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REVIEW

Modulation of cytoskeletal dynamics by mammalian nucleoside diphosphate kinase (NDPK) proteins

Natasha T. Snider · Peter J. Altshuler · M. Bishr Omary

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Abstract Nucleoside diphosphate kinase (NDPK) proteins comprise a family of ten human isoforms that participate in the regulation of multiple cellular processes via enzymatic and non-enzymatic functions. The major enzymatic function of NDPKs is the generation of nucleoside triphosphates, such as guanosine triphosphate (GTP). Mechanisms behind the nonenzymatic NDPK functions are not clear but likely involve context-dependent signaling roles of NDPK within multi-protein complexes. This is most evident for NDPK-A, which is encoded by the human *NME1* gene, the first tumor metastasis suppressor gene to be identified. Understanding which protein interactions are most relevant for the biological and metastasis-related functions of NDPK will be important in the potential utilization of NDPK as a disease target. Accumulating evidence suggests that NDPK interacts with and affects various components and regulators of the cytoskeleton, including actin-binding proteins, intermediate filaments, and cytoskeletal attachment structures (adherens junctions, desmosomes, and focal adhesions). We review the existing literature on this topic and highlight outstanding questions and potential future directions that should clarify the impact of NDPK on the different cytoskeletal systems.

Keywords Nucleoside diphosphate kinase · Cytoskeleton · Dynamics · Modulation · Microfilaments · Intermediate filaments · Microtubules

Introduction

Nucleoside diphosphate kinase (NDPK) proteins are multifunctional proteins expressed from bacteria to humans and are encoded by ten human genes, *NME1–9* and *RP2* (Boissan et al. 2009; Desvignes et al. 2009). Mammalian NDPKs (also known as Nm23) are classified into two groups based on their sequence identities. The *NME1–4* protein products, NDPK-A, NDPK-B, NDPK-C, and NDPK-D, belong to group I and share higher sequence identity (58–88 %) compared to the other family members, which belong to group II and share 22–44 % sequence identity (Boissan et al. 2009). The two groups also differ substantially in their intracellular distribution, with group I NDPKs exhibiting cytoplasmic (NDPK-A to NDPK-C) or mitochondrial (NDPK-D) localization and most of group II NDPKs being localized within cilia and flagella.

The major enzymatic function of NDPK-A to NDPK-D is to generate nucleoside triphosphates via transfer of a phosphate group from ATP to nucleoside diphosphates (Lacombe et al. 2000; Lascu and Gonin 2000). NDPKs can also function as mammalian histidine kinases (Attwood 2013) and possess 3′-5′-exonuclease activity (Kaetzel et al. 2006), which is important in DNA repair. These and other molecular functions of NDPKs remain to be clarified further since NDPKs are implicated in numerous cellular processes, including cell growth, differentiation, apoptosis, and migration (Boissan et al. 2009; Marino et al. 2012). Importantly, the human NDPK-A (Nm23-H1)-encoding gene, *NME1*, was the first metastasis suppressor gene identified (Steeg et al. 1988). Forced overexpression of NDPK in various cancer cell lines reduces their in vitro and in vivo migratory and invasive properties (Marino et al. 2012). Knockout of the mouse NDPK-A homologue (Nm23-M1) leads to augmented lung metastasis of primary hepatocellular carcinoma tumors (Boissan et al. 2005). There are reports for both a positive and negative correlation between NDPK-A expression and metastasis in the clinical setting, pointing out context-specific mechanisms. Numerous clinical studies have reported an inverse correlation between NDPK-A expression and the metastatic potential of epithelial tumors, including breast, liver, colon, ovarian, lung, and skin, while the opposite

N. T. Snider (✉) · P. J. Altshuler · M. B. Omary
Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
e-mail: nsnider@umich.edu

was found for hematopoietic malignancies, neuroblastoma, and osteosarcoma, where NDPK-A expression most often correlates with poor clinical outcome (Lacombe and Boissan 2013; Marino et al. 2012).

