Journal of Bioenergetics and Biomembranes, Vol. 35, No. 1, February 2003 (© 2003)

Nucleoside Diphosphate Kinases in Mammalian Signal Transduction Systems: Recent Development and Perspective

Narimichi Kimura,[1,2] Nobuko Shimada,[1] Yasushi Ishijima,[1] Mitsugu Fukuda,[1] Yohko Takagi,[1] and Naoshi Ishikawa[1]

Received August 16, 2002; accepted October 4, 2002

The role of nucleoside diphosphate (NDP) kinase with special reference to mammalian signal transduction systems was described. The interaction between NDP kinases and G proteins was reevaluated in view of their protein structural information and its significance was extended further on the basis of recent findings obtained with small molecular weight G proteins such as Rad, menin, and Rac. Meanwhile, observations suggesting involvement of NDP kinases in the regulation of cell growth and differentiation led to the realization that NDP kinases may play a crucial role in receptor tyrosine kinase signal transduction systems. In fact, a number of experimental results, particularly obtained with PC12 cells, implicate that NDP kinases appear to regulate differentiation marker proteins and cell-cycle-associated proteins cooperatively. Consequently, we propose a hypothesis that NDP kinases might act like a molecular switch to determine the cell fate toward proliferation or differentiation in response to environmental signals.

KEY WORDS: NDP kinase; nm23; signal transduction; G protein; nerve growth factor; PC12 cells.

A HISTORICAL VIEW

In signal transduction systems that are mediated by heteromeric GTP binding (G) proteins, ligand binding to its specific receptor triggers activation of G proteins and in turn effectors (Gilman, 1987). These systems absolutely require guanine nucleotides (GTP and GDP) for their ligand-dependent regulation (Kimura and Nagata, 1977; Kimura and Shimada, 1983). G proteins assume two states, inactive GDP-bound form and active GTP-bound form. The activated receptor acts as a guanine nucleotide exchange factor (GEF) to achieve G protein-GTP, whereas the intrinsic GTPase of G protein terminates the active state by hydrolyzing GTP (Bourne *et al.*, 1991).

The interaction between nucleoside diphosphate (NDP) kinase and signal transduction systems was explicitly described for hormone-dependent adenylyl cyclase systems in which the ligand binding to a corresponding receptor triggers flow of signal to the effector, adenylyl cyclase, through a trimeric G protein, Gs (Kimura, 1993; Kimura and Nagata, 1979; Kimura and Shimada, 1983). The hormonal activation of adenylyl cyclase taking place in the presence of GDP, as well as GTP, in in vitro assay system was well accounted for by conversion of GDP to GTP by the aid of NDP kinase. The interaction of NDP kinase with the hormone-dependent adenylyl cyclase system is unique in several points: (1) the GTP supplying mechanism via NDP kinase operates for Gs, not ADP-ribosylation factor, only when hormone receptor is activated (Kimura, 1993; Kimura and Shimada, 1985, 1986). Namely, this mechanism does not work under the unstimulated condition or receptor-independent activation by cholera toxin; (2) the membrane associated NDP kinase, in collaboration with activated receptor, can decrease the action of a competitive inhibitor, GDP ($K_i = 1\ \mu M$), by two orders of magnitude, which allows G protein to be activated by a small amount of GTP, at a conversion rate of 1% from the added GDP (Kimura, 1993; Kimura and Shimada, 1983); (3) The membrane associated NDP kinase exists in part as a complex with Gs, which is under the influence of receptor regulation

[1] Cellular Signaling Research Group, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173-0015, Japan.
[2] To whom correspondence should be addressed; e-mail: nkimura@tmig.or.jp.

0145-479X/03/0200-0041/0 © 2003 Plenum Publishing Corporation

(Kimura and Shimada, 1988a, 1988b; 1990). In bovine retinal rod outer segment membranes, however, a cytosolic form of NDP kinase (α, but not β, isoform) shows an equilibrium binding to the membrane through G protein (Gt) (Orlov *et al.*, 1996). Although it is believed that the GTP concentration surrounding G protein is sufficiently high compared with GDP to achieve G protein-GTP by a GDP-GTP exchange reaction, so far no convincing data have been presented in vivo. Hence, the discovery of membrane associated NDP kinase and its interaction with signal transduction systems have drawn arguments regarding the mode of G protein activation, particularly, as to whether NDP kinase directly phosphorylates GDP bound to G protein to form G protein-GTP or it functions to alter the GTP pool in the immediate vicinity of the G proteins, which allows effective formation of G protein-GTP by the exchange reaction (Kikkawa *et al.*, 1990; Kimura, 1993; Randazzo *et al.*, 1991, 1992). At any rate, the GTP supplying system operated by NDP kinase appears to guarantee rapid onset and effective exertion of the maximal capacity of the G protein coupled signal transduction systems.

