Association of *N-myc* Downregulated Gene 1 with Heat-Shock Cognate Protein 70 in Mast Cells

Toshihiko Sugiki, Yoshitaka Taketomi, Rei Kikuchi-Yanoshita, Makoto Murakami, and Ichiro Kudo*

Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University; 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–8555, Japan. Received January 7, 2004; accepted January 19, 2004

***N-Myc* downregulated gene (NDRG) 1 is markedly induced during *in vitro* maturation of mouse immature bone marrow-derived mast cells (BMMCs) into a mature connective tissue mast cell (CTMC)-like phenotype. However, cellular function of this unique cytosolic protein is currently obscure. In this study, we sought potential NDRG1-binding proteins using yeast two-hybrid analysis and found that NDRG1 is capable of binding to heat-shock cognate protein 70 (Hsc70) both *in vitro* and in mast cells. The expression of Hsc70 was markedly elevated during the *in vitro* maturation of BMMCs into CTMC-like cells in accordance with the increased expression of NDRG1. Deletion of the C-terminal hydrophilic tandem repeats from NDRG1 facilitated the interaction with Hsc70 *in vitro*. Interaction between NDRG1 and Hsc70 was constitutive in mast cells and was not altered following cell activation. Although NDRG1 undergoes phosphorylation (accompanying paper), the binding of NDRG1 to Hsc70 was not affected by this event. Interestingly, the NDRG1-Hsc70 complex transiently appeared in the nuclear fraction of activated mast cells.**

Key words mast cell; *N-myc* downregulated gene 1 (NDRG1); Hsc70; yeast two-hybrid

N-Myc downregulated gene (NDRG) 1 is a member of the NDRG family, to which NDRG2, -3 and -4 also belong.[1,2] NDRG1 is a 43-kDa cytosolic protein containing a NDRG core domain, which is conserved among the NDRG family members, and three unique tandem repeats of 10 hydrophilic amino acids near the C-terminus. The expression of NDRG1 is markedly induced in a variety of cells under various conditions such as cell differentiation, hypoxia, and exposure to heavy metals,[3—5] whereas its expression is downregulated by the Myc family transcription factors and after cellular transformation.[6] Mutations in the *NDRG1* gene are linked to hereditary motor and sensory neuropathy.[7] Because overexpression of NDRG1 in neoplastic cells results in inhibition of cell growth and promotion of cell differentiation,[8,9] its expression may be related to the regulation of cell differentiation and stress. However, its precise cellular functions and regulatory mechanisms remain elusive.

Mast cells, which express the high-affinity IgE receptor (FcεRI), are activated by sensitization with IgE followed by challenge with multivalent antigens or by several secretagogues to release a variety of bioactive mediators such as histamine, eicosanoids and cytokines.[10,11] We previously demonstrated that coculture of interleukin-3-dependent mouse bone marrow-derived mast cells (BMMCs), a relatively immature population of mast cells, with mesenchymal fibroblasts in the presence of stem cell factor (SCF) resulted in prompt morphologic and functional maturation toward a mature connective tissue mast cell (CTMC)-like phenotype.[12] We then performed cDNA subtraction to identify a panel of genes of which the expression was markedly elevated during this *in vitro* mast cell maturation process, and found that NDRG1 was the most frequently induced among such genes.[13] Moreover, overexpression of NDRG1 in mast cells resulted in an augmented exocytotic response,[13] suggesting that NDRG1 allows mast cells to be differentiated into a phenotype that is more susceptible to various secretagogues.

In this study, we searched for proteins that bind to NDGR1 using yeast two-hybrid screening and found that NDRG1 is constitutively associated with heat-shock cognate protein 70 (Hsc70). Elimination of the C-terminal tandem repeats facilitates the interaction between NDRG1 and Hsc70.

MATERIALS AND METHODS

***In Vitro* Differentiation of BMMCs into CTMC-Like Cells** The procedure for coculture of BALB/c mouse BMMCs with Swiss 3T3 fibroblasts in the presence of a soluble form of mouse SCF was described previously.[8—10] The cocultured BMMCs were safranin-positive (indicative of heparin biosynthesis), expressed mouse mast cell protease-4 (a CTMC marker), produced more prostaglandin D_2 after cell activation, and responded to compound 48/80 and substance P,[12] events reminiscent of those occurring in CTMCs.

