

Inhibition of Progesterone-induced *Xenopus* Oocyte Maturation by Nm23¹

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Abstract

The Nm23 protein has been implicated in a wide variety of biological processes, including suppression of metastasis, phytochrome responses in plants, and regulation of differentiation. Here we examine whether Nm23 is involved in *Xenopus laevis* oocyte maturation. We found that Nm23 is present in oocytes, indicating that it has the potential to be a regulator of maturation. Furthermore, modest overexpression of Nm23 inhibited progesterone-induced oocyte maturation. This maturation-inhibitory activity was shared by both the acidic Nm23-H1 isoform and the basic Nm23-H2 isoform and by Nm23 mutants that lack nucleoside diphosphate kinase activity (Nm23-H1 H118F and Nm23-H2 H118F). Expression of Nm23 proteins delayed the accumulation of Mos and the activation of p42 mitogen-activated protein kinase (MAPK) in progesterone-treated oocytes but had no discernible effect on Mos-induced p42 MAPK activation. Therefore, Nm23 appears to act upstream of the Mos/mitogen-activated protein/extracellular signal-regulated kinase kinase/p42 MAPK cascade. These findings suggest a novel biological role for Nm23.

Introduction

Nm23 was originally identified as a possible metastasis suppressor gene through the discovery that its expression is associated with low metastatic potential murine melanoma cell lines (1, 2). Nm23 is a member of a gene family that is highly conserved in both prokaryotes and in eukaryotes. Six human Nm23 family members have been identified: (a)

Nm23-H1 (3); (b) Nm23-H2 (4); (c) DR-nm23 (5); (d) Nm23-H4 (6); (e) Nm23-H5 (7); and (f) Nm23-H6 (8).

Several lines of evidence suggest that Nm23 has a role in regulating differentiation and development. Nm23 is a homologue of the *Drosophila awd* (abnormal wing disc) gene (9), and mutations in *awd* are associated with a variety of developmental defects. During mouse embryonic development, the accumulation of Nm23 protein coincides with differentiation of the embryonic nervous system, heart, and various epithelial tissues during mouse organogenesis (10), again pointing to a possible role in development. The identification of a differentiation-inhibiting factor (denoted "I factor") in cell lysates and conditioned media from mouse myeloid leukemia cells as a member of the Nm23 family suggests a role for Nm23 in suppressing differentiation (11). Nm23 expression decreases during the differentiation of hematopoietic cells (12), again suggesting a role as an antidifferentiation factor. In other contexts, Nm23 may promote differentiation. For example, overexpression of Nm23 promotes neurite outgrowth in PC12 cells (13, 14).

The best-characterized Nm23 proteins exist as homo- or hetero-hexamers of two 152-amino acid isoforms, the acidic Nm23-H1 protein and the basic Nm23-H2 protein (15). These and other Nm23 proteins possess NDP³ kinase activity, catalyzing the transfer of the terminal phosphate of nucleoside triphosphates to NDPs. In some contexts, the NDP kinase activity of Nm23 appears to be critical for its biological effects. For example, whereas wild-type Nm23 promotes neurite outgrowth, a point mutant of Nm23 (H118A) that lacks NDP kinase activity does not promote neurite outgrowth and, in fact, suppresses nerve growth factor-induced neurite outgrowth (13, 14).

However, in other contexts, the NDP kinase activity of Nm23 appears to be unimportant. Mutated Nm23 proteins lacking NDP kinase activity still score as the differentiation-inhibiting "I factor" mentioned above (16). In addition, Nm23-H2 can bind to a nuclease hypersensitive element on the human *c-myc* promoter and transactivate gene expression *in vitro* and *in vivo* (17, 18), and this transactivation is independent of NDP kinase activity (19). Thus, some Nm23 functions appear to be independent of its NDP kinase activity. Nm23 proteins also function as histidine/aspartic acid-specific protein kinases (20) and as serine/threonine-specific protein kinases (21) and have been proposed to link receptors to G proteins (22). However, it is not yet clear how relevant any of these activities are for the biological effects of Nm23, nor is it clear where Nm23 exerts its effects. It may act in the nucleus (as

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³ The abbreviations used are: NDP, nucleoside diphosphate; GVBD, germinal vesicle breakdown; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein/extracellular signal-regulated kinase; cAMP, cyclic AMP.

suggested by its identification as a *myc* promoter-binding protein), in the cytoplasm, or outside the cell (as suggested by its ability to inhibit differentiation when applied to cells).

