Volume 309, number 3, 358–362 FEBS 11534 September 1992

Molecular cloning and functional expression of the second mouse *nm*23/NDP kinase gene, *nm*23-M2

Takeshi Urano[a], Kogo Takamiya[a,b], Koichi Furukawa[a] and Hiroshi Shiku[a]

[a]*Department of Oncology* and [b]*First Department of Pathology, Nagasaki University School of Medicine, 12-4, Sakamoto-machi, Nagasaki 852, Japan*

Received 22 July 1992

A new murine cDNA of *nm*23/NDP kinase was isolated. A RT-PCR product was obtained from the normal mouse liver mRNA with primers designed for the human *nm*23-H2 gene. The product was used as a probe to screen a cDNA library from the murine melanoma cell line, B16, and two clones containing the entire open reading frame were obtained. It was predicted that the DNA sequence encoded 152 amino acids which was 98% identical to the *nm*23-H2 protein. The entire *nm*23-M1 and -M2 gene-coding regions were translated as fusion proteins with a glutathione *S*-transferase. These fusion proteins displayed NDP kinase activities.

*nm*23/nucleoside diphosphate kinase; Metastatic suppressor gene; *nm*23-M2 gene; Monoclonal antibody

1. INTRODUCTION

The *nm*23 gene, a potential suppressor of metastasis, was originally identified by differential hybridization between two murine melanoma sub-lines, one with a high and the other with a low metastatic capacity [1]. Highly metastatic sub-lines had a quantitative reduction in the *nm*23 gene mRNA when compared with less metastatic lines in several experimental tumor systems [1,2]. Subsequently, the deduced amino acid sequence of the product of the *nm*23 genes was found to share high homology with nucleoside diphosphate kinases (NDP kinase, EC 2.7.4.6) in a variety of species. Two polypeptides of human NDP kinase in erythrocytes have been sequenced [3]. The polypeptide chain, A, was identical to the *nm*23-H1 protein, a human homologue of the murine *nm*23 protein, whereas chain B was identical to the *nm*23-H2 protein, another human isotype of *nm*23. It has been claimed that the human NDP kinase is a hexameric enzyme consisting of these two polypeptides.

This evidence prompted us to look for a murine homologue of the *nm*23-H2 gene [4]. We isolated the cDNA of the *nm*23-M2, a new isoform of murine *nm*23, and determined the NDP kinase activity of its product. These two isoforms were also analyzed with the monoclonal antibody (mAb), M1-45, that is reactive with the *nm*23-M1 protein.

Correspondence address: H. Shiku, Department of Oncology, Nagasaki University School of Medicine, Nagasaki 852, Japan. Fax: (81) (958) 493 695.

Abbreviations: NDP kinase, nucleoside diphosphate kinase; mAb, monoclonal antibody; RT, reverse transcription; PCR, polymerase chain reaction; GST, glutathione *S*-transferase

2. MATERIALS AND METHODS

2.1. *RT-PCR*

Total RNA was extracted from the normal liver of C57BL/6 mice using guanidinium thiocyanate [5]. Single-stranded cDNA prepared from 3 μg of total RNA using Moloney murine leukemia virus reverse transcriptase (BRL) with an oligo(dT)$_{14}$ primer was used as a template for the polymerase chain reaction (PCR). The primers used for PCR of the whole *nm*23-M1 and *nm*23-M2 gene-coding regions were as follows: sense primers, M1-AX, 5'-GGGGATCCATGGCCAACAGTGAGC G-3'; M2-AX, 5'-GGGGATCCATGGCCAACC TCGAGCG-3' (each primer contained a *Bam*H1 site); antisense primers, M1-BX, 5'-GGGAATTCACTCATAGATCCAGTTC-3'; M2-BX, 5'-GGGAATTCTACTCGTACACCCAGTCA-3' (each primer contained an *Eco*R1 site). These primers and an oligo(dT)$_{14}$ primer were prepared using a 394 DNA synthesizer (Applied Biosystems). 35 cycles of denaturation (93°C, 1 min), annealing (50°C, 1.5 min), and extension (72°C, 1 min) were performed in a thermal cycler (Program Temp Control System PC-700, Astec Inc., Fukuoka, Japan). PCR products were analyzed by PAGE in 12% gels.