Mast Cell Activation Mast cells (5×10^4 cells/ml) were cultured for 2 d in 1 ml of culture medium, sensitized with 200 ng/ml IgE anti-dinitrophenyl (DNP) for 2 h, washed twice with culture medium, and then activated for 10 min at 37 °C with 10 ng/ml DNP-conjugated bovine serum albumin (Sigma) as an antigen (Ag) to assess β-hexosaminidase (β-HEX) release.[12,13]

Yeast Two-Hybrid Screening The yeast two-hybrid screening of putative NDRG1-binding proteins was performed using the Matchmaker GAL4 two-hybrid system 3 (Clontech) according to the manufacturer's instructions. Briefly, NDRG1 cDNA was subcloned into the bait vector pGBKT7 (Clontech). The resulting plasmid and the prey vector pACT2 containing a mouse kidney cDNA library (Clontech) were transfected into yeast cells (AH109; Clontech) by heat shock at 42 °C for 15 min. The cells were seeded onto SD/-Leu/-His/-Trp plates comprising yeast nitrogen base (Becton) and -Leu/-His/-Trp DO supplement (Clontech). After 1 week, the colonies were seeded onto SD/-Leu/-His/-Trp plates containing 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside and cultured for 1 week. Blue colonies were then transferred to SD/-Leu/-His/-Trp/-Ade plates and cul-

* To whom correspondence should be addressed. e-mail: kudo@pharm.showa-u.ac.jp

tured for 1 week. After three rounds of screening on the SD/-Leu/-His/-Trp/-Ade plates, plasmids were extracted from individual blue colonies and transfected into *Escherichia coli* (DH5α; Takara Biomedicals) by electroporation. The amplified plasmids were purified and the prey sequences were analyzed using an autofluorometric DNA sequencer (310 Genetic Analyzer, Applied Biosystems). The deduced sequences were analyzed using the BLASTN search.

RT-PCR Total RNA (1 μg) extracted from mast cells using Trizol reagent (Invitrogen) was reverse-transcribed using RNA PCR Kits (Takara Biomedicals). The primer sets (Greiner Japan, Tokyo, Japan) used for PCR reaction were as follows: for mouse NDRG1, 5′-ATGTCCCGACACGTACATGAC-3′ and 5′-TTACGACCAGAGGTGGATCCAG-3′; for mouse Hsc70, 5′-ATGTCTAAGGGACCTGCAGTTGG-3′ and 5′-TTAATCCACCTCTTCAATGGTGGGG-3′; and for mouse PICK-1 (for protein that interacts with C-kinase-1), 5′-ATGTTTGCAGACTTAGACTATGACATCG-3′ and 5′-TCAGGAGTCACACCAGCTTC-3′. The PCR was conducted with Advantage cDNA polymerase mix (Clontech) at 95 °C for 5 min, 23 to 30 cycles of 95 °C for 30 s and 68 °C for 3 min, and 68 °C for 3 min. The reaction products were electrophoresed on 1% agarose gels, and the bands were visualized with ethidium bromide staining.

Preparation of Recombinant Mouse Hsc70 Mouse Hsc70 cDNA obtained by RT-PCR as described above was subcloned into the pCR3.1 vector (Invitrogen) and transformed into *E. coli* (JM109, Takara Biomedicals). The obtained Hsc70/pCR3.1 construct was digested with EcoRI, and the insert was subcloned into the pGEX-2T vector (Amersham Bioscience) at the EcoRI site. The resulting plasmid was transformed into JM109 and cultured in the presence of 0.4 mM isopropyl-thio-β-D-galactoside to produce recombinant Hsc70 protein N-terminally fused with glutathione-*S*-transferase (GST). The GST-Hsc70 protein was purified from the bacterial homogenates on a glutathione-Sepharose column (Amersham Bioscience) according to the manufacturer's instructions. Elution of GST-Hsc70 protein was monitored by SDS-PAGE. Fractions containing pure GST-Hsc70 protein were dialyzed against 10 mM Tris–HCl (pH 7.4) containing 150 mM NaCl (TBS).

Preparation of Recombinant NDRG1 Protein cDNAs for the full-length NDRG1 and its truncated form, which lacks the three C-terminal tandem repeats, were prepared as described previously.[13] These cDNA inserts were subcloned into the pET-21c vector (Novagen) at the EcoRI site and transformed into *E. coli* (BL21(DE3); Invitrogen) and cultured in the presence of 0.4 mM isopropyl-thio-β-D-galactoside to produce recombinant His_6-tagged NDRG1 protein. The His_6-NDRG1 protein was purified from bacterial homogenates on a Ni-NTA column (QIAGEN) to near homogeneity according to the manufacturer's instructions and dialyzed against TBS.