Clarification of these mechanisms in future studies should reveal new directions for the design of metastasis-limiting strategies by selective targeting of NDPKs. To that end, it is now well appreciated that NDPKs exert their wide-ranging effects in part by forming complexes with proteins involved in multiple cellular functions, including nucleotide exchange factors, transcriptional regulators, cell growth regulators, and cytoskeletal components (Marino et al. 2011). The goal of this mini-review is to summarize the functional evidence supporting a role for NDPK in modulating cytoskeletal dynamics, in particular as it pertains to its metastasis suppressor function. We focus primarily on systems where functional connections have been established between NDPK and the various cytoskeletal systems, summarized in Table 1. Our discussion is focused on the actin cytoskeleton, intermediate filament cytoskeleton, and cytoskeletal attachment sites (cell junctions).

NDPK modulates actin filament dynamics by regulating actin-associated proteins

Actin filaments are the major cytoskeletal component that contributes to the directional motility of cells (Pollard and Cooper 2009). Actin filaments are composed of globular subunits that are organized in a head-to-tail manner, resulting in molecular polarity that includes a barbed end and a pointed end (Dominguez and Holmes 2011). During cell movement, actin filaments are added to the barbed end, which faces outward relative to the cell surface. As the actin polymers branch and grow just beneath the plasma membrane, physical force is generated to enable cell movement. The membrane curvature is dramatically altered during cell motility due to the presence of actin-based structures important for migration and invasion into the extracellular matrix (ECM), such as membrane ruffles, lamellipodia, invadopodia, and podosomes (Buccione et al. 2004). Numerous proteins associate with actin and modulate actin filament dynamics, such as branching, membrane anchoring, linking to other cytoskeletal components, stabilizing, severing, and capping (Winder and Ayscough 2005). As supported by several mechanistic studies, the effects of NDPK in cancer metastasis can, at least in part, be attributed to interactions with actin-associated proteins, including the GTPase dynamin (Gu et al. 2010) and the actin-severing/capping protein gelsolin (Sun et al. 1999) as highlighted below.

NPDK-dynamin interactions regulate endocytosis, cell adhesion, and cytokinesis Evidence from multiple studies suggests that a major function of NDPKs is to promote the activity of the GTPase dynamin, which is an important regulator of the actin cytoskeleton (Menon and Schafer 2013). There are three dynamin isoforms in mammals. Dynamins 1 and 3 are found predominantly in the brain, whereas dynamin 2 is ubiquitously

Table 1 Summary of known associations between mammalian NDPKs and cytoskeletal components

	Cytoskeleton-associated protein or structure	NDPK isoform (species)	Context-dependent mammalian NDPK function	References
Actin cytoskeleton	dynamin 2	NDPK-A and NDPK-B (human)	promotes membrane remodeling and endocytosis of cell surface receptors	Boissan et al. 2014
	dynamin 2	NDPK-A (human)	promotes cytokinesis and chromosome stability	Conery et al. 2010
	OPA1	NDPK-D (human)	promotes mitochondrial inner membrane fusion	Boissan et al. 2014
	gelsolin	NDPK-A (human, mouse)	antagonizes actin-severing activity of gelsolin	Marino et al. 2013
Intermediate filament cytoskeleton	keratin 8/18	NDPK-A and NDPK-B (mouse)	may protect against oxidative stress-induced keratin aggregation	Snider et al. 2011
	vimentin	NDPK-A (rat)	not known	Roymans et al. 2000
Microtubule cytoskeleton	β-tubulin	NDPK-A (human)	not known	Lombardi et al. 1995
	rat brain microtubules	Txl2 (human)	not known	Sadek et al. 2003
	porcine brain microtubles	NDK7 (mouse)	not known	Ikeda 2010
Cytoskeletal attachment sites	adherens junctions	NDPK-A (human)	promotes stability of adherens junctions through unknown mechanisms	Boissan et al. 2010
	desmosomes	NDPK-A and NDPK-B (human)	may promote desmosome formation (via plakoglobin association)	Aktary et al. 2010
	focal adhesions	NDPK-B (human)	may affect lamellipodia formation during cell spreading (via ICAP-1 association)	Fournier et al. 2002

expressed (Ferguson and De Camilli 2012). The major known function of dynamin is in the regulation of endocytosis, the process by which cellular components from the plasma membrane together with extracellular material are internalized (Ferguson and De Camilli 2012). With respect to its involvement in tumor cell biology, dynamin presence has been noted in focal adhesions, invadopodia, podosomes, and lamellipodia, and its activity has been attributed to the acquisition of migratory and invasive cell phenotypes (Baldassarre et al. 2003; Kruchten and McNiven 2006).