We have been studying the biochemical pathways that regulate *Xenopus* oocyte maturation. This process is interesting from a developmental perspective because maturation is the final, rate-limiting step in the production of a fertilizable egg. The study of oocyte maturation has also yielded important insights into cell cycle regulation, signal transduction cascades, and translational control. Fully grown stage VI *Xenopus* oocytes are naturally arrested at the G₂-M-phase transition of meiosis I. Release from the G₂-like arrest is stimulated by progesterone, which initiates a series of events that culminates in GVBD (or nuclear envelope breakdown) and the appearance of a white spot on animal pole of the oocyte (reviewed in Ref. 23). Early after progesterone exposure, there is a decrease in oocyte cAMP levels and inhibition of adenylyl cyclase and protein kinase A. After a lag of a few hours, polyadenylation of Mos mRNA occurs, resulting in an increase in the translation of Mos. Synthesis of Mos leads to the activation of MEK and p42/extracellular signal-regulated kinase 2 MAPK, which facilitates the activation of maturation-promoting factor, a protein kinase composed of Cdc2 and cyclin B. Together, p42 MAPK and Cdc2/cyclin B trigger the dramatic cell biological changes of oocyte maturation.

Here we examine whether the ectopic expression of Nm23 proteins can facilitate or inhibit oocyte maturation. We found that expression of modest concentrations of Nm23-H1 or Nm23-H2 (to ~3-fold the endogenous Nm23 level) is sufficient to substantially inhibit progesterone-induced oocyte maturation. Catalytically inactive Nm23 proteins also inhibited maturation, indicating that this effect does not depend on the NDP kinase activity of Nm23. Nm23 appears to exert its effect upstream of the Mos/MEK/MAPK cascade because it inhibited progesterone-induced Mos accumulation but did not measurably inhibit Mos-induced maturation. These findings show that Nm23 proteins have the potential to be maturation-inhibiting factors.

Results

Oocytes Constitutively Express a Nm23-like Protein. To determine whether Nm23 might be involved in the early development of *Xenopus laevis*, we first examined whether Nm23 proteins are present in *Xenopus* oocytes and eggs. Three *Xenopus* Nm23 cDNAs (X1, X2, and X3) have been identified and shown to be 82–87% identical to their human counterparts (24). We used antihuman Nm23 antibodies to look for a cross-reacting Nm23 protein in *Xenopus* oocytes. A putative Nm23 band with apparent molecular weight of M_r 24,000 was seen by immunoblotting (Fig. 1A). Its identification was supported by its binding to ATP-Sepharose, a characteristic of Nm23 proteins (25). There was no change in the intensity or apparent molecular weight of the endogenous Nm23 band during progesterone-induced oocyte maturation.

Overexpression of Wild-Type and Catalytically Inactive Mutant Nm23 Delays Progesterone-induced GVBD. Next we carried out microinjection studies to determine whether

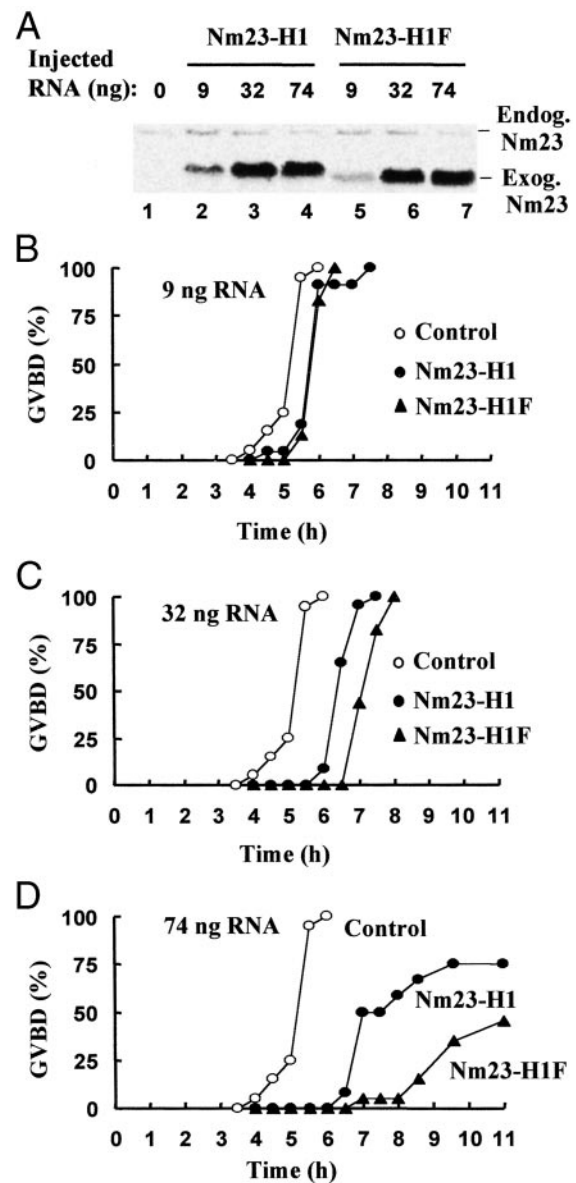


Fig. 1. Effects of Nm23-H1 and Nm23-H1F on maturation. A, Nm23 immunoblot showing the endogenous M_r ~24,000 Nm23 protein and the ectopic M_r ~19,000 human Nm23 proteins. Oocytes were microinjected with water (Lane 1) or synthetic mRNAs for Nm23-H1 (Lanes 2–4) or Nm23-H1F (Lanes 5–7). Oocytes were incubated for 20–24 h in the absence of progesterone to allow full expression of Nm23 and then incubated for 10 h in the presence of progesterone. B–D, time course of maturation in oocytes microinjected with water (Control, ○) or mRNAs for Nm23-H1 (●) or Nm23-H1F (▲). The amount of RNA injected ranged from 9–74 ng/oocyte, as indicated. GVBD was scored by the appearance of a white dot at the oocyte's animal pole. Ten to 20 oocytes were followed in each experimental group. Data shown are from one of two similar experiments.