GST Pull-Down Assay Recombinant GST-Hsc70 (10 μg) was incubated with 10 μl of glutathione-Sepharose beads for 1 h at 4 °C. After washing the beads with phosphate-buffered saline (PBS), recombinant His_6-tagged NDRG1 (10 μg) was added to the beads. After incubation for 12 h at 4 °C, the beads were washed with PBS, boiled in SDS-PAGE sample buffer, and subjected to SDS-PAGE followed by immunoblotting with anti-His_6 antibody (Amersham Bioscience).

Cell Fractionation RBL-2H3 cells cultured to subconfluence in 100-mm dishes were washed with PBS and suspended in 200 μl of cell lysis buffer composed of 10 mM HEPES buffer (pH 7.9) containing 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM PMSF and 10 μg/ml leupeptin. Then 12 μl of 10% NP-40 was added, and the resulting lysates were centrifuged for 30 s at 10000×g to separate into the supernatant (cytosol) and pellet (nucleus) fractions. The nuclear fractions were suspended in 200 μl of cell lysis buffer and spun down (this procedure was repeated three times). Finally, the nuclear fractions were resuspended in 200 μl of cell lysis buffer and sonicated for subsequent use.

Coimmunoprecipitation Mast cell homogenates were incubated with 10 μl of protein G-Sepharose (Amersham Bioscience) for 1 h at 4 °C to eliminate nonspecific absorption of proteins to the beads. After brief centrifugation, the supernatants were incubated for 3 h at 4 °C with protein G-Sepharose that had been incubated with 10 μg of anti-NDRG1 antibody[9] or anti-Hsc70 antibody (Santa Cruz) overnight at 4 °C. After washing with PBS, the beads were boiled in SDS-PAGE sample buffer and subjected to Western blotting with anti-Hsc70 or anti-NDRG1 antibody.

Treatment of Cells with Protein Kinase Inhibitors RBL-2H3 cells were incubated for 30 min with 10 μM H-89 (protein kinase A inhibitor) (Seikagaku Kogyo), 2 μM GF109203X [protein kinase C (PKC) inhibitor] (BIOMOL), 10 μM KN-62 (calcium-calmodulin kinase II inhibitor) (Sigma), 10 μM 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (DRB; casein kinase-II inhibitor) (Calbiochem), or vehicle control (dimethyl sulfoxide) in medium. Then the cells were lyzed and subjected to immunoprecipitation.

Other Procedures Protein concentrations were determined using a BCA protein assay kit (Pierce). The procedure for Western blotting was described previously.[12,13] Data were analyzed using Student's *t*-test.

RESULTS

Yeast Two-Hybrid Screening To identify potential NDRG1-binding proteins, we performed the yeast two-hybrid screening assay with full-length mouse NDRG1 cDNA as bait and a cDNA library prepared from mouse kidney, in which NDRG1 is the most abundantly expressed among mouse tissues,[1,6] as prey. The results of the screening are summarized in Table 1. Of 146 final isolates, two clones encoded Hsc70, a cytosolic molecular chaperone that has been implicated in various biological events including ATP-dependent protein folding, vesicular transport, and exocytosis.[14,15] Several clones encoded molecules that have been shown to function in membrane fusion, including p47[16,17] and PICK-1.[18,19] Four clones encoded aspartoacylase, a causal gene for Canavan disease.[20,21] In addition, several mitochondrial proteins, such as the mitochondrial anion channel VDAC1 and cytochrome C oxidase subunit II (Cox2), were detected in multiple clones (Table 1). The detection of mitochondrial proteins as potential NDRG1-binding partners is intriguing to note, since NDRG1 has a potential cytochrome c family heme-binding motif and a phosphopantethein attachment motif, which are compatible with mitochondrial location, and is often localized in the mitochondria.[22] Nonetheless, since