Evidence pointing to physical and functional associations between NDPK and dynamin was presented over a decade ago (Baillat et al. 2002; Krishnan et al. 2001). The functional relevance of this interaction to endocytosis was initially highlighted for dynamin-dependent synaptic vesicle recycling (Krishnan et al. 2001) and loss of cell surface-localized fibroblast growth factor receptor in *Drosophila melanogaster* (Dammai et al. 2003). Additional work provided further support that NDPK promotes dynamin function in endocytosis as well as in cell division. For example, NDPK was found to promote dynamin-mediated endocytosis of E-cadherin to induce loss of adherens junctions (Palacios et al. 2002). This process involves recruitment of NDPK to adherens junctions by guanosine triphosphate (GTP)-bound ARF6, which is a GTPase that functions in endocytic recycling and actin cytoskeleton remodeling. Upon recruitment, NDPK promotes endocytic vesicle fission and downstream inactivation of Rac1, which is another important regulator of cell migration and invasion (Lawson and Burridge 2014). Additionally, NPDK-A and dynamin associate during cytokinesis to promote chromosome stability. Loss of NDPK-A is linked to cytokinesis failure and tetraploidy, which is phenocopied by a loss of dynamin (Conery et al. 2010). The enzymatic activity of NDPK was found to be important for its association with dynamin during cytokinesis.

The significance of the GTP-generating function of NDPK and its ability to promote dynamin activity was convincingly demonstrated in elegant work by Boissan et al., which showed that NDPKs directly interact with and support the function of dynamin 2 and the mitochondrial dynamin-like GTPase OPA1 by generating high concentrations of GTP in a localized manner to promote membrane remodeling (Boissan et al. 2014). Using several approaches, the authors showed that NDPK-A and NDPK-B are bound to membrane-associated dynamin 2 to promote membrane fission, while loss of NDPK-A and NDPK-B led to inhibition of endocytosis and accumulation of clathrin-coated pits that was not attributed to a global reduction in GTP levels. These effects extended to the mitochondrial compartment, where NDPK-D was found to control mitochondrial fusion by promoting the function of the dynamin-related GTPase OPA1 (Boissan et al. 2014). Loss of NDPK-D phenocopied the loss of OPA1 with respect to the presence of increased number of cells with fragmented, as opposed to tubular, mitochondria.

Therefore, the recent studies in mammalian systems (Boissan et al. 2014; Conery et al. 2010) combined with the earlier work in *D. melanogaster* (Dammai et al. 2003; Krishnan et al. 2001) provide evidence for an evolutionarily conserved function of NDPKs in regulating the function of dynamin and dynamin-like GTPases.

NDPK-gelsolin interactions limit cell migration and tumor metastasis Another link between the actin cytoskeleton and NDPK is its interaction with gelsolin. The activation of gelsolin, an actin-severing and monomer-sequestering protein, results in the generation of shorter and more numerous actin filaments, which translates to alterations in cellular morphology, motility, and signaling pathways (Nag et al. 2013). There is in vitro evidence to support a promoting role for gelsolin in cancer cell invasion, and this is mediated through a number of oncogenic signaling effectors (Chen et al. 1996; De Corte et al. 2002), but the role of gelsolin in metastasis is not clear.

A recent proteomics-based study identified and functionally confirmed gelsolin as an interacting partner of NDPK (Marino et al. 2013), validating and expanding upon a prior study that reported this interaction as a component of the anti-metastatic function of NDPK (Garzia et al. 2006). Association between gelsolin and NDPK-A was demonstrated in several cancer cell lines and primary tumors (Marino et al. 2013). Importantly, NDPK reduced the actin depolymerizing activity of gelsolin, antagonized gelsolin-stimulated tumor cell motility in vitro, and attenuated the pro-metastatic function in an in vivo model of mammary tumor metastasis. Therefore, the anti-metastatic function of NDPK may be partially mediated by its ability to indirectly affect actin filament dynamics by modulating the activity of gelsolin.