Nm23 was a positive or negative regulator of progesterone-induced oocyte maturation. Immature oocytes were microinjected with various amounts of wild-type Nm23-H1 or catalytically inactive Nm23-H1F mRNA and incubated for 24 h to allow the Nm23 proteins to be expressed. The Nm23 mRNA did not cause the oocytes to mature and had no

obvious effect on oocyte viability. However, Nm23 expression did have a dramatic effect on the oocytes' responses to progesterone (5 $\mu\text{g}/\text{ml}$). As shown in Fig. 1, B–D, both Nm23-H1 and Nm23-H1F caused a dramatic, dose-dependent delay in progesterone-induced maturation. The delay was detectable at the lowest doses of RNA used (Fig. 1B) and increased with increasing RNA doses (Fig. 1, C and D). The catalytically inactive Nm23-H1F protein was more effective, not less effective, than wild-type Nm23-H1 (especially in Fig. 1, C and D). Therefore, the maturation-delaying effect of Nm23-H1 does not depend on the NDP kinase activity of Nm23.

Fig. 1A shows the final levels of Nm23-H1 and Nm23-H1F after 34 h of expression (24 h in the absence of progesterone followed by 10 h in the presence of progesterone). The lowest amount of injected RNA (9 ng/oocyte) resulted in an exogenous Nm23 band about three times the intensity of the endogenous Nm23 band (Fig. 1A). Thus, a relatively modest level of Nm23 overexpression produced a measurable effect on the kinetics of maturation (Fig. 1B), suggesting that the endogenous Nm23 protein may also function to restrain or oppose maturation. Higher amounts of Nm23 RNA produced higher levels of Nm23 protein (Fig. 1A) and had more dramatic effects on the timing of maturation (Fig. 1, C and D). Comparable concentrations (50 ng) of RNA for an irrelevant protein (catalytically inactive Xmkp-1) had no detectable effect on maturation,⁴ arguing that the inhibition of progesterone-induced maturation is a Nm23-specific effect.

Next we compared the basic Nm23-H2 and Nm23-H2F proteins with the acidic Nm23-H1 and Nm23-H1F proteins in terms of their effects on maturation. Oocytes were microinjected with 50 ng of mRNA for one of the four Nm23 proteins. About 24 h after injection, when the accumulation of Nm23 protein reached maximum levels, half of the microinjected oocytes were placed in 5 $\mu\text{g}/\text{ml}$ progesterone. As shown in Fig. 2, the H2 and H2F forms of Nm23 were similar to the H1 and H1F forms in their ability to delay progesterone-induced maturation (compare Fig. 2, A and B). Once again, the catalytically inactive form (Nm23-H2F) had a stronger inhibitory effect than the active NDP kinase (Nm23-H2; Fig. 2, A and B).

The concentration of progesterone used in the previous experiments (5 $\mu\text{g}/\text{ml}$) is sufficient to induce maximal maturation in most batches of oocytes. We also wished to determine the effect of Nm23 expression on maturation induced by lower concentrations of progesterone. Oocytes were injected with 50 ng of Nm23-H1 or Nm23-H1F mRNA or with water and treated with 5, 0.5, 0.1, and 0.01 $\mu\text{g}/\text{ml}$ progesterone, in which concentration 100% of control oocytes could be matured. As shown in Fig. 3, the inhibitory effect of the Nm23 mRNAs was more marked when lower doses of progesterone were used.

Nm23 Acts Upstream of Mos. Progesterone causes a rapid, transient decrease in cAMP levels in oocytes. This is followed by a lag period of a few hours that culminates in the polyadenylation and translation of the Mos message. Mos, in

