting trials. These trials are large, costly, and lengthy. Newer proposed trial designs may facilitate such testing (Steeg 2012).

Future perspectives: a potential unifying theme

The function of *Nme* protein as an endocytic factor was noted more than 10 years ago in *Drosophila* (Dammai et al. 2003; Krishnan et al. 2001). More recent genetic studies have further supported a more general role in vesicular transport (Feng et al. 2014; Hippe et al. 2011a; Hippe et al. 2011b; Hippe et al. 2009). It is therefore reasonable to contemplate that elucidating the molecular mechanism underlying such ancestral physiological function could help clarify many of the current seemingly endless arrays of cellular functions. Some recent findings may point the way. As mentioned, *Nme2* has been shown to be part of the COPII complex required for vesicle transport from the endoplasmic reticulum to the Golgi apparatus (Kapetanovich et al. 2005). The authors suggested that *Nme2* participates in the formation of a proteinaceous scaffold along which ER exit sites are organized. Another possibility is that ER-to-Golgi transport requires microtubules (Presley et al. 1997). Interestingly, one of the earliest observed properties of *Nme*, in this case *awd* and the bovine NDPK, was its association with microtubules (Biggs et al. 1990; Nickerson and Wells 1984). More directly related to endocytosis, it was recently demonstrated that *Nme1* (and *Nme2*) was localized at clathrin-coated pits and interacted with the proline-rich domain of dynamin. In vitro, *Nme1* and *Nme2* were recruited to dynamin-induced tubules, stimulated GTP loading on dynamin, and triggered fission in the presence of ATP and GDP (Boissan et al. 2014). This finding, combined with the intriguing property of *Nme4* (a mitochondria-specific *Nme* protein) in bridging two lipid bilayers (Schlattner et al. 2014; Schlattner et al. 2013), could suggest a scaffolding role of the *Nme* proteins to bring together proteins and lipid components that are involved in vesicle fusion and/or fission. In line with this interpretation, *Nme3* associated with membranous structures, most likely mitochondria (Negroni et al. 2000).

In a broader context, it is very likely that *Nme* proteins serve their myriad of cellular functions by playing the role of a scaffold. Depending of the partners in different tissue and subcellular contexts, the *Nme* scaffolds can exert different functions, which may include endocytosis, macromolecular transport, signal transduction, and various enzymatic functions including NTP supply, protein kinase, or nuclease activity. With this scaffold hypothesis, we also propose a potential unifying theme for many of the antimetastatic actions of *Nme* proteins consistent with their developmental roles: Likely, *Nme* proteins provide spatial and temporal control of signaling or modify the activity of other scaffold proteins. Thus, reports of *Nme* binding to multiple aspects of the cytoskeletal machinery may all involve a common motility-related activation scaffold. This raises the question whether the instability of metastatic tumor cells may be at least partially accounted for by the loss of organized scaffolding, setting established signaling pathways inherent in differentiation into disarray. If true, it will be important to understand the regulation of *Nme* expression levels as well as the properties which *Nme* proteins use to exert their scaffolding function. These hypotheses suggest a myriad of new studies that need to be undertaken with appropriate controls to address this potentially important *Nme* activity in vivo. As discussed herein for a variety of examples, developmental studies using model organisms with the help of advanced cell biology and proper systems biology may set the gold standard for future *Nme*-related research.

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