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

New Insights Into Nm23 Control of Cell Adhesion and Migration

Henri-Noël Fournier,[1] Corinne Albigès-Rizo,[1] and Marc R. Block[1,2]

Received July 24, 2002; accepted October 4, 2002

The molecular mechanisms underlying the role of Nm23/NDP kinase in controlling cell migration and metastasis have been investigated. The recent progress in our understanding of cell migration at a molecular level gives us some clues to the putative Nm23 function as a suppressor of metastasis. Screening of the literature indicates that NDP kinases have pleiotropic effects. By modifying cytoskeleton organization and protein trafficking, some NDP kinase isoforms may indirectly promote adhesion to the extracellular matrix in some cell types. Conversely, Nm23 regulates cell surface expression of integrin receptors and matrix metallo-proteases, and thus directly controls the cell adhesion machinery. Finally, the recent discovery of the interaction between Nm23-H2 and the negative regulator of $\beta 1$ integrin-mediated cell adhesion, ICAP-1, which targets the kinase to lamellipodia and cell protrusions, suggests that the Nm23-H2/ICAP-1 complex plays a role in integrin signaling, and exerts a fine-tuning between migration and spreading.

KEY WORDS: Nm23; integrin; ICAP-1; lamellipodia; migration; metastasis.

INTRODUCTION

Nucleoside diphosphate kinases (NDP kinases) are a family of highly conserved proteins in eukaryotes (Lacombe *et al.*, 2000) of which eight different genes (*nm23-H1* to *nm23-H8*) have been identified in humans. NDP kinases play a major role in cell metabolism since they transfer the terminal phosphate of a nucleoside triphosphate to a nucleoside diphosphate, thus equilibrating the NDP and NTP cellular pools independently of the nature of the purine or pyrimidine bases (Lascu and Gonin, 2000; Parks and Agarwal, 1973). The *nm23* tumor metastasis suppressor gene was found to encode a protein identical to NDP kinase (Steeg *et al.*, 1988). Nm23-H1 and Nm23-H2 tumor suppressor activities have been identified in a number of human cancers (Hartsough and Steeg, 2000; Martin, and Pilkington, 1998; Rusciano, 2000). In cancer cell lines, in vitro, expression of *nm23* reduces metastatic potential and cell motility (Baba, *et al.*, 1995; Kantor, *et al.*, 1993, Leone, *et al.*, 1991). Although this effect has been extensively described, the molecular mechanisms underlying the role of Nm23 in cancer is poorly understood. A number of data indicate that Nm23 is a multifunctional protein reportedly involved in a variety of cellular functions including differentiation, proliferation, and apoptosis (Amendola, *et al.*, 2001; Gervasi, *et al.*, 1996; Lombardi, *et al.*, 2000; Negroni, *et al.*, 2000; Otero, 2000). Moreover, a number of reports are controversial. This may be due in part to the selective actions of some Nm23 isoforms. For instance, the nuclease hypersensitive element of the c-*myc* and PDGF-A (Platelet-derived growth factor A) promoters have been shown to bind specifically to Nm23-H2 (Ma, *et al.*, 2002; Postel, *et al.*, 1993). In this review, we will focus on the possible interferences between Nm23- and integrin-mediated cell migration and differentiation. Three general mechanisms

Key to abbreviations: ICAP-1, Integrin Cytoplasmic-Associated Protein 1; Nm23, Nonmetastatic 23; Tiaml, Tumor Invasion And Metastasis 1; GEF, Guanine nucleotide Exchange Factor; ROCK, Rho-associated Kinase; Rho, Ras homolog protein; Rac1, Ras related C3 botulinum toxin substratel; Rad, Ras associated with diabetes; Gem, immediate early gene expressed in mitogen-stimulated T-cells; Cdc42, Cell division cycle protein 42.

[1]Laboratoire de la Différenciation et de l'Adherence Cellulaires, UMR CNRS/UJF 5538, Institut Albert Bonniot, Faculté de Médecine de Grenoble, F38706 La Tronche Cedex, France.

[2]To whom correspondence should be addressed; e-Email: marc.block@ujf-grenoble.fr.

0145-479X/03/0200-0081/0

may account for these effects. Either a direct interaction of Nm23 with some components of the machinery that triggers cell/extracellular matrix interactions, or an indirect effect due to Nm23 transactivation activity that could lead to up- or downregulation of the expression of some components involved in cell adhesion. Eventually, some interference with the cytoskeleton assembly may also impair or favor cell migration or spreading on extracellular matrix.