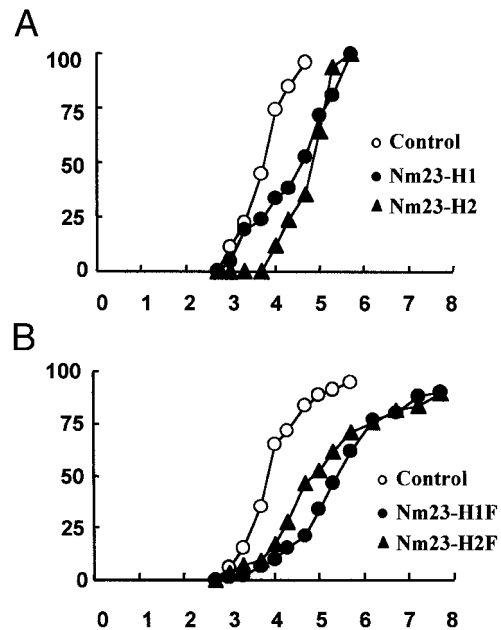


Fig. 2. The effects of acidic (Nm23-H1 and Nm23-H1F) and basic (Nm23-H2 and Nm23-H2F) isoforms of Nm23 on progesterone-induced GVBD. Oocytes were microinjected with mRNA (50 ng/oocyte) for Nm23-H1 (A, ●), Nm23-H2 (A, ▲), Nm23-H1F (B, ●), or Nm23-H2F (B, ▲) or microinjected with water (A and B, Control, ○). Oocytes were incubated for 20 or 24 h for full expression Nm23 and then stimulated with 5 $\mu\text{g}/\text{ml}$ progesterone. At least 20 microinjected oocytes were followed for each experimental group, and the results were repeated.

turn, activates the MAPK cascade proteins MEK1 and p42 MAPK, which brings about the activation of Cdc2. We wished to determine whether Nm23 inhibited maturation by delaying the synthesis of Mos, by inhibiting the activation of the MAPK cascade by Mos, or by inhibiting steps further downstream.

Accordingly, we examined the effect of Nm23 overexpression on the time course of Mos accumulation and p42 MAPK phosphorylation in progesterone-treated oocytes. Oocytes were microinjected with water or with Nm23-H1 and Nm23-H1F mRNAs (50 ng) and incubated for 20–24 h. As shown in Fig. 4, both Nm23-H1 and Nm23-H1F delayed the phosphorylation of p42 MAPK (Fig. 4A) and the accumulation of Mos (Fig. 4B). These findings suggest that Nm23 exerts its effects upstream of the Mos/MEK/p42 MAPK cascade.

In the same experiment, we also examined the effect of Nm23 overexpression on Mos-induced oocyte maturation. Control oocytes and Nm23-expressing oocytes were microinjected with purified malE-Mos protein (a fusion protein consisting of maltose-binding protein and *Xenopus* Mos; final concentration, 180 nM), and the time course of p42 MAPK phosphorylation was monitored. As shown in Fig. 4C, the time course of p42 MAPK phosphorylation was similar in all three sets of oocytes. These findings are again consistent with the hypothesis that Nm23 exerts its effects on oocyte maturation upstream of the Mos/MEK/p42 MAPK cascade.

⁴ M. L. Sohaskey, personal communication.

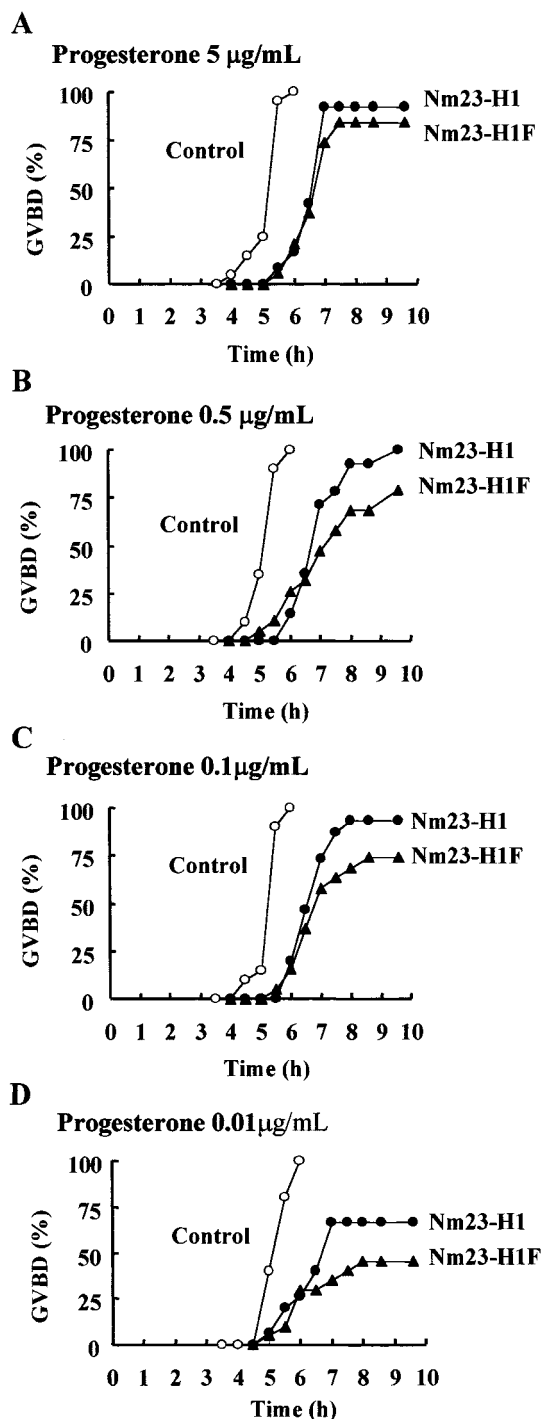


Fig. 3. The effects of Nm23-H1 and Nm23-H1F expression on maturation induced by various concentrations of progesterone. Oocytes were microinjected with water (Control, ○) or with Nm23-H1 (●) or Nm23-H1F (▲) mRNA (50 ng/oocyte) and incubated for 20–24 h. The oocytes were then incubated with (A) 5, (B) 0.5, (C) 0.1, and (D) 0.01 µg/ml progesterone as indicated and scored for GVBD. Data shown are from one of two similar experiments.