2.2. *Construction of plasmids pBSK-M2(H2), pBSK-M1, pBSK-M2, and DNA sequencing*

The PCR products were digested with *Bam*HI and *Eco*RI, separated by PAGE, purified by electroelution, and cloned into the *Bam*HI and *Eco*RI site of pBluescript II SK (Stratagene). DNA was sequenced using the Sequenase version 2.0 kit (USB) with [α-^{32}P]dCTP.

2.3. *Isolation of nm23-M2*

A λgt10 cDNA library made from the murine melanoma cell line, B16, was screened with the insert from pBSK-M2(H2). Two recombinant phages with overlapping restriction maps, mλ4-7 and mλ5-7, were isolated. A 0.8 kb *Eco*RI fragment of mλ5-7 was subcloned into pBluescript II SK (Stratagene). Double-stranded plasmid DNA was sequenced as described.

2.4. *Northern blotting*

Total RNA was prepared by acid guanidium thiocyanate/phenol/chloroform extraction [5]. Isolated RNA (10–20 μg) was electrophoretically separated on an 1% agarose gel containing formaldehyde and transferred to nylon membranes (GeneScreen plus, Du Pont).

Published by Elsevier Science Publishers B.V.

Hybridization and stringency of washing were performed according to the method recommended by the manufacturer. Probes were labeled with [α-^{32}P]dCTP using the multiprime labeling kit (Amersham). For hybridization studies, *Bam*HI–*Eco*RI fragments of pBSK-M1 and pBSK-M2 were used as probes.

2.5. *Construction of plasmid pGEX-M1 and pGEX-M2 for expression of nm23-M1 and nm23-M2 genes in E. coli*

Plasmids were constructed (pGEX-M1 and pGEX-M2) to express the *nm*23-M1 and *nm*23-M2 proteins fused with a 26 kDa glutathione *S*-transferase (GST) in *E. coli*, using the *Bam*HI–*Eco*RI fragments (463 bp) of pBSK-M1 and pBSK-M2. After digestion, the fragments were subcloned in the *Bam*HI and *Eco*RI site of pGEX2T (Pharmacia). DH5α *E. coli* bacteria were transformed with pGEX-M1 and pGEX-M2 or with pGEX2T as a control.

2.6. *Preparation and affinity purification of the bacterial extracts*

Bacterial extracts were prepared and purified essentially as described [6]. In brief, overnight cultures were diluted 1/10 (to 400 ml) in fresh medium and incubated for 2 h before addition of IPTG to a final concentration of 0.1 mM and a further 4 h incubation. The cells were then pelleted and resuspended in 10 ml MTPBS containing 1% Triton X-100. The cells were lysed on ice by mild sonication, then centrifuged at 10,000 × *g* for 5 min at 4°C. The supernatants were loaded onto a glutathione-Sepharose 4B column (Pharmacia). After washing the column twice with 5 bed vols. of MTPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4(pH 7.3)), the bound fractions were eluted with about 4 bed vols. of elution buffer (5 mM reduced glutathione (KOH)1N) in 50 mM Tris-HCl, pH 8.0). Protein purity was confirmed by SDS-PAGE and staining with Coomassie blue. The protein concentration was determined by the absorbance at 280 nm (A_{280} = 0.5 mg/ml).

2.7. *Assay for the phosphoenzyme formation of NDP kinase*

The reaction mixture (15 μl) contained standard buffer (20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.6 M KCl, 3 mM $CaCl_2$), 3 pM [γ-^{32}P]ATP and 0.1 μg of purified proteins. The mixture was incubated for 5 min in an ice bath and suspended in 15 μl of SDS sample buffer (125 mM Tris-HCl, pH 6.8, 20% glycerol, 4.6% SDS, 10% 2-mercaptoethanol, 0.2% Bromophenol blue). The mixture was boiled, then separated by SDS-PAGE (12%), followed by autoradiography.