Involvement of NDPK in the modulation of vimentin and keratin intermediate filaments

Intermediate filament (IF) proteins make up a major cytoskeletal component that is responsible for mechanoprotection and for regulation of growth- and stress-related signaling events (Eriksson et al. 2009; Kim and Coulombe 2007). Keratins, which represent the largest subfamily of IF proteins, are encoded by 54 human genes and expressed in an epithelial cell-specific manner (Schweizer et al. 2006). The other major cytoplasmic IF proteins include mesenchymal vimentin, myocyte desmin, neurofilaments, and glial fibrillary acidic protein, whereas A- and B-type lamin IFs form the nuclear lamina of all nucleated cells. IFs are also known to be involved in modulating cell migration (Chung et al. 2013) through

several mechanisms that involve mechanotransduction signaling, associated protein partners, and IF posttranslational modifications (Snider and Omary 2014), in particular, phosphorylation. In general, vimentin IFs promote cell migration, whereas keratin IFs have context-dependent effects that either promote or reduce cell migration (Chung et al. 2013; Ivaska et al. 2007).

Association of NDPK with vimentin IFs: potential role in epithelial-to-mesenchymal transition? Vimentin is normally expressed on cells of mesenchymal origin, such as leukocytes and vascular endothelial cells. This is contrast to epithelial cells, which express keratin IFs. During epithelial-to-mesenchymal transition (EMT), the process by which epithelial cells lose their polarity and intercellular adhesion properties while gaining a pro-migratory function (Kalluri and Weinberg 2009), keratins typically are downregulated while vimentin is upregulated. Therefore, vimentin is considered a marker for EMT but in recent years, it has become apparent that vimentin may also be an active promoter of EMT (Ivaska 2011). Specifically, vimentin can activate oncogenic kinases, such as the tyrosine kinase Axl1 (Vuoriluoto et al. 2011), and may promote the formation of lamellipodia (Helfand et al. 2011). An intact vimentin cytoskeleton is associated with decreased formation of lamellipodia, whereas disassembled vimentin filaments at the cell periphery is associated with increased cell motility (Helfand et al. 2011). This is supported by observations that targeted disassembly of vimentin filaments by microinjection of a vimentin peptide mimetic causes retraction of the filamentous vimentin network from the cell periphery and leads to membrane ruffling and formation of lamellipodia (Helfand et al. 2011).

The existing evidence points to a direct association between NDPK and vimentin (Otero 1997; Roymans et al. 2000), but not with keratin IFs (Snider et al. 2011), and the vimentin-NDPK interaction may be potentially relevant during EMT. The interaction with vimentin was demonstrated biochemically in frog lung, heart, and liver tissues (Otero 1997). Since IFs are highly abundant and may coprecipitate with other proteins nonspecifically, an important control was done with desmin, the major IF in the heart. This showed that, unlike vimentin, the more abundant desmin did not associate with NDPK in heart tissue. A subsequent independent study confirmed the biochemical association and demonstrated co-localization of NDPK with vimentin by indirect immunofluorescence in rat glioma cells (Roymans et al. 2000). Although the in vivo relevance of this interaction is not clear, NPDK promotes the assembly of vimentin filaments in vitro using purified proteins (Otero 2000). One possibility is that association of NDPK with vimentin may promote stabilization of vimentin filaments, thereby, inhibiting the formation of lamellipodia and attenuating cell migration. Since vimentin phosphorylation plays a critical role in filament assembly and disassembly (Hyder et al. 2008), it would also be of interest to investigate the role of NDPK histidine kinase activity in this context.