Discussion

Here we demonstrate that human Nm23 can influence the course of *Xenopus* oocyte maturation; overexpression of

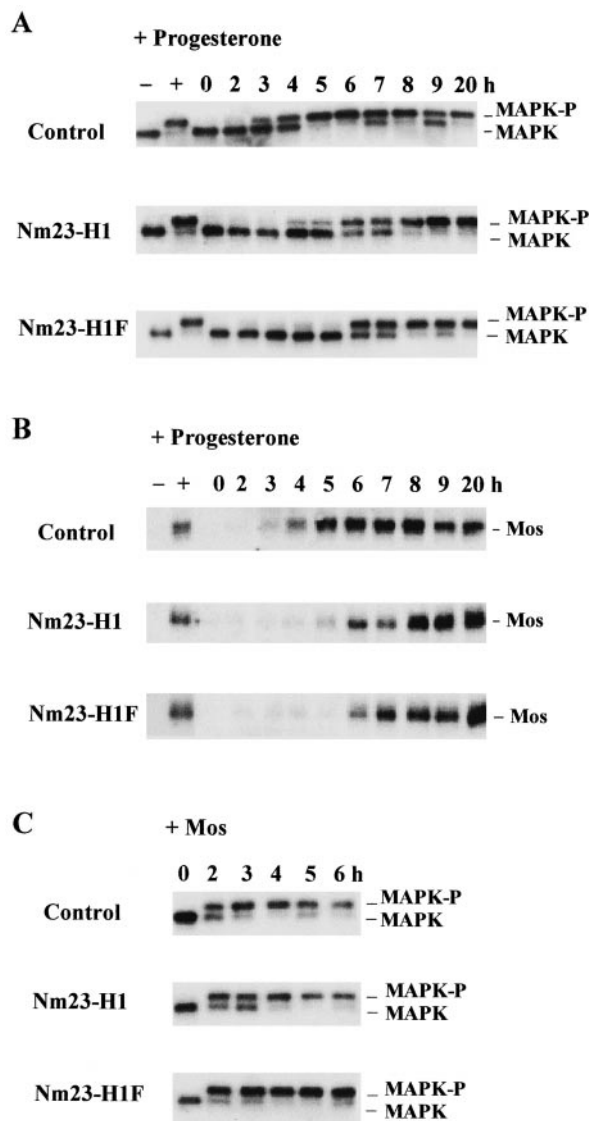


Fig. 4. Effects of Nm23-H1 expression on progesterone-induced p42 MAPK phosphorylation (A) and Mos accumulation (B) and on Mos-induced p42 MAPK phosphorylation (C). p42 MAPK (A) and Mos (B) immunoblots for progesterone-treated oocytes are shown. Oocytes were microinjected with 50 ng of mRNA encoding Nm23-H1 or Nm23-H1F or with water and incubated for 20 or 24 h. Oocytes were then incubated with progesterone (0.1 µg/ml) for the lengths of time indicated. Five oocytes were randomly collected, pooled, and lysed for each time point. The upper bands in A represent phosphorylated p42 MAPK. The first two lanes in A and B are samples of immature oocytes (-) and mature oocytes (+) run as standards. The remaining lanes are samples of five oocytes collected at various times after progesterone treatment and then pooled and lysed. C, p42 MAPK immunoblots of Mos-injected oocytes. Oocytes were microinjected with 50 ng of mRNA encoding Nm23-H1 or Nm23-H1F or with water and incubated for 20 or 24 h. Oocytes were then microinjected with purified recombinant Mos protein (final concentration, 180 nM) and incubated for the lengths of time indicated. Samples of five oocytes were pooled and lysed for each time point.

Nm23-H1 or Nm23-H2 delays progesterone-induced GVBD. The effect depended on the amount of Nm23 mRNA microinjected and the concentration of progesterone used to initiate maturation. Substantial delays were seen under condi-

tions in which Nm23 was only modestly overexpressed, suggesting that the endogenous Nm23 protein may also act to oppose or restrain oocyte maturation.

The enzymatically inactive mutants Nm23-H1F and Nm23-H2F were found to inhibit progesterone-induced maturation even more effectively than the wild-type proteins. This finding indicates that NDP kinase activity is not required for Nm23 to negatively regulate progesterone-induced oocyte maturation. Kinase-independent effects of Nm23 have been found in other contexts as well (16, 19). However, in some contexts, the NDP kinase activity of Nm23 does appear to be critical for biological function. For example, neurite outgrowth in PC12 cells is promoted by Nm23, but not by kinase-inactive forms of Nm23 (14). The present results suggest that the mutants may mimic the active rather than inactive conformation of the wild-type proteins in some respects. For example, the mutants might more readily undergo serine phosphorylation than the wild-type proteins. This hypothesis awaits further investigation.