The crystallographic studies revealed that the guanine nucleotide in trimeric G protein α subunit is cradled between the GTPase domain resembling those of monomeric G proteins and the helical domain not found in other GTPases (Lambright *et al.*, 1994; Noel *et al.*, 1993; Sprang, 1997; Wall *et al.*, 1995). On the other hand, the substrate-binding site of NDP kinase (*Myxococcus xanthus* NDP kinase, human nm23-H2 and rat NDP kinase α) exists in a cleft between head region and Kpn loop (Matsuzaki *et al.*, unpublished data; Webb *et al.*, 1995; Williams *et al.*, 1993). Taking these 3-D structures of both proteins harboring nucleotide into account, it seems unlikely that GDP bound to G protein in situ can be recognized as the substrate of NDP kinase as well. In light of these recent studies the direct transphosphorylation by NDP kinase from G protein-GDP to G protein-GTP, though not conclusive, seems unlikely to occur. The scheme of direct transphosphorylation, however, only highlights a facet of the interaction between NDP kinase and G protein coupled signal transduction systems. For example, there still exists the possibility that the interaction between G protein and NDP kinase may form a unique compartmentalized environment advantageous for the conversion of G protein-GDP to G protein-GTP, or vice versa, as suggested in a number of recent studies (see later). Further, more intricate phenomena taking place in membranes, such as activated receptor-dependent coupling between NDP kinase and signal transduction systems, and activated receptor-induced decrease of apparent affinity for GDP, remain unanswered (Kimura, 1993). In addition, the physiological significance and molecular characteristics of membrane associated NDP kinase await further studies.

G PROTEINS AND NDP KINASES: AN ASSESSMENT OF RECENT STUDIES

G Proteins Associated With Endocrine Diseases and NDP Kinase

A number of diseases ranging from endocrine system disorders and tumors are associated with altered expression and/or mutations occurring in G protein superfamilies. The most notable responses induced by constitutively active Ras because of abrogated GTPase activity are the induction of serum-independent cell proliferation and anchorage-independent growth in fibroblastic cells, and malignant transformation in human cases (Bos, 2000). Mutations occurring in trimeric G proteins cause a broader spectrum of diseases including endocrine systems (Iiri *et al.*, 1998; Mumby, 2000). A subset of pituitary adenomas and thyroid tumors resulting from mutations in Gs (*gsp*) and another tumors derived from mutations in Gi (*gip2*) are both related to reduction of GTPase activity of their own. McCune-Albright syndrome, which is charcterized by hyperfunction of multiple endocrine glands (e.g., pituitary somatotrophs, adrenal cortex, and gonads) coupled with café-au-lair skin hyperpigmentation and polyostotic fibrous dysplasia, is associated with missense mutation of Gs (*gsp*). Pseudohypoparathyroidism Ia accompanying phenotypic features, such as obesity and short stature, is caused by a loss-of-function mutations leading to decreased Gs function. In view of the potential regulatory function of NDP kinase toward G proteins, the role of NDP kinase in signal transduction systems may be envisioned in relation to particular cases of G protein-related disorders. In this context, the recently discovered new classes of G protein, whose altered expressions and/or mutations cause diseases in endocrine tissues, are of great interest, since these G proteins are likely to be functionally coupled with NDP kinase/nm23 (see later). These studies should provide us with important clues for understanding the role of NDP kinase in the regulation of G proteins under physiological and pathological conditions.

Rad and NDP Kinase

Rad (Ras associated with diabetes) is a new number of the Ras superfamily with a molecular size of 35 kDa, which is overexpressed in muscle of Type II diabetic humans (Reynet and Kahn, 1993). The expression of Rad

was high in skeletal muscle, cardiac muscle, and lung but almost undetectable in liver, brain, and pancreas. Rad protein is localized mostly in the cytosol; in contrast to Ras, it undergoes neither fatty acid modification nor isoprenylation (Bilan *et al.*, 1998). Zhu *et al.* found the GTPase activating protein (GAP) activity for Rad in the cell cytosol and identified it to be NDP kinase/nm23 (Zhu *et al.*, 1995, 1999).

GST-Rad alone slowly hydrolyzed GTP, while GST-nm23 exhibited no detectable GTP hydrolysis. Interestingly, incubation of both proteins increased dramatically the rate of GTP hydrolysis. The seeming GAP activity of NDPK/nm23 was specific toward Rad; other small GTPases such as Ral, Ran, Ras, and Rho were unaffected. The differential property of these G proteins is not unexpected since the nucleotide α- and β-phosphate recognition loop and the guanine base recognition loop are similar in structure, while the Mg^{2+} and GTP γ-phosphate binding site differ among these Ras family proteins (Sprang, 1997). Whether the increased GTP hydrolysis by nm23 occurs without dissociation of GTP from Rad-GTP was challenged by covalently cross-linking GTP to Rad. The result apparently supported the direct conversion of GTP bound to Rad to GDP by nm23/NDP kinase in situ (Zhu *et al.*, 1999).

The GAP activity was lost by use of either S105N-Rad, which was able to bind GDP but not GTP, or the kinase negative H118F-nm23-H1 (Zhu *et al.*, 1999). The latter finding, however, seems contradictory to the generally accepted nature of GAP since crystallographic analysis revealed that GAP supplies a catalytic residue (arginine or lysine) into the active site of the G protein, resulting in activation of its GTPase activity (Wittinghofer, 2000). Therefore, in the particular case of Rad, nm23/NDP kinase itself might have executed the hydrolysis of GTP in part, rather than stimulating GTPase activity of Rad as a typical GAP.