NDPKs may limit pathologic aggregation of keratin IFs by attenuating the levels of reactive oxygen species Based on a 2-D difference in gel electrophoresis (2D-DIGE) proteomic analysis, mouse NDPK-A and NDPK-B were identified as candidate proteins that confer resistance to pathologic aggregation of keratin 8/18 IFs in the liver (Snider et al. 2011). Several chronic liver diseases, including alcoholic and nonalcoholic steatohepatitis, are characterized by the presence of keratin 8/18-containing cytoplasmic aggregates in hepatocytes termed Mallory-Denk bodies (MDBs). Elevated oxidative stress and deficient proteasome/autophagy pathways underlie MDB-related liver pathology (Zatloukal et al. 2007). There is a significant genetic and sex component to MDB formation in humans and in mice (Hanada et al. 2008, 2010; Snider et al. 2011). Comparison of C57BL6/J and C3H/HeN, the most and least MDB-susceptible mouse strain, respectively, revealed that hepatocyte and liver NDPK expression and activity were significantly lower in the C57BL6/J mouse livers and hepatocytes (Snider et al. 2011). Furthermore, knockdown of NDPK-A/B in primary hepatocytes led to significant accumulation of reactive oxygen species upon treatment with an MDB-inducing agent. Although NDPK exhibited a filamentous distribution pattern in primary hepatocytes and partially co-localized with keratins 8/18, co-immunoprecipitation analysis under basal conditions did not reveal an association (Snider et al. 2011). The possibility that NDPK associates dynamically with keratins under a specific context (e.g., during stress induction) cannot be excluded. Irrespective of a direct association, NDPK likely functions to limit keratin aggregation by protecting against oxidative stress-induced damage in hepatocytes via mechanisms that are currently not known.

NDPKs are microtubule-binding proteins in vitro

One of the first functions ascribed to NDPKs was their ability to bind microtubules, which was demonstrated 40 years ago with the co-purification of NDPK with a preparation of microtubules from bovine brain (Nickerson and Wells 1984). Since then, links between NDPKs and microtubules have been established in several in vitro situations but evidence that NDPKs can modulate the activity of microtubules is lacking. It was demonstrated that in rat C6 glioma cells, catalytically active NDPK-A was a component of the centrosome and associated with γ-tubulin (Roymans et al. 2001). Additionally, mouse NDPK-A associates with β-tubulin in C2C12 myotubes, and this association was more apparent

upon induction of myoblast fusion and the formation of multinucleated fibers (Lombardi et al. 1995). Therefore, the association between NDPK-A and β-tubulin appears to be dynamic. Mouse NDK7, which belongs to the group II NDPKs and is primarily expressed in tissues with motile axonemes (including testis, oviduct, lunch, and trachea), was also identified as a microtubule-binding protein. Purified recombinant mouse NDK7 associated in vitro in a co-sedimentation assay with taxol-stabilized microtubules from porcine brain (Ikeda 2010). Lastly, the thioredoxin-like 2 protein Txl2, which has an N-terminal thioredoxin domain and a C-terminal NDPK domain and is encoded by the *NME9* gene, binds microtubule preparations in vitro (Sadek et al. 2003). Therefore associations between NDPK and microtubules may be most relevant to the group II NDPKs, but the in vivo occurrence and significance of these associations remain to be established.

NDPK activity modulates the dynamics of cytoskeletal attachment sites

Some of the mechanisms behind the anti-metastatic effects of NDPK appear to be linked to its ability to modulate cell adhesions, including adherens junctions, desmosomes, and focal adhesions. Adherens junctions and desmosomes are intercellular attachment sites composed of three major protein families: cadherins, armadillo proteins, and cytoskeletal adaptors (Green et al. 2010). In the case of adherens junctions, the cytoplasmic tail of E-cadherin is linked to the actin cytoskeleton via the armadillo protein family member β-catenin. In desmosomes, on the other hand, cadherins communicate with IFs via another armadillo family protein, plakoglobin (also known as γ-catenin). Whereas adherens junctions are considered more dynamic and involved in epithelial reorganization, desmosomes form strong adhesions that enable mechanical integration of cells within tissues (Dusek and Attardi 2011; Green et al. 2010). Focal adhesions are cell-substrate attachment structures that anchor actin filaments and can also serve as signaling platforms (Geiger et al. 2009). They are formed by integrin receptors, which are part of the cellular sensing machinery for environmental chemo- and mechanosensing. Dysfunction of any of these junctional complexes is associated with increased metastasis and cancer progression (Dusek and Attardi 2011; Knights et al. 2012) so this system is highly relevant to the effects of NDPK in cancer metastasis. For example, nuclear translocation of β-catenin orchestrates an oncogenic program by binding to LEF/TCF transcription factors and promoting Wnt signaling, while E-cadherin-mediated cell adhesion is lost during tumor progression (Berx and van Roy 2009). As discussed below, there is evidence for co-regulation as well as direct associations between NDPK and junctional proteins, highlighting several potential mechanisms for NDPK in regulating cytoskeletal attachment sites.