2.8. *[^{32}P]GTP formation from the phosphoenzyme intermediates and GDP*

Phosphoenzyme intermediates (0.1 μg) were added to reaction mixtures (50 μl) containing 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 3 mM $CaCl_2$, 10 μM GDP and 0.1 M KCl. After the mixtures were incubated for 3 min in an ice bath, the enzyme reaction was arrested by the addition of 0.5 M EDTA (final concentration 30 mM). Aliquots of 5 μl were spotted onto PEI-cellulose F thin-layer plates (Merck). The [^{32}P]GTP formed was detected by development in 0.75 M KH_2PO_4 (pH 3.65) at room temperature, followed by autoradiography.

2.9. *Monoclonal antibody*

A rat was immunized three times with *nm*23-M1 fusion protein at two week intervals: the first time subcutaneously with 50 μg of protein and complete Freund's adjuvant, the second time subcutaneously with 100 μg of protein and incomplete Freund's adjuvant, and the third time intraperitoneally with 100 μg of fusion protein alone. Spleen cells were obtained from the rat and fused with the murine myeloma cell line NS-1. The hybridoma culture supernatants were assayed for reactivity with *nm*23-M1 protein using an enzyme-linked immunosorbent assay and immunoblotting. The limiting dilution of positive cultures was performed 3 times to obtain monoclonality. The mAb, M1–45, specific for the *nm*23-M1 protein, was obtained.

2.10. *Immunoprecipitation*

Cells were lysed in 0.01 M Tris buffer (pH 7.3), 0.15 M $MgCl_2$, 0.5% Nonidet P-40, 1 mM phenylmethylsulphonylfluoride. The lysates were cleared by centrifugation at 10,000 × *g* in a microcentrifuge, then incubated with the mAb, M1–45, for 1 h at room temperature. The mixture was incubated with rabbit anti-rat IgG (ZYMED) at room temperature for 1 h. The immunocomplex was precipitated by protein A-Sepharose (Pharmacia).

2.11. *Immunoblotting*

The proteins separated on 15% SDS-PAGE were electrophoretically transferred onto an Immobilon membrane (Millipore). The membrane was incubated with the mAb, M1–45, and bands were detected using a VECTASTAIN ABC kit (Vector Laboratories) according to the manufacturers' instructions. The proteins were visualized using an immunostaining HRP kit (Konica).

3. RESULTS

3.1. *Identification of the second mouse* nm23 *gene*

The previously reported murine *nm*23 gene shared 98% homology with human *nm*23-H1 and 88% with *nm*23-H2 [4]. PCR was therefore performed on the normal liver cDNA from C57BL/6 mice using primers for human *nm*23-H2, H2-AX and H2-BX [7]. We inserted this amplified product into the *Bam*HI–*Eco*RI site of pBluescript II SK, generating pBSK-M2(H2) and sequenced it. This product shared significant homology with *nm*23-H2 and was used as a probe to screen a λgt10 cDNA library made from the murine melanoma cell line, B16. Seven clones were isolated, two of which contained the entire open reading frame, including the poly(A) tail. The entire sequence of this gene, designated *nm*23-M2, is shown in Fig. 1. Northern blotting revealed that this gene encoded a 0.8 kb mRNA which was distinct from *nm*23-M1, which had a 1.2 as well as a 0.8 kb mRNA (Fig. 2).