MECHANISMS OF CELL ADHESION AND MIGRATION

Cell migration is an integrated, multistep process that requires the continuous and coordinated formation and disassembly of adhesive structures (Webb, *et al.*, 2002). It involves stable attachment of the cell to the extracellular matrix at the leading edge which requires transmembrane receptors of the integrin family (Martin, *et al.*, 2002). On these adhesion sites strong forces transmitted by the cytoskeleton move the cell body forward while at the rear, the release of adhesion and retraction is observed. The extension of the lamellipodia is driven by actin polymerization induced by the recruitment of the Arp2/3 complex, the VASP/Zyxin complex, and the Wiscott–Aldrich syndrome protein (WASP) (Bernheim-Groswasser, *et al.*, 2002; Le Clainche, *et al.*, 2001; Pantaloni, *et al.*, 2001). Near the leading edge, the activated G protein Racl and its effectors localize and might control actin polymerization and assembly of small substrate anchorage sites named focal complexes (Nobes and Hall, 1995; Rottner, *et al.*, 1999). At the ventral face of the cell body, focal complexes are maturated into larger structures, the focal adhesions that may differ in protein composition (Table I). Focal adhesion assembly is directed by activation of the G protein RhoA and by mechanical forces (Chrzanowska–Wodnicka and Burridge, 1996; Rottner, *et al.*, 1999; Riveline, *et al.*, 2001). Indeed, actomyosin contraction is controlled through the inhibition by phosphorylation of MLC phosphatase by the serine threonine kinase ROCK, an effector of RhoA (Totsukawa, *et al.*, 2000). Although a sequential activation of Cdc42, Rac1, and finally RhoA was initially described in Swiss 3T3 (Nobes and Hall, 1995), the most common feature observed in many biological systems is an antagonistic action of Rac1 and RhoA (Rottner, *et al.*, 1999). While Rac1 and Cdc42 promote lamellipodia and filipodia respectively, RhoA stimulates focal adhesion and stress fiber assembly.

Conversely, at the rear of the cell, weakening of the cell/matrix interaction may be due to a dispersal of the integrin receptors, possibly because of calpain cleavage (Dourdin, *et al.*, 2001; Palecek, *et al.*, 1998). While nonmotile cells exhibit large focal adhesions and few cell protrusions, highly motile cells have large lamellipodia and do not assemble focal adhesions (Duband, *et al.*, 1988).

Nm23 INTERFERENCE WITH THE CELL ADHESION MACHINERY MAY CONTROL CELL MIGRATION

Modification of Cell Surface Integrin Expression

The simpler explanation for the effect of Nm23 on cell adhesion is via direct action on integrin receptors. Upregulation of $\beta 1$ integrins at the cell surface has been described in neuroblastoma cells after transfection with a plasmid encoding the DR-Nm23 isoform (Amendola, *et al.*, 1997). This was correlated with an increase in cell adhesion on collagen type I. Whether this increase in integrin expression represents an externalization of preexisting receptors or neosynthesis remains a matter of debate. Variation of integrin cell surface expression can account by itself for the antimetastatic effect of DR-Nm23 and may not be restricted to the DR isoform. For instance, the PuF transcription factor of c-*myc* was found identical to Nm23-H2 (Postel, *et al.*, 1993, 1996). Since c-*myc* downregulates $\beta 1$ integrin expression in epithelial cells (Waikel, *et al.*, 2001), it is possible that Nm23-H2 could modify integrin expression at the surface of these cells.

Protein Trafficking and Cell Migration

A more indirect action of Nm23 could be on cell-substratum adhesion assembly as a result of a control of intracellular protein trafficking. Recent genetic evidence in *Drosophila* indicates that Abnormal wing disc (Awd), an ortholog of NDPK, enhances endocytosis of synaptic vesicles by acting as a supplier of GTP to the G protein dynamin which is involved in the fission of clathrin-coated vesicles (Krishnan, *et al.*, 2001). Thus, Nm23 may contribute to the stimulation of intracellular protein transport. Increasing evidence suggests that integrin-containing vesicles move from the rear of the migrating cells to a perinuclear region, and from this perinuclear region to lamellipodia (Webb, *et al.*, 2002). One can therefore postulate that vesicular transport provides a significant supply of material at the cell front. On the other hand the involvement of some regulatory components in vesicular transport, such as small G proteins of the ARF family or ARF-GTPase Activating Protein (GAP), strongly suggests that vesicular transport also contributes to the recruitment and

targeting of signaling proteins to focal adhesions. Indeed, ARF1 and ASAP1, an ARF-GAP, mediate paxillin and focal adhesion kinase (FAK) recruitment to focal adhesions in Swiss 3T3 fibroblasts and REF 52 cells, respectively (Liu, *et al.*, 2002; Norman, *et al.*, 1998). Both paxillin and FAK are major regulatory players in the control of focal adhesion assembly and signaling. Therefore, modulation of protein trafficking by Nm23 could significantly modify cell adhesion to the extracellular matrix.