NDPK deficiency is linked to loss of adherens junctions The acquisition of an invasive phenotype in the human hepatocellular carcinoma (HCC) cell line HepG2 upon silencing of NDPK-A was mechanistically linked to mislocalization of E-cadherin, loss of adherens junctions, and rearrangement of the actin cytoskeleton in the form of filopodia-like structures (Boissan et al. 2010). The loss of adherens junctions upon NDPK-A silencing was not only limited to HepG2 cells but also observed in the human colon carcinoma HCT8/S11 cells, thereby, suggesting a possible general effect. These changes were paralleled by nuclear translocation of β-catenin and subsequent hyperactivation of Wnt signaling and specifically attributed to the loss of NDPK-A, but not NDPK-B expression. The authors also examined a limited set of primary HCC and colorectal cancer tumors and noted a loss of NDPK-A expression along with nuclear β-catenin accumulation in the invasive fronts of some of the tumors, pointing out a potential clinical relevance of these findings. Although the exact mechanisms by which NDPK-A loss leads to loss of adherens junctions remains to be elucidated, it appears that enzymatic activity may not be a critical factor in this case since the authors noted only a 20 % loss of total cellular NDPK activity upon NDPK-A knockdown (Boissan et al. 2010). One possibility is that NDPK may serve as a molecular linker in adherens junctions under specific contexts.

Positive co-regulation and association between NDPK and the desmosomal protein plakoglobin In recent years, dysfunction of desmosomal components has also been linked to cancer progression via a number of mechanisms (Dusek and Attardi 2011). For example, the desmosomal protein plakoglobin can directly compete with β-catenin for assembly into adherens junctions, which leads to accumulation of β-catenin in the nucleus (Zhurinsky et al. 2000). Furthermore, plakoglobin can translocate to the nucleus and upregulate LEF/TCF target genes and potentially other oncogenic factors independently of its competition with β-catenin in adherens junctions (Conacci-Sorrell et al. 2002). In contrast to these apparent tumor-promoting functions of plakoglobin, loss of plakoglobin expression has also been linked to cell invasiveness (Bailey et al. 2012; Holen et al. 2012), thus, pointing to a context-dependent effect.

Ectopic expression of plakoglobin in the human tongue squamous cell carcinoma SCC9 cell line (which lack endogenous plakoglobin) facilitates desmosome formation and an epidermoid phenotype characterized by contact-dependent growth inhibition and limited in vitro migration and invasion (Parker et al. 1998). Therefore, in this context, plakoglobin expression and desmosome formation exert antitumor properties. This phenotypic change in the SCC9 cells was

accompanied by elevated expression of several proteins, including NDPK-A and NDPK-B (Aktary et al. 2010). Regulation of NDPK-A was at the transcriptional level and was mediated by a plakoglobin-induced decrease in the expression of the oncogenic chromatin remodeling SATB1, which appears to function as a negative regulator of *NME1* expression (Aktary and Pasdar 2013). Immunofluorescence staining analysis revealed partial plasma membrane junctional localization of NDPK-A/B, whereas co-immunoprecipitation of desmosomal component proteins suggested an association between NDPK-A/B and plakoglobin, as well as α-catenin and E-cadherin (Aktary et al. 2010). The biochemical findings extended to other epithelial cell lines, including MCF-7, MCF-10-2A, MDCK, and SW620. Previously reported findings that NDPK promotes adhesion of the oral squamous cell carcinoma of the tongue (CAL 27) cells may be rooted in the same mechanisms (Bago et al. 2009). The outstanding questions from this work pertain to the specific roles of NDPK in the formation and/or stabilization of desmosomes and in mediating the differential effects of plakoglobin in cancer.