On the other hand, the GEF activity of NDP kinase/nm23 towards Rad, Gem, and Ras is also demonstrated by the conversion of GDP covalently cross-linked to these small G proteins to GTP although the net GTP synthesis by Rad was smaller than that by Gem or Ras because of the GTP hydrolysis stimulating activity of nm23 for Rad-GTP (Zhu *et al.*, 1999). Again, whether NDP kinase/nm23 acts as a GEF must be rigorously assessed. Accumulating data demonstrate that the protein structures of GEFs are homologous within but differ among G protein famlies. Nevertheless, they share common properties: GEFs increase the dissociation rate of guanine nucleotide from its binding site according to the following sequences; GEF forms a ternary complex with G protein-GDP, resulting in a change of the nucleotide binding pocket from a tight to a loose binding conformation, which enables a fast release of the bound nucleotide (Wittinghofer, 2000). The outcome of the reaction is determined solely by the relative nucleotide affinities and the concentration of GDP versus GTP. The association of G protein-GTP with its effectors facilitates the reaction toward activation.

Together, although NDP kinase/nm23 seemingly acts as not only GAP but also GEF towards Rad, its biochemical properties are entirely different from those of typical GAPs and GEFs. NDP kinase/nm23, owing to its NDP kinase enzyme activity, is likely to enhance the turnover rate between G protein-GDP and G protein-GTP, which may allow cells to accommodate flexibly to the surrounding milieus. This provides a novel aspect of NDP kinase/nm23 in terms of G protein regulation. In addition, Rad enhances not only anchorage-dependent but also anchorage-independent cell growth and stimulates tumorigenesis of a human breast cancer cells (MDA-MB231), all of which are antagonized by nm23/NDP kinase (Tseng *et al.*, 2001). Either cell clone, transfected with Rad or nm23 or both, formed tumors with morphology of medullary breast carcinoma when implanted into nude mice. Collectivly, Rad behaves as an oncogenic protein in breast cells, whereas NDP kinase/nm23 appears to participate in cell growth and tumorigenesis as a negative regulator for Rad.

Menin and NDP Kinase

Multiple endocrine neoplasia-type 1 (MEN1) is an autosomal dominant familial cancer syndrome characterized by tumors in parathyroids, enteropancreatic endocrine tissues, and the anterior pituitary (Chandrasekharappa *et al.*, 1997). The *MEN1* gene encodes a ubiquitously expressed 610-amino acid protein, termed menin specifically localized in the nuclei (Guru *et al.*, 1998). Menin forms a complex with JunD and represses JunD-activated transcription in its complexed form (Agarwal *et al.*, 1999). This protein was revealed to be associated with NDP kinase/nm23 in a yeast two-hybrid system using the *MEN1* cDNA fragment as bait (Ohkura *et al.*, 2001). Very interestingly, Yaguchi *et al.* recently found that menin contains several sequence motifs similar to those present in GTPases (G1-G5) (Yaguchi *et al.*, in press). Menin in fact possessed an ability to bind GTP and exhibited GTP hydrolytic activity in vitro when coexisted with NDP kinase A/nm23-H1, although neither menin nor NDP kinase/nm23 alone showed detectable GTPase activity. Further, when menin and nm23 were expressed in HEK293 cells, both were immunoprecipitated by respective antibodies and the immunoprecipitated

complex showed GTP hydrolytic activity (Yaguchi *et al.*, in press). By analogy with NDP kinase towards Rad, NDP kinase/nm23 appears to facilitate the menin activation cycle although no available data is provided for GEF activity of NDP kinase.

Rac and NDP Kinase

NDP kinase/nm23 has an ability to affect cell motility in response to various extracellular signals, which is believed to account for its metastasis suppressor activity (Hartsough and Steeg, 2000; Ouatas *et al.*, 2003; Steeg *et al.*, 1988). MDA-MB-435 (Leone *et al.*, 1993) and MDA-MB-231 (Russel *et al.*, 1998) human breast carcinoma cells and K1735 mouse melanoma cells (Leone *et al.*, 1991) lose their cell motility towards fetal bovine serum and some defined factors by nm23-H1 and its mouse homolog, respectively. HD3 colon carcinoma cell line shows inverse correlation between cell motility and *nm23* expression manipulated by antisense oligonucleotides (Hsu *et al.*, 1995). An *nm23-H1*-transfected oral squamous cell carcinoma cell shows a reduced cell motility towards fibroblast-derived chemotactic factor (Khan *et al.*, 2001). We recently found that NDP kinase β drastically decreased chemokinetic (random) cell motility of a rat mammary adenocarcinoma cell line MTLn3 (Fukuda *et al.*, in press), whereas NDP kinase α rather suppresed the chemotactic cell motility towards the lung extract in vitro (Fukuda *et al.*, submitted for publication). The latter phenomenon significantly paralleled the metastasis suppressive activity of NDP kinase α (Fukuda *et al.*, 1996). These result postulate the differential participation of NDP kinase isoforms in the process of cell motility and suggest their roles in the signaling pathways rather than the cell motility machineries per se.