The DNA sequence predicted a single open reading frame of 152 amino acids encoding a protein with a

```
ACCCACCGGCTTTCGGACCATGGCCAACCTCGAGCGTACCTTCATTGCCATCAAGCCAGA
                  M  A  N  L  E  R  T  F  I  A  I  K  P  D

TGGCGTGCAGCGCGGCCTGGTGGGCGAGATCATCAAACGGTTCGAGCAGAAGGGGTTCCG
 G  V  Q  R  G  L  V  G  E  I  I  K  R  F  E  Q  K  G  F  R

CCTGGTGGCCATGAAGTTCCTTCGGGCCTCTGAAGAACACCTGAAGCAGCATTACATCGA
 L  V  A  M  K  F  L  R  A  S  E  E  H  L  K  Q  H  Y  I  D

CCTGAAAGACCGTCCTTTCTTCCCGGGGCTGGTGAAGTACATGAACTCGGGGCCCGTGGT
 L  K  D  R  P  F  F  P  G  L  V  K  Y  M  N  S  G  P  V  V

GGCCATGGTCTGGGAGGGGCTCAATGTGGTGAAAACGGGCCGAGTGATGCTGGGGGAGAC
 A  M  V  W  E  G  L  N  V  V  K  T  G  R  V  M  L  G  E  T

CAATCCAGCTGATTCAAAACCAGGCACCATCCGTGGGGATTTCTGCATTCAAGTTGGCAG
 N  P  A  D  S  K  P  G  T  I  R  G  D  F  C  I  Q  V  G  R

GAACATCATTCATGGCAGTGATTCAGTGGAGAGTGCTGAGAAAGAGATCCATCTGTGGTT
 N  I  I  H  G  S  D  S  V  E  S  A  E  K  E  I  H  L  W  F

TAAGCCCGAAGAACTGATCGACTACAAGTCTTGTGCCCATGACTGGGTGTACGAGTAGAC
 K  P  E  E  L  I  D  Y  K  S  C  A  H  D  W  V  Y  E

ATGAAGAAACCAGAATCCTTTTCAGCACTACTGATGGGTTTCTGGACAGAGCTCTTCATC

CCACTGACAAGATGGATCATCTTTTCTAAAACAATAAAGACTTTGGAACTGAAAAAAAAA

AAAAAAAAAAA
```

Fig. 1. Nucleotide sequence and deduced amino acid sequence of *nm*23-M2 cDNA. The polyadenylation signal (AATAAA) is underlined.

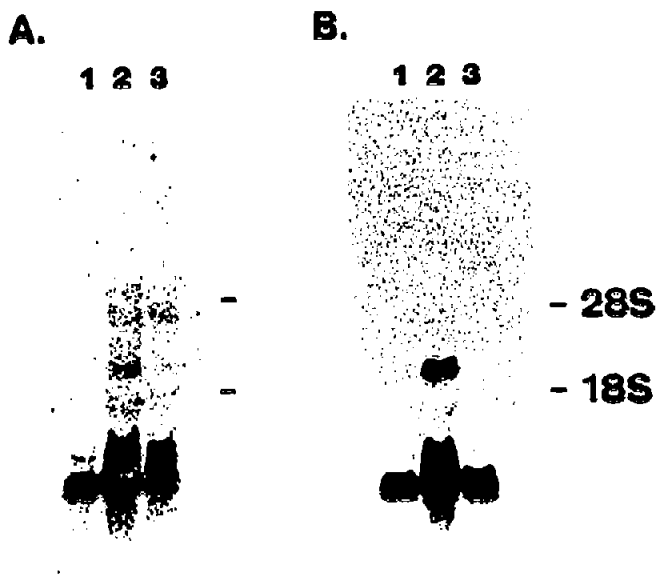

Fig. 2. Expression of *nm*23-M1 and -M2 mRNA. Northern blotting of the murine myeloma cell line, NS-1 (lane 1), the melanoma cell line, B16 (lane 2), and the 3-methylcholanthrene-induced fibrosarcoma cell line, CMS7 (lane 3), using probes of the *Bam*HI–*Eco*RI fragment of the pBSK-M1 (A) or the pBSK-M2 vector (B). The positions of 18 S and 28 S rRNA are indicated on the right.