NDPK may regulate integrin signaling via its association with integrin cytoplasmic domain-associated protein 1 Integrin activation is required for focal adhesion formation. Conformational changes of the integrin heterodimer lead to increased affinity of integrins for extracellular matrix components (Geiger et al. 2009). Several proteins are known to modulate integrin activation in a positive and negative manner. Two classes of proteins, the talins and the kindlins, link the cytoplasmic domains of β-integrins to the actin

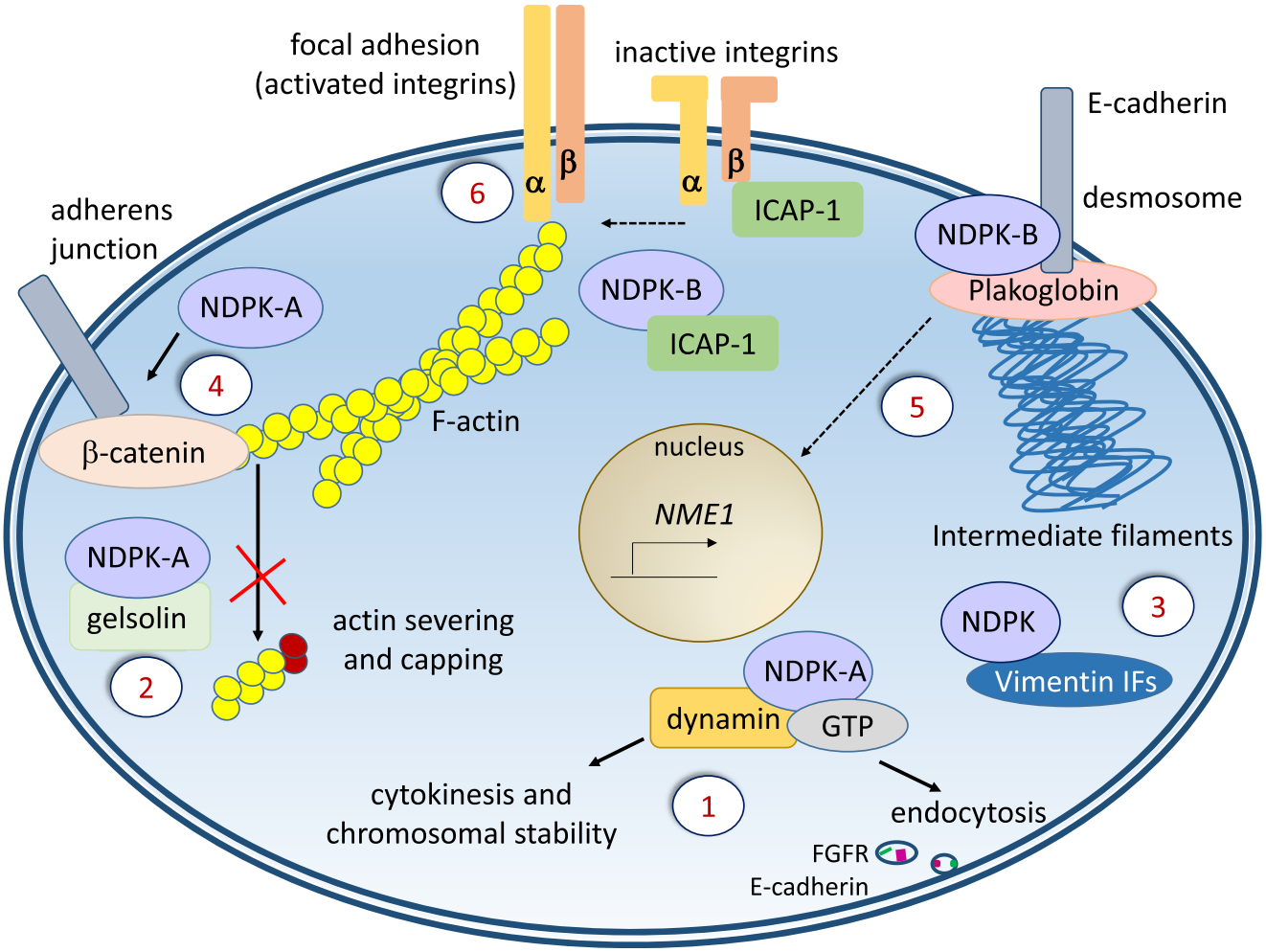

Fig. 1 Involvement of NDPK in modulating cytoskeletal dynamics. *1*. NDPK associates with and promotes the activity of the GTPase dynamin in endocytosis of fibroblast growth factor receptor and E-cadherin. NDPK also promotes dynamin function by providing GTP during cytokinesis to promote chromosomal stability. *2*. NDPK associates with gelsolin and inhibits its actin-severing/capping function. *3*. NDPK associates with vimentin intermediate filaments in multiple tissues and promotes vimentin filament assembly in vitro. *4*. NDPK supports adherens junction formation through unknown mechanisms. Loss of NDPK leads to loss of adherens junctions and nuclear accumulation of β-catenin. *5*. Multiple components of desmosomal junctions, including E-cadherin and plakoglobin, associate with NDPK. Plakoglobin also upregulates NDPK protein levels via induction of *NME1* gene expression. *6*. NDPK associates with ICAP-1, which is a negative regulator of integrin signaling. A potential relevance of this association is to sequester ICAP-1 to allow integrin activation and formation of focal adhesions