The motility of cells depends on a complex interplay among molecules that regulate cytoskeletal components such as actin and myosin. Members of Rho family proteins, RhoA, Rac, and Cdc42, play important roles in this phenomenon. Rac and Cdc42 promote protrusive events at the leading edge of cells, whereas RhoA induces retraction of the leading edge (Burridge, 1999; Hall, 1998). Otsuki *et al.* recently reported that Rac was regulated by NDP kinase/nm23 through interaction with a specific GEF, Tiam1 (Otsuki *et al.*, 2001). nm23-H1 interacts with the amino-terminal region containing proline rich region of Tiam1, resulting in inhibition of the Tiam1-induced production of GTP bound Rac and abolition of Jun N-terminal kinase (JNK) activation. This action of nm23-H1 was reproduced by a kinase-inactive mutant protein (H118C). Although nm23-H1 had an ability to convert the covalently crosslinked Rac-GDP into Rac-GTP, neither direct association with Rac nor the GAP activity for Rac was shown in vitro. Further, Rac-GTP formation was extremely low in nm23-H1-overexpressed cells in vivo, demonstrating that nm23-H1 may mainly act through inhibiting Tiam1. When nm23-H1 (or mutant nm23-H1(H118C)) was overexpressed in Rat1 cells, the number of F-actin containing ruffles formed by the adhesion to a matrix component was reduced. Taken together, nm23-H1 appears to negatively regulate Tiam1 and in turn Rac in vivo. Whether the behavior of nm23-H1 depicted in these experiments well explains its proposed metastasis suppressor activity remains to be further examined. Especially, whether nm23 acts in a unidirectional way in terms of cell motility or whether it can regulate negatively as well as positively depending on sorts of signals must be clearly discriminated. Furthermore, the finding that recombinant nm23-H1 and Tiam1 do not interact directly in vitro raises the important question as to the mechanism by which the two are linked. In any case, as exemplified in this study, when the action of nm23-H1 is bidirectional or elicited at multiple sites, the assessment of the physiological role of nm23/NDP kinase in a given biological system must be carefully done.

NDP KINASES IN OTHER SIGNAL TRANSDUCTION SYSTEMS

NDP Kinases in Cell Growth and Differentiation

The increased expression of the NDP kinase/*nm23* gene often occurs in association with enhanced cell growth (Caligo *et al.*, 1995; Creanor and Mitchinson, 1989; Igawa *et al.*, 1994; Keim *et al.*, 1992), tumorigenesis (Lacombe and Jacobs, 1992), and immortalization (Ohneda *et al.*, 1994). Inhibition of cell division by microinjection of an anti-nm23 antibody into cultured cells (Sorscher *et al.*, 1993) and cell growth suppression by treatment with antisense oligonucleotides or antisense mRNA of NDP kinase/*nm23* (Cipollini *et al.*, 1997; Kimura, 2000) are more direct support for the involvement of NDP kinase in cell growth. On the other hand, a number of literatures describe that NDP kinase may be positively involved during the development and differentiation of cells and tissues as well (Dabernat *et al.*, 1999; Lakso *et al.*, 1992; Ouatas *et al.*, 1998; Timmons and Shearn, 2000). High expression levels of NDP kinases are seen in postmitotic tissues (Kimura *et al.*, 1990; Shimada *et al.*, 1993): NDP kinase α, a major form of the rat, is particularly high in the heart, kidney, and skeletal muscle, whereas NDP kinase β is abundantly present in the brain and testis.

A link between NDP kinase and cell growth/differentiation signal transduction systems has been

presented in a small panel. NDP kinase/*nm23*-transfected mouse melanoma and human breast carcinoma cells accompany diminished sensitivity to tranforming growth factor-β (TGF-β) in soft agar colonization (Leone *et al.*, 1991, 1993), reduced cell motility towards insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and serum (Kantor *et al.*, 1993). Upon transfection of *nm23-H2*/NDP kinase-B, a human oral squamous cell carcinoma cell, LMF4, displayed a higher growth rate with reduced sensitivity to PDGF, IGF-1, and insulin in culture (Miyazaki *et al.*, 1999). Unexpectedly, however, when the transfected LMF4 cells were injected into nude mice intravenously, they showed a differentiated morphology at the lung metastatic sites, which may account for the decreased metastatic potential of the transfected cells. It follows that nm23/NDP kinase appears to act in a bi-directional way in the cell: one is growth-promoting under a given condition, whereas the other is differentiation-promoting under certain other conditions.

PC12 Cells: A Good Model for NDP Kinase Research?