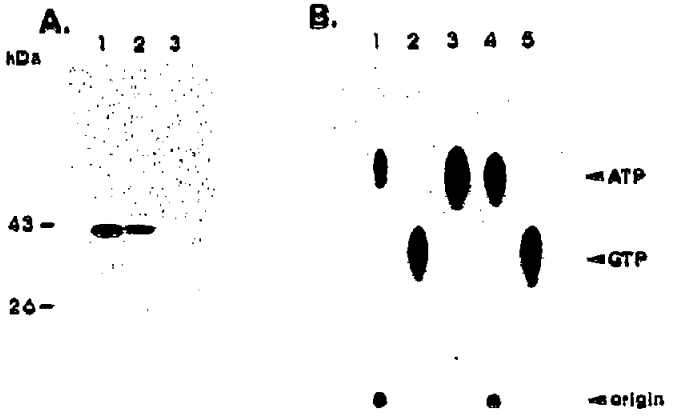

Fig. 3. NDP kinase activity of the *nm*23 proteins. (A) Detection of the phosphoenzyme intermediates by autoradiography. The purified proteins used for phosphoenzyme formation were *nm*23-M1 (lane 1), *nm*23-M2 (lane 2) and GST (lane 3). (B) [^{32}P]GTP formation from the phosphoenzyme intermediate and cold GDP; (lane 1) *nm*23-M1 phosphoenzyme intermediate alone; (lane 2) *nm*23-M1 phosphoenzyme intermediate incubated with cold GDP; (lane 3) [γ-^{32}P]ATP incubated with cold GDP without protein; (lane 4), *nm*23-M2 phosphoenzyme intermediate alone; (lane 5), *nm*23-M2 phosphoenzyme intermediate incubated with cold GDP. The migration of ATP and GTP is indicated on the right.

predicted molecular mass of 17.3 kDa. The deduced protein was 98% identical to the *nm*23-H2 protein.

3.2. *Production of the* nm*23-M1 and* nm*23-M2 proteins in* E.coli *and their NDP kinase activities*

The *Bam*HI–*Eco*RI fragments of pBSK-M1 and pBSK-M2 were then cloned into the *Bam*HI and *Eco*RI site of the pGEX2T expression vector generating pGEX-M1 and pGEX-M2, respectively. The entire *nm*23-M1 and *nm*23-M2 gene-coding regions were translated as fusion proteins with a 26 kDa GST. Both proteins were digested with thrombin at 25°C for 30 min, fractionated by SDS-PAGE and stained with Coomassie blue. *nm*23-M1 and -M2 proteins migrated at 17 and 17.5 kDa, respectively (Fig. 4a).

The affinity-purified fusion proteins (both *nm*23-M1 and *nm*23-M2) formed phosphate-incorporating proteins (phosphoenzyme intermediates) when incubated with [γ-^{32}P]ATP under standard conditions (Fig. 3a).

Thin-layer chromatography followed by autoradiography showed that [^{32}P]GTP was formed from the phosphoenzyme intermediates of both *nm*23-M1 and *nm*23-M2 proteins and cold GDP (Fig. 3b). Also, [^{32}P]GTP was formed from [γ-^{32}P]ATP and cold GDP by both *nm*23-M1 and *nm*23-M2 fusion proteins (data not shown).

3.3. *Reactivity with the mAb, M1–45, specific for* nm*23-M1 protein*

We generated the mAb, M1–45, by immunizing a rat with the *nm*23-M1 fusion protein, as described in Materials and Methods. The mAb, M1–45, was reactive with *nm*23-M1 protein but not with *nm*23-M2 protein by immunoblotting (Fig. 4b). The mAb, M1–45, however, immunoprecipitated two bands from NS-1 lysates, one corresponding to the *nm*23-M1 protein and the other to the *nm*23-M2 protein (Fig. 4a). Both proteins precipitated from NS-1 lysates by mAb M1–45 showed NDP kinase activity (Fig. 4c).

4. DISCUSSION

The new murine cDNA isolated in this study is highly homologous to the mammalian *nm*23/NDP kinase cDNAs which have been previously identified. The deduced amino acid sequence of this gene has 88.2, 88.2, 98 and 99.3% identity with *nm*23-M1, -H1, -H2, and rat NDP kinase, respectively [1,4,8]. It also shows an apparent homology with other NDP kinases from non-mammalian species; 77.5, 46.8, 44.9 and 64.7% identity on an amino acid basis with NDP kinases of *Drosophila melanogaster*, *Myxococcus xanthus*, *E. coli* and *Dictyostelium discoideum*, respectively [9–12]. In particular, the high homology of this cDNA with that of human *nm*23-H2 indicates that it is another isotype of murine *nm*23 cDNA corresponding to the *nm*23-H2 cDNA in human. It is therefore very likely that both mouse and human