Two lines of evidence indicate that Nm23 acts upstream of Mos to inhibit oocyte maturation: (a) Nm23 expression delays the accumulation of Mos; and (b) Nm23 does not affect Mos-induced p42 MAPK activation. Although the signal transduction pathways upstream of Mos are still poorly understood, previous work suggests two points in the process where Nm23 might exert its antimaturation effect. Nm23 can interact physically with the G_s protein (26, 27) and has been proposed to participate in G protein activation (22). Thus, Nm23 might increase basal G_s activity and cAMP levels in the oocyte, accounting for its ability to inhibit progesterone-induced maturation. Nm23 has also been proposed to inhibit polyadenylation of its own message by binding to sequences similar to the *c-myc* nuclease hypersensitive element in its 3'-untranslated region (24). Cytoplasmic polyadenylation of Mos and other mRNAs is critical for progesterone-induced oocyte maturation (28–31). Thus, Nm23 might inhibit maturation by interfering with polyadenylation. Studies are under way in our laboratory to test these hypotheses. In addition, the identification of Nm23-interacting proteins may provide clues as to how Nm23 impinges on cell signaling in *Xenopus* oocytes and in other systems as well.

Materials and Methods

Materials. Plasmids containing wild-type Nm23 were provided by Dr. P. S. Steeg [National Cancer Institute, Bethesda, MD (21, 32)], and mutants were provided by Dr. H. S. Lee (Kangwon National University, Chuncheon, Korea). The *Xenopus* expression vector pRD67 (33), which is derived from pST64T and pcDNA, was a kind gift from Dr. J. H. Kim (Paichai University, Taejon, Korea). For expression of Nm23 isoforms in *Xenopus* oocytes, the *EcoRI*-*SalI* fragments from pT9H1 and pT9H2 (34) were inserted into the *EcoRI* and *XhoI* sites of pRD67. The Nm23-H1 H118F (Nm23-H1F) and Nm23-H2 H118F (Nm23-H2F) mutants were inserted into pRD67 in a similar way and confirmed by sequencing. A fusion protein consisting of maltose-binding protein and *Xenopus* Mos fusion (malE-Mos) was expressed in bacteria from a plasmid provided by Dr. G. Vande Woude (Frederick Cancer Research and Development Center, Frederick, MD) and purified as described previously (35). Nm23 antibody was prepared using recombinant Nm23 expressed in *Escherichia coli* and purified by ATP affinity chromatography (25).

Oocyte Manipulation. Female *Xenopus laevis* were injected with 67 units of pregnant mare serum gonadotropin (Sigma) 1–2 weeks before surgery to stimulate development of stage VI oocytes. Pieces of ovary

were surgically removed from frogs anesthetized with tricaine (Sigma). Fully grown stage VI *Xenopus* oocytes were sorted manually after defolliculation of the oocyte for 1–1.5 h with 2 mg/ml collagenase (Sigma) in calcium-free modified Barth's solution [88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 10 mM HEPES (pH 7.5)]. The oocytes were washed several times with modified Barth's solution and then allowed to recover overnight in OR2 solution [82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM Na_2HPO_4 , and 5 mM HEPES (pH 7.5)] supplemented with 1 mg/ml BSA and 50 μ g/ml gentamicin.

In Vitro Transcription and Microinjection. Nm23 mRNA was prepared using an *in vitro* SP6 transcription kit (Ambion) and stored in aliquots at $-80^\circ C$. Oocytes were microinjected with 50 nl of mRNA (50 ng) around the equator of the oocyte, transferred, and incubated for 1 day to allow Nm23 protein accumulation to reach maximal levels. Meiotic maturation was induced by treating oocytes with 0.1–5 μ g/ml progesterone (Sigma), and GVBD was assessed after various incubation times. In some experiments, Nm23-expressing oocytes were subjected to a second microinjection with malE-Mos protein (final concentration, 180 nM) to induce maturation.

Oocyte Lysis and Immunoblotting. Oocytes were lysed by pipetting through a 200- μ l pipette tip in 60 μ l of ice-cold lysis buffer (100 mM NaCl, 50 mM 2-glycerolphosphate, 10 mM EDTA, 2 mM NaF, 1 mM Na_3VO_4 , 10 mg/ml leupeptin, 10 mg/ml chymostatin, and 10 mg/ml pepstatin) and centrifuged for 2 min in a Beckman E microcentrifuge with a right angle rotor to remove lipid and yolk protein. Cytoplasm was added immediately to 0.2 volume of 6 \times Laemmli gel sample buffer and boiled for 5 min at $90^\circ C$. Samples were separated by electrophoresis on 10.5% (for MAPK) or 15% (for Nm23) SDS-polyacrylamide gels (acrylamide:bisacrylamide, 100:1) and transferred to polyvinylidene difluoride membranes. Nm23 was detected with polyclonal antiserum (1:1000) and alkaline phosphatase-conjugated secondary antibody using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega) as substrate. For p42 MAPK and Mos, the blotting membrane was incubated with polyclonal X15 anti-MAPK serum (1:1000; Ref. 36) or anti-Mos serum (Santa Cruz Biotechnology; 1:500) and detected with enhanced chemiluminescence (Amersham).