cytoskeleton and are critical for integrin activation (Geiger et al. 2009). On the other hand, the integrin cytoplasmic domain-associated protein 1 (ICAP-1) slows focal adhesion assembly by antagonizing talin and kindlin functions (Millon-Fremillon et al. 2008).

Although the functional importance and mechanisms of ICAP-1-mediated inhibition of integrin activation have only recently become appreciated, the association between ICAP-1 and NDPK-B was documented over a decade ago (Fournier et al. 2002). A yeast two-hybrid analysis identified NDPK-B as an interacting partner of ICAP-1. The association between NDPK-B and ICAP-1 was confirmed biochemically by using recombinant proteins in vitro and in cell culture using co-immunoprecipitation and indirect immunofluorescence analysis. Although NDPK-A was also found to co-localize with ICAP-1 within lamellipodia at the initiation of cell spreading, there is no biochemical evidence for a direct interaction between them (Fournier et al. 2003). The biochemical association studies revealed that the C terminus of ICAP-1, which contains a protein tyrosine phosphatase (PTP) domain, is required for binding to NDPK-B. Additionally, crystallization of ICAP-1 bound to β1-integrin revealed that the PTP domain is also involved in this interaction (Liu et al. 2013). This indicates that the interaction of ICAP-1 with NDPK-B may preclude association between ICAP-1 and β1-integrin, and in that sense, NDPK-B could function as a negative regulator of ICAP-1 to allow β1-integrin activation. In support of that, ICAP-1 and NDPK-B co-localize in lamellipodia during early stages of cell spreading, and this process precedes formation of focal adhesions that lack both proteins (Fournier et al. 2002).

One possibility for the functional significance of this interaction could be that, upon NDPK-B binding, ICAP-1 is sequestered intracellularly to allow for integrin activation and focal adhesion formation to proceed. These mechanisms would be analogous to that of another protein, Krev/Rap1 interaction trapped 1 (KRIP1), an antagonist of ICAP-1 that is important for integrin activation (Liu et al. 2013). Therefore, the interaction of NDPK with specific components of the environmental sensing machinery will need to be reevaluated in functional and in vivo studies to determine if it is a significant contributor to NDPK-mediated metastasis suppression.

Conclusions

The most well-established function of NDPK proteins is the tumor metastasis suppressor role of NDPK-A. However, the mechanisms behind this process are still unclear, mostly because the enzymatic activities of NDPKs are not fully understood and because NDPKs appear to mainly work in a complex with other cellular proteins. NDPKs interact directly or indirectly with all of the major cytoskeletal elements and are likely to impact their cellular functions. The interactions of NDPKs with components of the cytoskeletal machinery are highly relevant, given the well-established roles of the cytoskeleton in cell motility. Although few mechanistic studies have examined the ability of NDPK to regulate cytoskeletal dynamics (summarized in Fig. 1), there are important directions that may be considered in future studies. These include a clarification for the isoform-specific roles of NDPK in mediating actin-based cell motility in vivo, effects of NDPK on vimentin and potentially other IF protein assembly and disassembly properties in vivo and the potential consequences of this process to EMT and other cellular responses, and the functional role of NDPK in modulating the formation and/or stability of cell-cell and cell-substrate adhesions. Discriminating between enzymatic and nonenzymatic NDPK functions in all of these processes will also be essential. The use of available genetic mouse models, such as NDPK−/− mice, and generating conditional knockouts to probe NDPK function in a cell-specific manner should significantly aid progress in this field. Ultimately, novel pharmacological approaches targeting specific interactions between NDPK and proteins involved in cytoskeleton remodeling may offer treatment strategies for metastatic cancer in the appropriate patient populations.

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