Interference with Integrin-Mediated Signaling and Cytoskeleton Organization

The metastasis suppressor activity of Nm23 may also be due to the interference with G proteins of the Rho family that control cytoskeleton organization and cell/matrix contact assembly. It has recently been described that the Rac1 exchange factor Tiam1 binds Nm23-H1 (Otsuki, *et al.*, 2001). This may result in the sequestration and/or inactivation of this factor, thus inducing a decrease in activated Rac1 bound to GTP. Inactivation of Rac1 is likely to favor the activation of RhoA that would switch the cell from a motile to a fully spread phenotype. Alternatively, Nm23 has been described to interact directly with the monomeric G protein Rad. Nm23 exhibits a specific Rad-GAP activity. However, in the presence of ATP, GDP-Rad was also converted into GTP-Rad by NDP kinase activity (Zhu, *et al.*, 1999). This latter effect might not be relevant to a physiological process since high levels of expression of Rad in breast cancer cell lines are associated with an increase in invasiveness and growth rate that can be inhibited by coexpression of Rad and Nm23 (Tseng, *et al.*, 2001). Overexpression of Rad or the close member of this GTPase family Gem seems to be associated with a reorganization of the cytoskeleton and neurite extentions in neuroblastoma. Recent data suggest that this effect is via a direct interaction of the isoforms ROCKα and ROCKβ with Rad and GEM respectively, independently of RhoA, (the major ROCK activator), leading to the inhibition of the kinase activities. This provides a novel mechanism that favors the migratory phenotype versus the spreading phenotype (Ward, *et al.*, 2002). Through this new signaling pathway controlling cytoskeletal organization and cell adhesion, Nm23 could reduce cell migration.

Another way by which Nm23 could modulate cell migration and adhesion is via a modulation of cytoskeletal dynamics. The association of Nm23-H1 and Nm23-H2 with microtubules points toward the hypothesis that these NDP kinases would supply GTP in the proximity of microtubules which favors tubulin polymerization (Biggs, *et al.*, 1990; Lombardi, *et al.*, 1995). Microtubule dynamics contributes to the directional locomotion of many cell types (Mikhailov and Gundersen, 1998). Moreover, targeting of microtubules to cell-substrate contacts may promote the dissociation of focal adhesions (Kaverina, *et al.*, 1998, 1999), although the underlying mechanism remains unknown. This might be particulary important for cell tail retraction.

Nm23 and Expression of Matrix Metallo Proteinases

The degradation of basement membranes and stromal extracellular matrix is crucial for invasion and metastasis of malignant cells. This degradation is initiated by proteinases secreted by different cell types participating in tumor cell invasion, and increased expression and/or activity of every known class of proteinases (metallo-, serine-, aspartic-, and cysteine-proteinases) has been linked to malignancy and invasion of tumor cells (Westermarck and Kahari, 1999). In vitro, overexpressed *nm23-*β, (the Rat ortholog of human *nm23-H1*), binds to the RE-1 enhancer element flanking the 5′ flanking region of the rat and human gelatinase *A/MMP2* genes. By direct competition for binding with the transactivator YB-1, Nm23 represses the protease transcription (Cheng, *et al.*, 2002). This downregulation may result in a decrease of invasiveness of the cells.

Nm23/ICAP-1 INTERACTION: A NEW DIRECTION IN THE ELUCIDATION OF Nm23 TUMOR SUPPRESSOR ACTIVITY

ICAP-1, A Negative Regulator of Cell Adhesion Mediated by β1 Integrin

Integrin cytoplasmic domain associated protein 1 (ICAP-1), a 200 amino acid long peptide, was initially found as a partner of the β1 integrin subunit cytoplasmic tail in a two-hybrid screen (Chang, *et al.*, 1997). Two variants of *ICAP-1* named α and β have been described on the basis of DNA sequence analysis. The β isoform corresponds to a skip of exon 6 (D. Bouvard and R. Fässler, personal communication) and does not bind to the integrins. However, no evidence for the expression of *ICAP-1*β in vivo have been provided to date and only the properties of the full length protein will be discussed hereafter. A growing number of small proteins interacting with specific integrin cytoplasmic tails have been recently characterized, despite the similarity of the intracellular domains of β integrin chains. This suggests that specific integrins are coupled to distinct signaling pathways. Overexpression of *ICAP-1* in COS7 or CHO cells stimulates cell migration

in Boyden chambers (Zhang and Hemler, 1999). ICAP-1 is a phosphoprotein within eukaryotic cells (Zhang and Hemler, 1999). The phosphorylation sites include, PKA, PKC, and CamKII consensus phosphorylation motifs, all located in the N-terminal half of the protein. The T38D mutant of ICAP-1 that mimics the phosphorylated form of the protein strongly impairs CHO cell spreading on fibronectin, suggesting that ICAP-1 behaves as a negative regulator of β1 integrin-mediated cell adhesion under the control of protein phosphorylation (Bouvard and Block, 1998). ICAP-1 interacts specifically with the C-terminal NPXY motif of the β1 integrin cytoplasmic domain. Alanine scanning mutagenesis of this region reveals that Val(787), Val(790), and (792)NPKY(795) are critical for ICAP-1 binding. The NPXY motif is a known binding site for phosphotyrosine binding (PTB) domain proteins and, computational modeling reveals that amino acids 58–200 can fold into a PTB motif (Chang, *et al.*, 2002).