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References

1. Steeg, P. S., Bevilacqua, G., Kopper, L., Thorgerisson, U. P., Talmadge, J. E., Liotta, L. A., and Sobel, M. E. Evidence for a novel gene associated with low tumor metastatic potential. *J. Natl. Cancer Inst.*, 80: 200–204, 1988.
2. Freije, J. M., MacDonald, N. J., and Steeg, P. S. Differential gene expression in tumor metastasis: Nm23. *Curr. Top. Microbiol. Immunol.*, 273: 215–232, 1996.
3. Rosengard, A. M., Krutzsch, H. C., Shearn, A., Biggs, J. R., Barker, E., Margulies, I. M., King, C. R., Liotta, L. A., and Steeg, P. S. Reduced Nm23/Awd protein in tumour metastasis and aberrant *Drosophila* development. *Nature (Lond.)*, 342: 177–180, 1989.
4. Stahl, J. A., Leone, A., Rosengard, A. M., Porter, L., King, C. R., and Steeg, P. S. Identification of a second human nm23 gene, nm23-H2. *Cancer Res.*, 51: 445–449, 1991.
5. Venturelli, D., Martinez, R., Melotti, P., Casella, I., Peschle, C., Cucco, C., Spampinato, G., Darzynkiewicz, Z., and Calabretta, B. Overexpression of DR-nm23, a protein encoded by a member of the nm23 gene family, inhibits granulocyte differentiation and induces apoptosis in 32Dc13 myeloid cells. *Proc. Natl. Acad. Sci. USA*, 92: 7435–7439, 1995.
6. Milon, L., Rousseau-Merck, M. F., Munier, A., Erent, M., Lascu, I., Capeau, J., and Lacombe, M. L. nm23-H4, a new member of the family of human nm23/nucleoside diphosphate kinase genes localised on chromosome 16p13. *Hum. Genet.*, 99: 550–557, 1997.
7. Munier, A., Feral, C., Milon, L., Pinon, V. P., Gyapay, G., Capeau, J., Guellaen, G., and Lacombe, M. L. A new human nm23 homologue (nm23-H5) specifically expressed in testis germinal cells. *FEBS Lett.*, 434: 289–294, 1998.