PC12 cells, derived from rat adrenal pheochromocytoma, cease to proliferate and begin to extend neurites like sympathetic neurons in response to nerve growth factor (NGF) (Greene and Tischler, 1976). In contrast, epidermal growth factor (EGF) enhances cell proliferation and does not result in morphological changes (Huff *et al.*, 1981). These two growth factors, both of which interact with respective tyrosine kinase receptors, share signaling pathways in many respects, including Ras and mitogen-activated protein (MAP) kinase activation, but result in different states of the cells (Chao, 1992). Although precise molecular mechanisms remain unsettled, accumulating data suggest that duration of extracellular signal-regulated kinase (ERK) activation is critical for the cell signalings to determine cell fates: as a whole, sustained activation of ERK (induced by NGF) leads to cell differentiation, whereas its transient activation (triggered by EGF) results in cell proliferation in PC12 cells (Marshall, 1995).

The cell fate of PC12 cells seems to eventually depend on whether a given signal can lead to cessation of cell division or not. For example, upon NGF treatment, cell cycle associated proteins including cyclin-dependent kinases (CDKs) (CDK1, CDK2, CDK4, and CDK6) and their associated kinase activities decline, whereas cyclin D1 and p21, a CDK inhibitor protein, are up-regulated in PC12 cells (Yan and Ziff, 1995). Overexpression of CDK2 inhibits NGF-induced growth suppression and differentiation (Dobashi *et al.*, 1995), while a mixture of antisense oligonucleotides for CDK1 and CDK2, resulting in down-regulation of both kinase activities, is required to provoke neurite outgrowth (Dobashi *et al.*, 2000).

Gervasi *et al.* investigated the role of NDP kinase/nm23 in relation to proliferation and differentiation of PC12 cells using sense (S-nm23-M1) and antisense (AS-nm23-M1) of mouse *nm23-M1* full length cDNA (Gervasi *et al.*, 1996). In the presence of NGF, S-nm23-M1 transfectant showed delayed cell growth, earlier onset of neurite outgrowth, and increased differentiation marker proteins such as neurofilaments (NF-H, NF-M, and NF-L) and β-tubulin, whereas AS-nm23-M1 enhanced cell growth and suppressed neuronal differentiation. Neither sense nor antisense cDNA affected the proliferative rate in growth medium without NGF.

To further understand the nm23-M1 (nm23-H1) action, Lombardi *et al.* performed transfection experiments, in which sense and antisense nm23-H1 and mutant forms of nm23-H1 was overexpressed in PC12 cells, and monitored cell morphology and the gene expression of Rb2/p130, an RB family protein which, as an effector downstream of AP-2, plays a major role in maintaining the differentiated state (Lombardi *et al.*, 2001). The pRb2/p130 was unaffected by NGF in control cells but increased in its underphosphorylated form in the S-nm23-M1 clone. In the AS-transfected clone not only unstimulated but also NGF-stimulated pRb2/p130 levels were decreased. Both wild type nm23-H1 and H118F mutant enhanced the NGF-induced neuronal differentiation of PC12 cells, whereas S120G and P96S mutant nm23-H1 suppressed the NGF-induced morphological change. The former observation implicates that the enzyme activity of NDP kinase is unrelated to this effect. The two other mutant nm23-H1 decreased the Rb2/p130 protein level although their actions towards Rb2/p130 promoter activity did not parallel well. Considering the fact that Rb2/p130 per se is capable of triggering the onset of differentiation (Paggi *et al.*, 2001), nm23/NDP kinase, independently of its enzyme activity, may modulate the NGF-induced differentiation of PC12 cells through affecting the Rb2/p130 transcription. In these studies, however, using mouse or human *nm23* homologous gene for the analysis of rat cells needs to be validated.

NDP Kinases: A Molecular Switch to Determine Cell Fate of PC12D Cells?

The observation that NDP kinase β and its mouse homolog is highly expressed in neural tissues (Arnaud-Dabernat, *et al.*, 2003; Dabernat *et al.*, 1999; Shimada *et al.*, 1993; see also a cover photograph) prompted us to investigate its role in neuronal cells using a subline of PC12 cells, PC12D, which shows early onset of