Although it is clear that ICAP-1 plays important roles in the regulation of cell adhesion, the mechanism of ICAP-1 function in the signaling pathways has not yet been completely elucidated. Direct competition of ICAP-1 with talin for binding to the β1 cytoplasmic domain has been observed in vitro (our unpublished results). In vivo, while ICAP-1 is located in lamellipodia of spreading cells, it is not present in focal adhesions of fully spread cells (Fig. 1). Reciprocally, talin is a marker of focal adhesions that might not be present in lamellipodia (Reddy, *et al.*, 2001). Since the colocalization of ICAP-1 and talin has never been observed, while β1 integrins are found in both lamellipodia and focal adhesions this strongly suggests that the competition between talin and ICAP-1 binding on integrin cytosolic tails also occurs in vivo. This is substantiated by the fact that talin head binds to integrin using a PTB like motif of the FERM domain (Calderwood, *et al.*, 2002). Alternatively, it has been reported that ICAP-1 binds Cdc42 and Rac1 (Degani, *et al.*, 2002). This interaction could interfere with Rac1 activation, resulting in the modification of cell adhesion and migration.

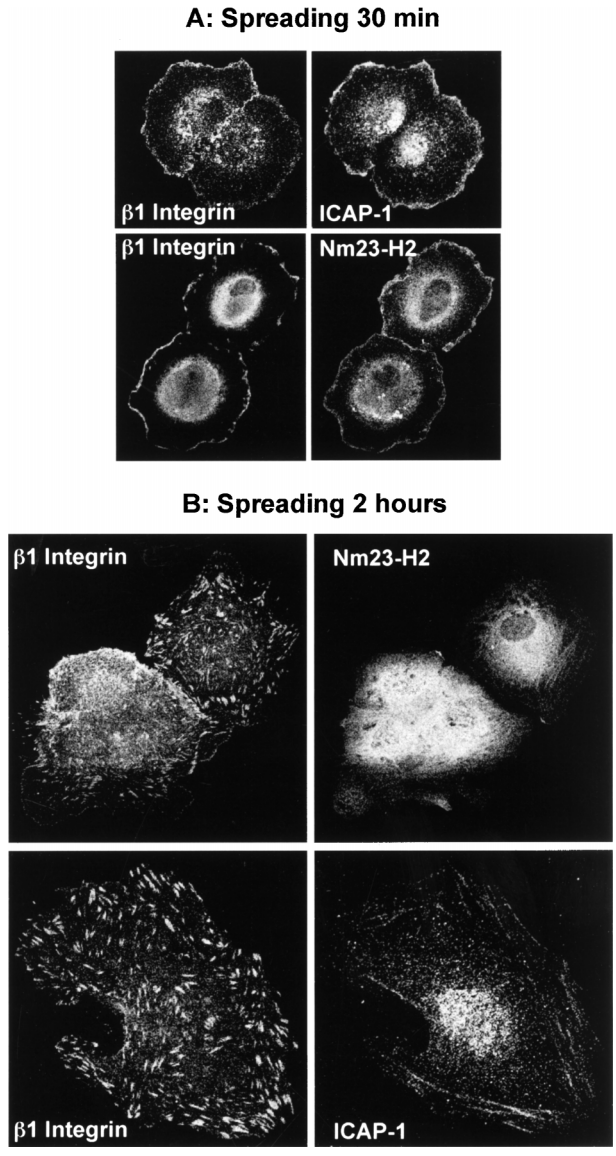

Fig. 1. ICAP-1 and Nm23-H2 are colocalized with β1 integrins at the cell edges in lamellipodia. Fibroblast cells were plated on coverslips coated with fibronectin, fixed after 30 min or 2 h, and stained with polyclonal anti-ICAP-1 or Nm23-H2 antibodies and monoclonal anti-β1 integrin antibody. ICAP-1 and Nm23-H2 proteins colocalize with β1 integrins only during the early stages of spreading (30 min). Focal adhesion observed at 2 h of spreading contained neither ICAP-1 nor Nm23-H2. Visualization of a section and image capture were carried out with a confocal microscope.

Nm23-H2, a New Partner of ICAP-1

A two-hybrid screen revealed the interaction of ICAP-1 with Nm23-H2. In vitro, pull-down assays indicate that Nm23 H2 binds the C-terminal moiety of ICAP-1 (Fournier, *et al.*, 2002) while little, if any, interaction was observed with Nm23-H1 or DR-Nm23. Within the cells, colocalization of ICAP-1 and Nm23-H2 together with β1 integrins was observed in lamellipodia during the early stages of fibroblast spreading on fibronectin (Fig. 1A). It is noteworthy that, despite of the lack of a biochemical interaction between ICAP-1 and nNm23-H1, the latter protein was also found in lamellipodia at the early stages of spreading (Fig. 2). This localization may reflect the heteromeric nature of Nm23 hexamers in the cytosol with both Nm23-H1 and H2 present in the same oligomer.