8. Mehus, J. G., Deloukas, P., and Lambeth, D. O. NME6: a new member of the nm23/nucleoside diphosphate kinase gene family located on human chromosome 3p21.3. *Hum. Genet.*, *104*: 454–459, 1999.
9. Biggs, J., Hersperger, E., Steeg, P. S., Liotta, L. A., and Shearn, A. A *Drosophila* gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. *Cell*, *63*: 933–940, 1990.
10. Lakso, M., Steeg, P. S., and Westphal, H. Embryonic expression of nm23 during mouse organogenesis. *Cell Growth Differ.*, *3*: 873–879, 1992.
11. Okabe-Kado, J., Kasukabe, T., Honma, Y., Hayashi, M., Henzel, W. J., and Hozumi, M. Identity of a differentiation inhibiting factor for mouse myeloid leukemia cells with NM23/nucleoside diphosphate kinase. *Biochem. Biophys. Res. Commun.*, *182*: 987–994, 1992.
12. Willems, R., Van Bockstaele, D. R., Lardon, F., Lenjou, M., Nijs, G., Snoeck, H. W., Berneman, Z. N., and Slegers, H. Decrease in nucleoside diphosphate kinase (NDPK/nm23) expression during hematopoietic maturation. *J. Biol. Chem.*, *273*: 13663–13668, 1998.
13. Gervasi, F., D'Agnano, I., Vossio, S., Zupi, G., Sacchi, A., and Lombardi, D. nm23 influences proliferation and differentiation of PC12 cells in response to nerve growth factor. *Cell Growth Differ.*, *7*: 1689–1695, 1996.
14. Ishijima, Y., Shimada, N., Fukuda, M., Miyazaki, H., Orlov, N. Y., Orlova, T. G., Yamada, T., and Kimura, N. Overexpression of nucleoside diphosphate kinases induces neurite outgrowth and their substitution to inactive forms leads to suppression of nerve growth factor- and dibutyryl cyclic AMP-induced effects in PC12D cells. *FEBS Lett.*, *445*: 155–159, 1999.
15. Gilles, A. M., Presecan, E., Vonica, A., and Lascu, I. Nucleoside diphosphate kinase from human erythrocytes. Structural characterization of the two polypeptide chains responsible for heterogeneity of the hexameric enzyme. *J. Biol. Chem.*, *266*: 8784–8789, 1991.
16. Okabe-Kado, J., Kasukabe, T., Hozumi, M., Honma, Y., Kimura, N., Baba, H., Urano, T., and Shiku, H. A new function of Nm23/NDP kinase as a differentiation inhibitory factor, which does not require its kinase activity. *FEBS Lett.*, *363*: 311–315, 1995.
17. Postel, E. H., Berberich, S. J., Flint, S. J., and Ferrone, C. A. Human c-myc transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis. *Science (Washington DC)*, *261*: 478–480, 1993.
18. Postel, E. H. NM23-NDP kinase. *Int. J. Biochem. Cell Biol.*, *30*: 1291–1295, 1998.
19. Postel, E. H., and Ferrone, C. A. Nucleoside diphosphate kinase enzyme activity of NM23-H2/PuF is not required for its DNA binding and *in vitro* transcriptional functions. *J. Biol. Chem.*, *269*: 8627–8630, 1994.
20. Wagner, P. D., Steeg, P. S., and Vu, N. D. Two-component kinase-like activity of nm23 correlates with its motility-suppressing activity. *Proc. Natl. Acad. Sci. USA*, *94*: 9000–9005, 1997.
21. MacDonald, N. J., De la Rosa, A., Benedict, M. A., Freije, J. M., Krutsch, H., and Steeg, P. S. A serine phosphorylation of Nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. *J. Biol. Chem.*, *268*: 25780–25789, 1993.
22. Bominaar, A. A., Molijn, A. C., Pestel, M., Veron, M., and Van Haastert, P. J. Activation of G-proteins by receptor-stimulated nucleoside diphosphate kinase in *Dictyostelium*. *EMBO J.*, *12*: 2275–2279, 1993.
23. Ferrell, J. E., Jr. *Xenopus* oocyte maturation: new lessons from a good egg. *Bioessays*, *21*: 833–842, 1999.
24. Ouatas, T., Abdallah, B., Gasmli, L., Bourdais, J., Postel, E., and Mazabraud, A. Three different genes encode NM23/nucleoside diphosphate kinases in *Xenopus laevis*. *Gene (Amst.)*, *194*: 215–225, 1997.
25. Kim, S. Y., Chang, K. H., Doh, H. J., Jung, J. A., Kim, E., Sim, C. J., and Lee, K.-J. Rapid purification and characterization of nucleoside diphosphate kinase isoforms using ATP-Sepharose affinity column chromatography. *Mol. Cells*, *7*: 630–634, 1997.
26. Kimura, N., and Shimada, N. Evidence for complex formation between GTP binding protein (Gs) and membrane-associated nucleoside diphosphate kinase. *Biochem. Biophys. Res. Commun.*, *168*: 99–106, 1990.
27. Kimura, N., and Shimada, N. Direct interaction between membrane-associated nucleoside diphosphate kinase and GTP-binding protein (Gs), and its regulation by hormones and guanine nucleotides. *Biochem. Biophys. Res. Commun.*, *151*: 248–256, 1988.
28. Fox, C. A., Sheets, M. D., and Wickens, M. P. Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUU. *Genes Dev.*, *3*: 2151–2162, 1989.
29. Barkoff, A., Ballantyne, S., and Wickens, M. Meiotic maturation in *Xenopus* requires polyadenylation of multiple mRNAs. *EMBO J.*, *17*: 3168–3175, 1998.
30. Gebauer, F., and Richter, J. D. The control switch of vertebrate oocyte meiosis. *Bioessays*, *19*: 23–28, 1997.
31. de Moor, C. H., and Richter, J. D. The Mos pathway regulates cytoplasmic polyadenylation in *Xenopus* oocytes. *Mol. Cell. Biol.*, *17*: 6419–6426, 1997.
32. Randazzo, P. A., Northup, J. K., and Kahn, R. A. Activation of a small GTP-binding protein by nucleoside diphosphate kinase. *Science (Washington DC)*, *254*: 850–853, 1991.
33. Davey, R. A., Hamson, C. A., Healey, J. J., and Cunningham, J. M. *In vitro* binding of purified murine ecotropic retrovirus envelope surface protein to its receptor, MCAT-1. *J. Virol.*, *71*: 8096–8102, 1997.
34. Chae, S. K., Lee, N. S., Lee, K.-J., and Kim, E. Transactivation potential of the C-terminus of human Nm23-H1. *FEBS Lett.*, *423*: 235–238, 1998.
35. Yew, N., Mellini, M. L., and Vande Woude, G. F. Meiotic initiation by the mos protein in *Xenopus*. *Nature (Lond.)*, *355*: 649–652, 1992.
36. Hsiao, K.-M., Chou, S.-y., Shih, S.-J., and Ferrell, J. E., Jr. Evidence that inactive p42 mitogen-activated protein kinase and inactive Rsk exist as a heterodimer *in vivo*. *Proc. Natl. Acad. Sci. USA*, *91*: 5480–5484, 1994.