morphological change (within 2 days) upon NGF treatment (Katoh-Semba *et al.*, 1987). To address this issue, we took advantage of the potential usefulness of dominant negative form of NDP kinases. This strategy relies on the assumption that the endogenous NDP kinases, particularly when they function as a member of signal transduction systems, appear to exhibit their functions through their enzyme activity during interacting with a downstream target protein(s). PC12D clones overexpressing NDP kinase β, but not NDP kinase α or mutant NDP kinases H118A, were able to extend neurites without any differentiation inducers and showed a prolonged doubling time in a growth medium (Ishijima *et al.*, 1999). In the presence of NGF, while cells overexpressing NDP kinase α or NDP kinase β extended neurites similar to the control clones, those expressing inactive NDP kinase, NDP kinase αH118A, or NDP kinase βH118A, did not (Ishijima *et al.*, 1999). In contrast to a study done by other laboratory (Lombardi *et al.*, 2001), inactive mutant NDP kinases did unambiguously exhibit their dominant negative effect toward the NGF signaling. It should be noted, however, that the effects of exogenous NDP kinases were clearly observed in the stably transfected clones but only marginal in transiently transfected cells (Ishijima *et al.*, 1999). Although there has been no clear explanation accounting for the delayed appearance of the dominant negative effect, it might be reasoned that the exogenous mutant forms need a while to mix up completely with the endogenous NDP kinase or to be translocated to the site where the endogenous NDP kinases should lie. The dominant negative NDP kinases affected neither activation kinetics of MAP kinases nor induction of a couple of immediate early genes (Ishijima *et al.*, 2001). However, they abolished a number of NGF-induced alterations. These include not only the increase of differentiation marker proteins (NFs and βIII-tubulin) but also fluctuations of a set of CDKs and cyclins (Ishijima *et al.*, 2001). Therefore, NDP kinases appear to function at a critical point, downstream of MAP kinase cascade and immediate early genes, where they cooperatively regulate the cell cycle machineries through their enzyme activity, NDP kinase activity, or protein kinase activity. Namely, NDP kinases might act like a molecular switch to determine the cell fate towards proliferation or differentiation in response to environmental signals. Although many of the important questions as to whether two major NDP kinase isoforms share the same target site(s) and how they modulate such cell cycle regulatory proteins remain to be elucidated, there have been a couple of intriguing literatures to speculate the NDP kinase function; major NDP kinase isoforms, either of them or both, may function as a transcription factor as described for PuF (Postel, *et al.*, 1993; Postel, this issue) or play as a possible modulator of transcription regulatory factors as suggested for ROR/RZR nuclear receptor subfamily (Parabicini *et al.*, 1996), EBNA-3C (Subramanian *et al.*, 2001), and menin (Guru *et al.*, 1998; Yaguchi *et al.*, in press), all of which undoubtedly exhibit respective functions in the nuclei. A novel paradigm for the regulatory mechanism of cell proliferation and differentiation is anticipated through investigating how NDP kinase/nm23 determines the cell fate in PC12 cells.

REFERENCES

Agarwal, S. K., Guru, S. C., Heppner, C., Erdos, M. R., Collins, R. M., Park, S. Y., Saggar, S., Chandrasekharappa, S. C., Collins, F. S., Spiegel, A. M., Marx, S. J., and Burns, A. L. (1999). *Cell* **96**, 143–152.

Arnaud-Dabernat, S., Bourbon, P. M., Dierich, A., Meur, M. Le, and Daniel, J.-Y. (2003). *J. Bioenerg. Biomembr.* **35**(1), XX–XX.

Bilan, P. J., Moyers, J. S., and Kahn, C. R. (1998). *Exp. Cell Res.* **242**, 391–400.

Bos, J. L. (2000). In *Frontiers in Molecular Bioligy, GTPases* (Hall, A., ed.), Oxford University Press, New York, pp. 67–88.

Bourne, H. R., Sanders, D. A., and McCormick, F. (1991). *Nature* **349**, 117–127.

Burridge, K. (1999). *Science* **283**, 2028–2029.

Caligo, M. A., Cipollini, G., Fiore, L., Calvo, S., Basolo, F., Collecchi, P., Ciardiello, F., Pepe, S., Petrini, M., and Bevilacqua, G. (1995). *Int. J. Cancer* **60**, 837–842.

Chandrasekharappa, S. C., Guru, S. C., Manickam, P., Olufemi, S.-E., Collins, F. S., Emmert-Buck, M., Debelenko, L. V., Zhuang, Z., Lubensky, I. A., Liotta, L. A., Crabtree, J. S., Wang, Y., Roe, B. A., Weisemann, J., Boguski, M. S., Agarwal, S. K., Kester, M. B., Kim, Y. S., Heppner, C., Dong, Q., Spiegel, A. M., Burns, A. L., and Marx, S. J. (1997). *Science* **276**, 404–407.

Chao, M. V. (1992). *Cell* **68**, 995–997.

Cipollini, G., Berti, A., Fiore, L., Rainaldi, G., Basolo, F., Merlo, G., Bevilacqua, G., and Caligo, M. A. (1997). *Int. J. Cancer* **73**, 297–302.

Creanor, J., and Mitchison, J. M. (1989) *J. Cell Sci.* **93**, 185–189.

Dabernat, S., Larou, M., Masse, K., Dobremez, E., Landry, M., Mathieu, C., and Daniel, J.-Y. (1999). *Gene* **236**, 221–230.

Dobashi, Y., Kudoh, T., Matsumine, A., Toyoshima, K., and Akiyama, T. (1995). *J. Biol. Chem.* **270**, 23031–23037.

Dobashi, Y., Shoji, M., Kitagawa, M., Noguchi, T., and Kameya, T. (2000). *J. Biol. Chem.* **275**, 12572–12580.

Fukuda, M., Ishii, A., Yasutomo, Y., Shimada, N., Ishikawa, N., Hanai, N., Nagata, N., Irimura, T., Nicolson, G. L., and Kimura, N. (1996). *Int. J. Cancer* **65**, 531–537.

Fukuda, M., Miyazaki, H., Shimada, N., Ishikawa, N., Ishii, A., Takagi, Y., Ishijima, Y., Kurama, H., and Kimura, N. *Membrane and Cell Biology*, in press.

Fukuda, M., Ishii, A., Koga, J., Ishijima, Y., Shimada, N., Ishikawa, N., and Kimura, N. submitted for publication.