Fully spread cells exhibited well-organized focal adhesions containing β1 integrins, but neither ICAP-1 nor

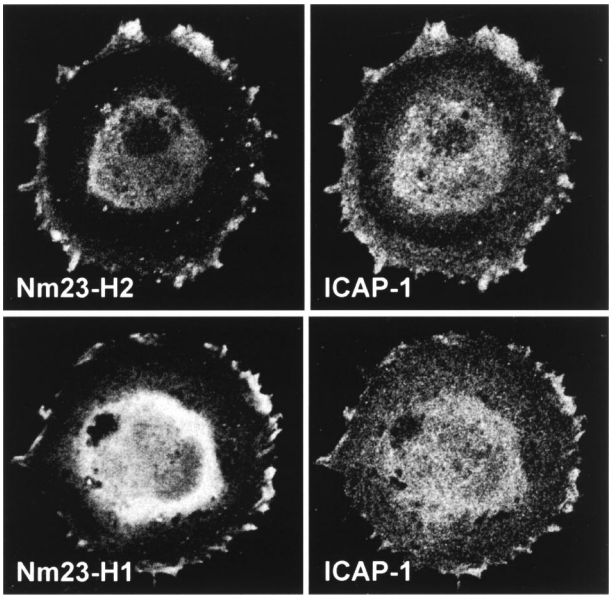

Fig. 2. Nm23-H1 and Nm23-H2 are colocalized with ICAP-1 in peripheral ruffles in spreading fibroblast cells. Fibroblast cells were plated on fibronectin 30 min before performing costaining with polyclonal anti ICAP-1 antibodies and monoclonal anti Nm23-H1 or H2 antibodies. Visualization of a section and image capture were carried out with a confocal microscope.

Nm23-H2 were colocalized in these structures (Fig. 1B), reflecting the dynamic nature of this interaction.

Physiological Significance of Nm23-H2/ICAP-1 Interaction

The colocalization and association of Nm23-H2 and ICAP-1 during cell adhesion suggest that this interaction is relevant to a physiological process. For the first time a direct link between the metastatic suppressor *nm23* and cell adhesion and migration machinery has been characterized. It is another example of the presence of chemically distinct cell-substratum adhesion sites, all involving integrins but with different specific connections to the cytoskeleton. Whereas some cytoplasmic components are found in both focal adhesions and focal complexes near lamellipodia, others are specifically found in one or the other of these structures (summarized in Table I). Nm23-H2 and H1 localize specifically in early cell-substratum contacts which engage $\beta 1$ integrins. Indeed, such localization is not observed in cells spreading on extracellular matrixes that recruit $\beta 3$ integrins such as vitronectin (Fournier, *et al.*, 2002). What could be the role of Nm23 in lamellipodia? Since Nm23-H2 interacts directly with ICAP-1, a negative regulator of cell adhesion that favors migration, one could hypothesize that Nm23-H2 sequesters ICAP-1, therefore reducing its action and favoring the interaction of talin with integrin and the assembly of new focal adhesions at the rear of lamellipodia (Fig. 3).

Alternatively, ICAP-1 may play the role of an adaptator protein that recruits Nm23-H2 at the leading edge of the cell. Here, a ternary complex ICAP-1/Nm23-H2/$\beta 1$ integrin may also target the small G proteins Rac1/Cdc42 (Degani *et al.*, 2002). Therefore, the Nm23-H2/ICAP-1 complex may participate to actin polymerization in lamellipodia. Since Nm23-H1 binds and negatively regulates Tiam1, a Rac1 specific nucleotide exchange factor (Otsuki, *et al.*, 2001), it may also decrease Rac1 activity. On the other hand, Rac1 activation was described to reduce RhoA-activity (Rottner, *et al.*, 1999). Thus, through Rac1 inactivation, Nm23 is likely to favor RhoA-dependent focal adhesion assembly and stable spreading of the cells. This effect could be synergistic with a possible inactivation of ROCK (a major effector of RhoA) through Rad/Gem

Table I. Protein Composition of Early Focal Complexes in Lamellipodia versus Focal Adhesions

Protein	Focal complexes in lamellipodia	Focal adhesions	References
Talin	−	+	(Reddy *et al.*, 2001)
α-Actinin	+	±	(Edlund *et al.*, 2001; Reddy *et al.*, 2001)
Vinculin	+	+	(Goldmann and Ingber, 2002)
Paxillin	+	+	(Woods *et al.*, 2002)
Focal adhesion kinase	+	+	(Parsons *et al.*, 2000)
F actin	+	+	(Small *et al.*, 2002)
VASP	+	+	(Rottner *et al.*, 2001)
ICAP-1	+	−	(Fournier *et al.*, 2002)
Nm23-H1, H2	+	−	(Fournier *et al.*, 2002)
Rac 1	+	−	(Nobes and Hall, 1995)
Arp2/3	+	−	(Weed *et al.*, 2000)
Skelemin	+	−	(Reddy *et al.*, 2001)