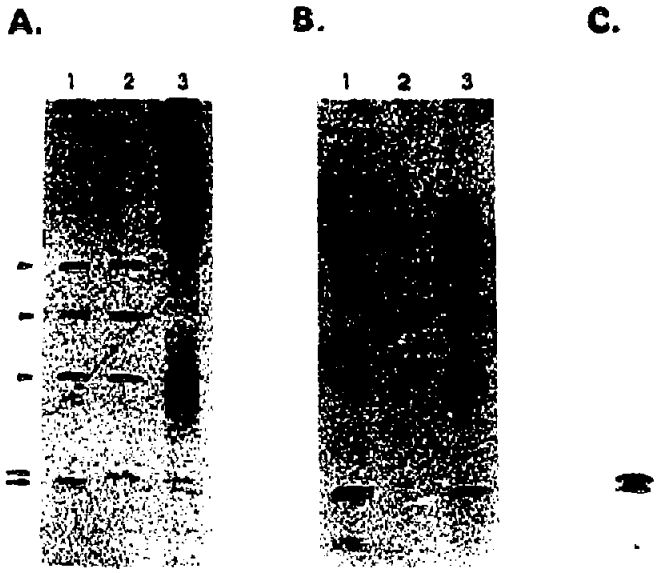

Fig. 4. Analysis of *nm*23-M1 and -M2 proteins with mAb M1-45. (A) NS-1 lysate was immunoprecipitated by the mAb, M1-45, specific for *nm*23-M1 protein, as described in Materials and Methods. Samples were fractionated by SDS-PAGE (15%) and stained with Coomassie blue: (lanes 1 and 2) thrombin-digested *nm*23-M1 and -M2 proteins, respectively; (lane 3) immunoprecipitates from NS-1 lysate with the mAb, M1-45. the mAb, M1-45, precipitated two molecules corresponding to *nm*23-M1 and -M2 proteins. Arrows indicate the position of the 17- (*nm*23-M1) and 17.5 kDa (*nm*23-M2) proteins. Arrowheads indicate the positions of the fusion proteins, thrombin and GST. (B) Immunoblotting of samples described in (A) by the mAb, M1-45, lanes 1-3 are as described in A. The mAb, M1-45, detected only *nm*23-M1 protein. (C) Detection of the phosphoenzyme intermediates by autoradiography. The immunoprecipitate from NS-1 lysate with the mAb, M1-45, was analyzed for phosphoenzyme formation as described in Materials and Methods. Both *nm*23-M1 and -M2 proteins formed phosphoenzyme intermediates.

have two isotypes of *nm*23/NDP kinase with high mutual homology, as previously suggested.

We have prepared both *nm*23-M1 and -M2 proteins as fusion proteins with a 26 kDa GST. Both formed phosphoenzyme intermediates and also GTP from GDP and [γ-^{32}P]ATP. We previously reported that, using the same approach, similar NDP kinase activity of *nm*23-H1 and -H2 proteins could be demonstrated [7]. Gilles et al. [3] reported that human NDP kinase is a hexameric molecule consisting of randomly associated *nm*23-H1 and -H2 proteins in various proportions. These hexameric forms of both proteins display NDP kinase activity. Our results, however, clearly indicate that all of these molecules also have NDP kinase activity as a monomeric peptide. The mAb, M1-45, which is reactive with *nm*23-M1 but not *nm*23-M2 protein by immunoblotting, consistently precipitated two bands with NDP kinase activity in the immunoprecipitation from NS-1 lysates. The precipitated bands were precisely coincident by SDS-PAGE analysis with the *nm*23 proteins produced in *E. coli*, which indicated that they are *nm*23-M1 and -M2.

The possibility that the mAb, M1-45, was reactive with *nm*23-M1 as well as *nm*23-M2 proteins in the immunoprecipitation cannot yet be neglected. A similar analysis of human *nm*23 proteins with a mAb specific for *nm*23-H1 or -H2 proteins, however, also constantly co-precipitated the partner isotype of *nm*23 protein (manuscript in preparation). Thus, murine and human *nm*23-NDP kinase associate with their partner isotypes in a very similar manner, although their precise molecular basis remains unknown.