Gervasi, F., D'Agnano, I., Vossio, S., Zupi, G., Sacchi, A., and Lombardi, D. (1996). *Cell Growth Diff.* **7**, 1689–1695.

Gilman, A. G. (1987). *Annu. Rev. Biochem.* **56**, 615–649.

Greene, L. A., and Tischler, A. S. (1976). *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2424–2428.

Guru, S. C., Goldsmith, P. K., Burns, A. L., Marx, S. J., Spiegel, A. M., Collins, F. S., and Chandrasekharappa, S. C. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 1630–1634

Hall, A. (1998). *Science* **279**, 509–514.

Hartsough, M. T., and Steeg, P. S. (2000). *J. Bioenerg. Biomembr* **32**, 301–308.

Hsu, S., Huang, F., Ossowski, L., and Friedman, E. (1995). *Caricinogenesis* **16**, 2259–2262.
Huff, K., End, D., and Guroff, G. (1981). *J. Cell Biol.* **88**, 189–198.
Igawa, M., Rukstalis, D. B., Tanabe, T., and Chodak, G. W. (1994). *Cancer Res.* **54**, 1313–1318.
Iiri, T., Farfel, Z., and Bourne, H. R. (1998). *Nature* **394**, 35–38.
Ishijima, Y., Fukuda, M., Shimada, N., Ishikawa, N., and Kimura, N. (2001). In *Abstract of The Fourth International Congress of the Genetics, Biochemistry and Physiology of NDP kinase/nm23/awd.* T18, Tokyo, Japan.
Ishijima, Y., Shimada, N., Fukuda, M., Miyazaki, H., Orlov, N. Y., Orlova, T. G., Yamada, T., and Kimura, N. (1999). *FEBS Lett.* **445**, 155–159.
Kantor, J. D., McCormick, B., Steeg, P. S., and Zetter B. R. (1993). *Cancer Res.* **55**, 1971–1973.
Katoh-Semba, R., Kitajima, S., Yamazaki, Y., and Sano M. (1987). *J. Neurosci. Res.* **17**, 36–44.
Keim, D., Melhem, R., Zhu, X. X., Lascu, I., Veron, M., and Strahler, J. R. (1992). *J. Clin. Invest.* **89**, 919–924.
Khan, M. H., Yasuda, M., Higashino, F., Haque, S., Kohgo, T., Nakamura, M., and Shindoh, M. (2001). *Am. J. Pathol.* **158**, 1785–1791.
Kikkawa, S., Takahashi, K., Takahashi, K., Shimada, N., Ui, M., Kimura, N., and Katada, T. (1990). *J. Biol. Chem.* **265**, 21536–21540.
Kimura, N. (1993). In *Handbook of Expt. Pharmacol: GTPases in Biology II, Vol. 108/II* (Dickey, B. F., and Birnbaumer, L., eds.), Springer-Verlag, Berlin, pp. 485–498.
Kimura, N. (2000). *J. Bioenerg. Biomembr.* **32**, 309–315.
Kimura, N., and Nagata, N. (1977). *J. Biol. Chem.* **252**, 3829–3835.
Kimura, N., and Nagata, N. (1979). *J. Biol. Chem.* **254**, 3451–3457.
Kimura, N., and Shimada, N. (1983). *J. Biol. Chem.* **258**, 2278–2283.
Kimura, N., and Shimada, N. (1985). *Biochem. Biophys. Res. Commun.* **131**, 199–206.
Kimura, N., and Shimada, N. (1986). *Biochem. Biophys. Res. Commun.* **134**, 928–936.
Kimura, N., and Shimada, N. (1988a). *J. Biol. Chem.* **263**, 4647–4653.
Kimura, N., and Shimada, N. (1988b). *Biochem. Biophys. Res. Commun.* **151**, 248–256.
Kimura, N., and Shimada, N. (1990). *Biochem. Biophys. Res. Commun.* **168**, 99–106.
Kimura, N., Shimada, N., Nomura, K., and Watanabe, K. (1990). *J. Biol. Chem.* **265**, 15744–15749.
Lacombe, M. L., and Jakobs, K. H. (1992). *Trends Pharmacol. Sci.* **13**, 46–48.
Lakso, M., Steeg, P. S., and Westphal, H. (1992). *Cell Growth Diff.* **3**, 873–879.
Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994). *Nature* **369**, 621–628.
Leone, A., Flatow, U., King, C. R., Sandeen, M. A., Margulies, I. M. K., Liotta, L. A., and Steeg, P. S. (1991). *Cell* **65**, 25–35.
Leone, A., Flatow, U., VanHoutte, K., and Steeg, P. S. (1993). *Oncogene* **8**, 2325–2333.
Lombardi, D., Palescandolo, E., Giordano, A., and Paggi, M. G. (2001). *Cell. Death. Diff.* **8**, 470–476.
Marshall, C. J. (1995). *Cell* **80**, 179–185.