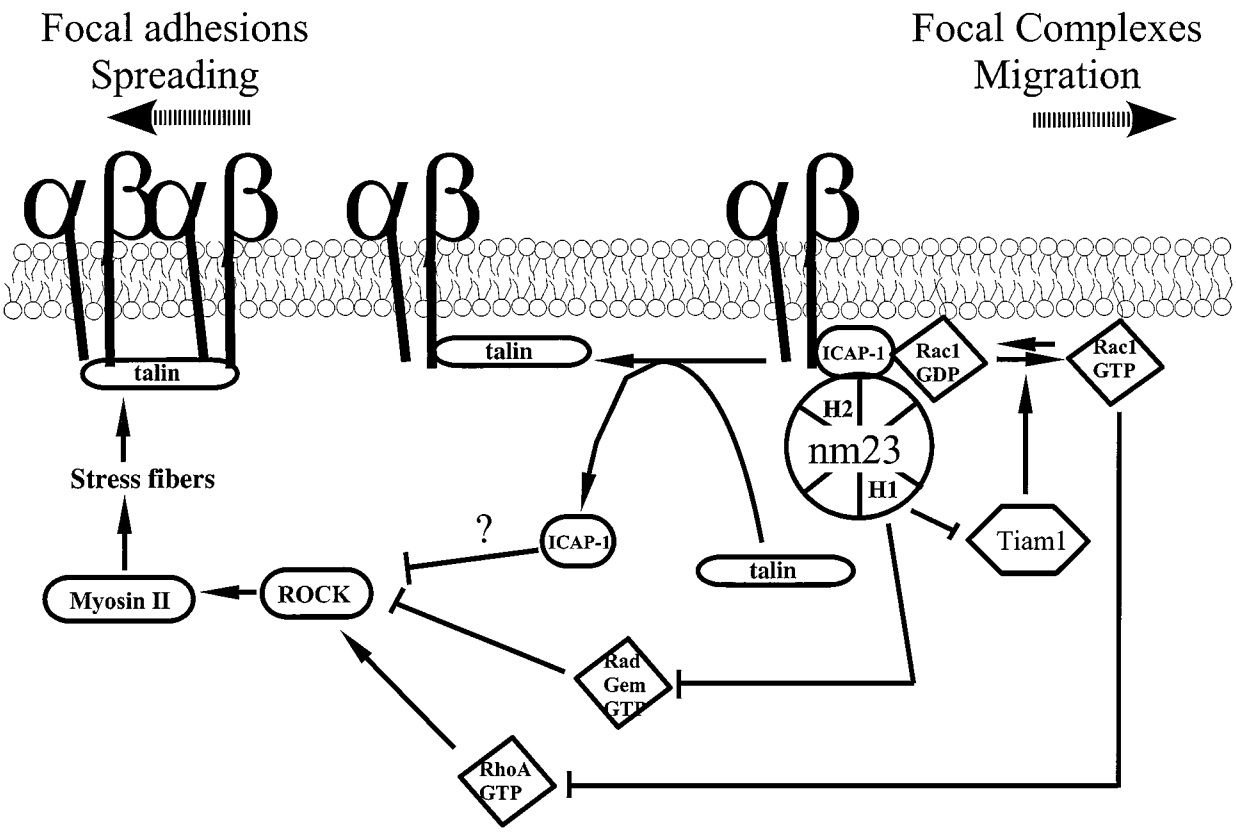

Fig. 3. Potential role of ICAP-1 and Nm23 proteins in the control of the adhesion and migration signaling pathway. During cell migration or during the early stages of cell spreading, the progress of the cell front is associated with the development of a new set of focal complexes at the base of the budding lamellipodia. In this context, the integrin-associated protein ICAP-1 could recruit Nm23 in the vicinity of integrins. Direct recruitment of Rac1 by ICAP-1 could result in the increase in Rac1 activity necessary for lamellipodia extension. Conversely, the inhibition of Tiaml, a GEF for Rac1 by Nm23 might favor the maturation of focal complexes into focal adhesions mediated by the inhibition of Rac1 activity and the subsequent activation of RhoA and its effector, Rho Kinase (ROCK). ROCK activity is required for stress fibers assembly and contractility. ICAP-1 as well as Nm23 could interfere with ROCK activity by direct interaction or through Rad inhibition, respectively. Moreover, the diversity of cell-matrix adhesions is associated with dynamic changes in their molecular composition. Indeed we presume that the cytoplasmic tail of $\beta 1$ integrin could interact with ICAP-1 in focal complexes, and with talin in focal adhesions.

and the recruitment of ROCK by ICAP-1. This interaction has been characterized in a two-hybrid system and by coimmunoprecipitation (Stroeken and E. Roos, personal communication).