All previously identified *nm*23-NDP kinases, except that in bacteria, share the tripeptide, Arg-Gly-Asp, which is the RGD consensus sequence for the recognition of integrin family members [13], as shown in Fig. 5. The *nm*23-M2 protein also has this tripeptide sequence. It is intriguing to speculate that *nm*3-NDP kinase functions as a ligand for an adhesive molecule of the integrin family. During the preparation of this manuscript, Okabe et al. [14] reported the partial amino acid sequence of the I-factor, which inhibited the induction of differentiation of the murine myeloid leukemia cell line, M1. Since the cyanogen bromide fragments of the I-factor exhibited 98% amino acid identity with *nm*23-H2, 91% to murine *nm*23 and 89% to *nm*23-H1, they anticipated that the I-factor is a putative murine *nm*23. In fact, the reported sequence is identical to our current sequence of *nm*23- M2 except for one at codon 69. It is most likely that the I-factor is indeed *nm*23-M2. The I-factor is detectable in the membrane fraction as well as in the conditioned medium of M1 cells. The I-factor activity in culture medium and the presence of the RGD consensus sequence of *nm*23-M2 may reveal a totally new extracellular role of *nm*23-NDP kinase in addition to the several distinct intracellular functions so far pro-

*nm*23-M2	91	LGETNPADSK	PGTIRGDFCI	QVGRNIIHGS	120
*nm*23-H2	91	----------	----------	----------	120
NDPK, rat	91	----------	----------	----------	120
*nm*23-M1	91	----------	----------	----------	120
*nm*23-H1	91	----------	----------	----------	120
awd	92	--A------L	----------	----------	121
NDPK, *Dictyostelium*	95	I-V---LA-A	--S-----GV	D---------	124
NDPK, *M. xanthus*	90	M-A----QAA	E----K--AT	SIDK-TV---	119
NDPK, *E. coli*	90	--A----NAL	A--L-A-YAD	SLTE-GT---	119

Fig. 5. Conservation of the RGD consensus sequence in NDP kinases from different species. Amino acid residues are listed using the single letter code. The dashes in the sequence are identical to *nm*23-M2. The stippled box indicates the RGD consensus sequence.

posed. It may also provide a direct interpretation of the participation of these proteins in the control of tumor metastasis.

Acknowledgements: We thank M. Taniguchi for kindly providing a λgt10 cDNA library made from the murine melanoma cell line, B16, and H. Yamaguchi for technical support. This work was supported by a Grant-in-Aid for Special Project Research on Cancer Bio-Science from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] 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.
[2] Steeg, P.S., Bevilacqua, G., Pozzatti, R., Liotta, L.A. and Sobel, M.E. (1988) Cancer Res. 48, 6550–6554.
[3] Gilles, A.-M., Presecan, E., Vonica, A. and Lascu, I. (1991) J. Biol. Chem. 266, 8784–8789.
[4] Stahl, J.A., Leone, A., Rosengard, A.M., Porter, L., King, C.R. and Steeg, P.S. (1991) Cancer Res. 51, 445–449.
[5] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[6] Smith, D.B. and Johnson, K.S. (1988) Gene 67, 31–40.
[7] Urano, T., Fushida, S., Furukawa, K. and Shiku, H. (1992) Int. J. Oncol. (in press).
[8] Kimura, N., Shimada, N., Nomura, K. and Watanabe, K. (1990) J. Biol. Chem. 265, 15744–15749.
[9] Biggs, J., Tripoulas, N., Hersperger, E., Dearolf, C. and Shearn, A. (1988) Genes Dev. 2, 1333–1343.
[10] Muñoz-Dorado, J., Inouye, M. and Inouye, S. (1990) J. Biol. Chem. 265, 2702–2706.
[11] Hama, H., Almaula, N., Lerner, C.G., Inouye, S. and Inouye, M. (1991) Gene 105, 31–36.
[12] Lacombe, M.-L., Wallet, V., Troll, H. and Véron, M. (1990) J. Biol. Chem. 265, 10012–10018.
[13] Hynes, R.O. (1992) Cell 69, 11–25.
[14] Okabe-Kado, J., Kasukabe, T., Honma, Y., Hayashi, M., Henzel, W.J. and Hozumi, M. (1992) Biochem. Biophys. Res. Commun. 182, 987–994.