Miyazaki, H., Fukuda, M., Ishijima, Y., Takagi, Y., Iimura, T., Negishi, A., Hirayama, R., Ishikawa, N., Amagasa, T., and Kimura, N. (1999). *Clin. Cancer Res.* **5**, 4301–4307.
Mumby, S. M. (2000). In *Frontiers in Molecular Bioligy, GTPases* (Hall, A., ed.), Oxford University Press, New York, pp. 1–34.
Noel, J. P., Hamm, H. E., and Sigler, P. B. (1993). *Nature* **366**, 654–663.
Ohkura, N., Kishi, M., Tsukada, T., and Yamaguchi, K. (2001). *Biochem. Biophys. Res. Commun.* **282**, 1206–1210.
Ohneda, K., Fukuda, M., Shimada, N., Ishikawa, N., Icho, T., Kaji, K., Toyota, T., and Kimura, N. (1994). *FEBS Lett.* **384**, 273–277.
Orlov, N. Y., Orlova, T. G., Nomura, K., Hanai, N., and Kimura, N. (1996). *FEBS Lett.* **389**, 186–190.
Otsuki, Y., Tanaka, M., Yoshii, S., Kawazoe, N., Nakaya, K., and Sugimura, H. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4385–4390.
Ouatas, T., Salerno, M., Palmieri, D., and Steeg, P. S. (2003). *J. Bioenerg. Biomembr.* **35**(1), XX–XX.
Ouatas, T., Selo, M., Sadji, Z., Hourdry, J., Denis, H., and Mazabraud, A. (1998). *Int. J. Dev. Biol.* **42**, 43–52.
Paggi, M. G., Bonetto, F., Sererino, A., Baldi, A., Battista, T., Bucci, F., Felsani, A., Lombardi, D., and Giordano, A. (2001). *Oncogene* **20**, 2570–2578.
Parabicini, G., Steinmayr, M., Andre, E., and Becker-Andre, M. (1996). *Biochem. Biophys. Res. Commun.* **227**, 82–87.
Postel, E. H., Berberich, S. J., Flint, S. J., and Ferrone, C. A. (1993), *Science* **261**, 478–480.
Randazzo, P. A., Northup, J. K., and Kahn, R. A. (1991). *Science* **254**, 850–853.
Randazzo, P. A., Northup, J. K., and Kahn, R. A. (1992). *J. Biol. Chem.* **267**, 18182–18189.
Reynet, C., and Kahn, C. R. (1993). *Science* **262**, 1441–1444.
Russel, R. L., Pedersen, A. N., Kantor, J., Geisinger, K., Long, R., Zbieranski, N., Townsend, A., Shelton, B., Brunner, N., and Kute, T. E. (1998). *Br. J. Cancer* **78**, 710–717.
Shimada, N., Ishikawa, N., Munakata, Y., Toda, T., Watanabe, K., and Kimura, N. (1993). *J. Biol. Chem.* **268**, 2583–2589.
Sorscher, S. M., Steeg, P., Feramisco, J. R., Buckmaster, C., Boss, G. R., and Meinkoth, J. (1993). *Biochem. Biophys. Res. Commun.* **195**, 336–345.
Sprang, S. R. (1997). *Annu. Rev. Biochem.* **66**, 639–678.
Steeg, P. S., Bevilacqua, G., Kopper, L., Thogeirsson, U. P., Talmadge, J. E., Liotta, L. A., and Sobel, M. E. (1988). *J. Natl. Cancer Inst.* **80**, 200–204.
Subramanian, C., Cotter II, M. A., and Robertson, E. S. (2001). *Nature Med.* **7**, 350–355.
Timmons, L., and Shearn, A. (2000). *J. Bioenerg. Biomembr.* **32**, 293–300.
Tseng, Y.-H., Vicent, D., Zhu, J., Niu, Y., Adeyinka, A., Moyers, J. S., Watson, P. H., and Kahn, C. R. (2001). *Cancer Res.* **61**, 2071–2079.
Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1995). *Cell* **83**, 1047–1058.
Webb, P. A., Perisic, O., Mendola, C. E., Backer, J. M., and Williams, R. L. (1995). *J. Mol. Biol.* **251**, 574–587.
Williams, R. L., Oren, D. A., Munoz-Dorado, J., Inouye, S., Inouye, M., and Arnold, E. (1993). *J. Mol. Biol.* **234**, 1230–1247.
Wittinghofer, A. (2000). In *Frontiers in Molecular Bioligy, GTPases* (Hall, A., ed.), Oxford University Press, New York, pp. 244–310.
Yaguchi, H., Ohkura, N., Tsukada, T., and Yamaguchi, K. (2002). *J. Biol. Chem.* **277**, 38197–38204.
Yan, G.-Z., and Ziff, E. B. (1995). *J. Neurosci.* **15**, 6200–6212.
Zhu, J., Reynet, C., Caldwell, J. S., and Kahn, C. R. (1995). *J. Biol. Chem.* **270**, 4805–4812.
Zhu, J., Tseng, Y.-H., Kantor, J. D., Rhodes, C. J., Zetter, B. R., Moyers, J. S., and Kahn, C. R. (1999). *Proc. Natl. Acad. Sci.* **96**, 14911–14918.