CONCLUDING REMARKS

The antimetastatic effect of Nm23 has been an enigma for more that 10 years and little is known about the molecular mechanisms underlying its role in cell physiology. Although Nm23 is a small protein, it has become obvious that it is multifunctional. The eight human isoforms add to the complexity, since some of Nm23 functions are specific to a subset of Nm23 isoforms. In this context, a number of described functions of NDP kinases suggest direct and indirect interference with the cell/extracellular matrix machinery. It is remarkable that all these effects point to the reduction of cell motility and the increase in cell spreading (Fig. 3). With its specific location in lamellipodia and cell protrusions the ICAP-1/Nm23 complex may carry out a fine tuning between cell protrusions and spreading. These findings open new directions for future research in this field.

Note

During the time course of the review of our manuscript, Palacios *et al.* Nature Cell Biol. 4: 929–936 (2002) describes that ARF-6 GTP that mediates endocytosis of E cadherin also recruits Nm23-H1 that provides

a source of GTP for dynamin-dependent fission of coated vesicles during endocytosis. This recruitment of NM-23-H1 to cell junctions is accompanied by a decrease in the cellular levels of Rac1-GTP.

ACKNOWLEDGMENTS

We are grateful to Dr loan Lascu and Marie-Lise Lacombe for their precious help and advices. We thank Sandra Dupé-Manet for her skillful assistance in immunomicroscopy studies. This work was supported in part by a grant from Association pour le Recherche contre le Cancer, La Ligue Nationale contre le Cancer and Fondation Espoir.

REFERENCES

Amendola, R., Martinez, R., Negroni, A., Venturelli, D., Tanno, B., Calabretta, B., and Raschella, G. (1997). *J. Natl. Cancer Inst.* **89**, 1300–1310.

Amendola, R., Martinez, R., Negroni, A., Venturelli, D., Tanno, B., Calabretta, B., and Raschella, G. (2001). *Med. Pediatr. Oncol.* **36**, 93–96.

Baba, H., Urano, T., Okada, K., Furukawa, K., Nakayama, E., Tanaka, H., Iwasaki, K., and Shiku, H. (1995). *Cancer Res.* **55**, 1977–1981.

Bernheim-Groswasser, A., Wiesner, S., Golsteyn, R. M., Carlier, M. F., and Sykes, C. (2002). *Nature* **417**, 308–311.

Biggs, J., Hersperger, E., Steeg, P. S., Liotta, L. A., and Shearn, A. (1990). *Cell* **63**, 933–940.

Bouvard, D., and Block, M. R. (1998). *Biochem. Biophys. Res. Commun.* **252**, 46–50.

Calderwood, D. A., Yan, B., De Pereda, J. M., Alvarez, B. G., Fujioka, Y., Liddington, R. C., and Ginsberg, M. H. (2002). *J. Biol. Chem.* **277**, 21749–21758.

Chang, D. D., Hoang, B. Q., Liu, J., and Springer, T. A. (2002). *J. Biol. Chem.* **277**, 8140–8145.

Chang, D. D., Wong, C., Smith, H., and Liu, J. (1997). *J. Cell. Biol.* **138**, 1149–1157.

Cheng, S., Alfonso-Jaume, M. A., Mertens, P. R., and Lovett, D. H. (2002). *Biochem. J.* **366**, 8007–8016.

Chrzanowska-Wodnicka, M., and Burridge, K. (1996). *J. Cell. Biol.* **133**, 1403–1415.

Degani, S., Balzac, F., Brancaccio, M., Guazzone, S., Retta, S. F., Silengo, L., Eva, A., and Tarone, G. (2002). *J. Cell. Biol.* **156**, 377–387.

Dourdin, N., Bhatt, A. K., Dutt, P., Greer, P. A., Arthur, J. S., Elce, J. S., and Huttenlocher, A. (2001). *J. Biol. Chem.* **276**, 48382–48388.

Duband, J. L., Nuckolls, G. H., Ishihara, A., Hasegawa, T., Yamada, K. M., Thiery, J. P., and Jacobson, K. (1988). *J. Cell. Biol.* **107**, 1385–1396.

Fournier, H. N., Dupe-Manet, S., Bouvard, D., Lacombe, M. L., Marie, C., Block, M. R., and Albiges-Rizo, C. (2002). *J. Biol. Chem.* **277**, 20895–20902.

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

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

Kantor, J. D., Mccormick, B., Steeg, P. S., and Zetter, B. R. (1993). *Cancer Res.* **53**, 1971–1973.

Kaverina, I., Krylyshkina, O., and Small, J. V. (1999). *J. Cell. Biol.* **146**, 1033–1044.

Kaverina, I., Rottner, K., and Small, J. V. (1998). *J. Cell. Biol.* **142**, 181–190.

Krishnan, K. S., Rikhy, R., Rao, S., Shivalkar, M., Mosko, M., Narayanan, R., Etter, P., Estes, P. S., and Ramaswami, M. (2001). *Neuron* **30**, 197–210.

Lacombe, M. L., Milon, L., Munier, A., Mehus, J. G., and Lambeth, D. O. (2000). *J. Bioenerg. Biomembr.* **32**, 247–258.

Lascu, I., and Gonin, P. (2000). *J. Bioenerg. Biomembr.* **32**, 237–246.

Le Clainche, C., Didry, D., Carlier, M. F., and Pantaloni, D. (2001). *J. Biol. Chem.* **276**, 46689–46692.

Leone, A., Flatow, U., King, C. R., Sandeen, M. A., Margulies, I. M., Liotta, L. A., and Steeg, P. S. (1991). *Cell* **65**, 25–35.

Liu, Y., Loijens, J. C., Martin, K. H., Karginov, A. V., and Parsons, J. T. (2002). *Mol. Biol. Cell* **13**, 2147–2156.

Lombardi, D., Lacombe, M. L., and Paggi, M. G. (2000). *J. Cell Physiol.* **182**, 144–149.

Lombardi, D., Sacchi, A., D'agostino, G., and Tibursi, G. (1995). *Exp. Cell Res.* **217**, 267–271.

Ma, D., Xing, Z., Liu, B., Pedigo, N. G., Zimmer, S. G., Bai, Z., Postel, E. H., and Kaetzel, D. M. (2002). *J. Biol. Chem.* **277**, 1560–1567.

Martin, K. H., Slack, J. K., Boerner, S. A., Martin, C. C., and Parsons, J. T. (2002). *Science* **296**, 1652–1653.

Martin, K. K., and Pilkington, G. J. (1998). *Anticancer Res.* **18**, 919–926.

Mikhailov, A., and Gundersen, G. G. (1998). *Cell Motil. Cytoskeleton* **41**, 325–340.

Negroni, A., Venturelli, D., Tanno, B., Amendola, R., Ransac, S., Cesi, V., Calabretta, B., and Raschella, G. (2000). *Cell Death Differ.* **7**, 843–850.

Nobes, C. D., and Hall, A. (1995). *Cell* **81**, 53–62.

Norman, J. C., Jones, D., Barry, S. T., Holt, M. R., Cockcroft, S., and Critchley, D. R. (1998). *J. Cell Biol.* **143**, 1981–1995.

Otero, A. S. (2000). *J. Bioenerg. Biomembr.* **32**, 269–275.

Otsuki, Y., Tanaka, M., Yoshii, S., Kawazoe, N., Nakaya, K., and Sugimura, H. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4385–4390.

Palecek, S. P., Huttenlocher, A., Horwitz, A. F., and Lauffenburger, D. A. (1998). *J. Cell Sci.* **111(7)**, 929–940.

Pantaloni, D., Le Clainche, C., and Carlier, M. F. (2001). *Science* **292**, 1502–1506.

Parks, R. E., Jr. and Agarwal, R. P. (1973), *Enzymes* **8**(Boyer P.D. Ed), 307–333.

Postel, E. H., Berberich, S. J., Flint, S. J., and Ferrone, C. A. (1993). *Science* **261**, 478–480.

Postel, E. H., Weiss, V. H., Beneken, J., and Kirtane, A. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93**, 6892–6897.

Reddy, K. B., Bialkowska, K., and Fox, J. E. (2001). *J. Biol. Chem.* **276**, 28300–28308.

Riveline, D., Zamir, E., Balaban, N. Q., Schwarz, U. S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A. D. (2001). *J. Cell. Biol.* **153**, 1175–1186.

Rottner, K., Hall, A., and Small, J. V. (1999). *Curr. Biol.* **9**, 640–648.

Rusciano, D. (2000). *Crit. Rev. Oncog.* **11**, 147–163.

Steeg, P. S., Bevilacqua, G., Kopper, L., Thorgeirsson, U. P., Talmadge, J. E., Liotta, L. A., and Sobel, M. E. (1988). *J. Natl. Cancer Inst.* **80**, 200–204.

Totsukawa, G., Yamakita, Y., Yamashiro, S., Hartshorne, D. J., Sasaki, Y., and Matsumura, F. (2000). *J. Cell Biol.* **150**, 797–806.

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.

Waikel, R. L., Kawachi, Y., Waikel, P. A., Wang, X. J., and Roop, D. R. (2001). *Nat. Genet.* **28**, 165–168.

Ward, Y., Yap, S. F., Ravichandran, V., Matsumura, F., Ito, M., Spinelli, B., and Kelly, K. (2002). *J. Cell Biol.* **157**, 291–302.

Webb, D. J., Parsons, J. T., and Horwitz, A. F. (2002). *Nat. Cell Biol.* **4**, E97–E100.

Westermarck, J., and Kahari, V. M. (1999). *FASEb J.* **13**, 781–792.

Zhang, X. A., and Hemler, M. E. (1999). *J. Biol. Chem.* **274**0, 11–19.

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. U.S.A.* **96**, 14911–14918.