Table 1. Yeast Two-Hybrid Screen of Potential NDRG1-Binding Proteins

Clones	No.	Accession no.	Properties
Hsc70	2	U73744	Heat-shock protein
PICK1	1	Z46720	Regulation of membrane fusion
p47	1	Y11684	Regulation of membrane fusion
Aspartoacylase-3	3	AF356879	Aspartoacylase
Aminoacylase-2	1	BC024934	Aspartoacylase
Voltage-dependent anion channel 1 (VDAC1)	2	U30840	Mitochondrial protein
Cytochrome c oxidase subunit II (Cox2)	2	AF378830	Mitochondrial protein
ATPase subunit 6	2	AF093677	Mitochondrial protein

Representative clones identified from 146 final isolates of the yeast two-hybrid screening are summarized.

we previously demonstrated that overexpression of NDRG1 in mast cells results in enhanced degranulation,[13] we were interested here in particular sets of proteins which have been implicated in the regulation of exocytosis (*i.e.*, Hsc70, PICK-1, and p47).

Interaction of NDRG1 with Hsc70 in Mast Cells We conducted RT-PCR with primers for Hsc70, PICK-1, and p47 using total RNAs obtained from BMMCs before and after 4 d of coculture with fibroblasts in the presence of SCF (a condition under which BMMCs are readily differentiated into CTMC-like cells[12,13]) as well as from RBL-2H3 cells, a rat mast cell line. NDRG1 was poorly expressed in interleukin-3-maintained BMMCs and was markedly increased after coculture (Fig. 1), as reported in our previous paper.[13] Similarly, the expression of Hsc70 was faint in BMMCs and greatly elevated after coculture (Fig. 1). The expression of PICK-1 was fairly low (Fig. 1) and that of p47 was undetectable (data not shown) in BMMCs before and after coculture. In RBL-2H3 cells, NDRG1 was significantly detected (although at a lower level than in cocultured BMMCs), as reported previously.[13] Hsc70 expression in RBL-2H3 cells was comparable to and PICK-1 expression was modestly higher than those in BMMCs after coculture (Fig. 1). Given these expression profiles, we focused on the interaction between NDRG1 and Hsc70 in subsequent studies.

To evaluate the interaction between NDRG1 and Hsc70, we next determined whether both proteins were able to associate directly *in vitro*. Incubation of recombinant NDRG1 with GST-Hsc70 and subsequent precipitation with glutathione-Sepharose demonstrated the presence of associated NDRG1 (Fig. 2), implying their direct physical binding. No precipitation of NDRG1 was observed when the pull-down assay was performed with control Sepharose beads (data not shown). However, the interaction between the full-length NDRG1 and Hsc70 *in vitro* appeared weak, since only a small fraction of NDRG1 (relative to 10 μg of NDRG1 input) was coprecipitated with Hsc70. Interestingly, an NDRG1 mutant, which lacked the three C-terminal tandem repeats, was more efficiently coprecipitated with GST-Hsc70 than was the full-length NDRG1 (Fig. 2). These results suggest that the C-terminal tandem repeats have an inhibitory effect on the NDRG1-Hsc70 interaction.

To assess whether the interaction between NDRG1 and Hsc70 takes place in mast cells, the lysate of RBL-2H3 cells, in which both NDRG1 and Hsc70 were expressed (Fig. 1), was subjected to immunoprecipitation with anti-NDRG1 antibody and then to immunoblotting with anti-Hsc70 antibody. As shown in Fig. 3A, a 70-kDa band was clearly detected in the immunoprecipitate. This band was not detected when the anti-NDRG1 antibody immunoprecipitate was blotted with control antibody or when the immunoprecipitation was carried out with control antibody. Conversely, anti-Hsc70 antibody, but not control antibody, was capable of pulling down a 43-kDa NDRG1 (Fig. 3B). Reprobing the same membranes

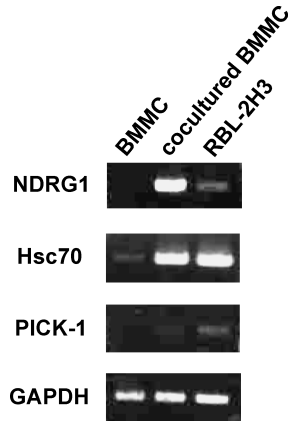

Fig. 1. Expression of NDRG1 and Its Potential Binding Proteins in Mast Cells

Total RNAs obtained from BMMCs maintained in IL-3, those cocultured with Swiss 3T3 cells in the presence of SCF for 4 d, and RBL-2H3 cells were subjected to RT-PCR for NDRG1, Hsc70, PICK-1, and GAPDH as described in Materials and Methods. Amplified bands were visualized in agarose gels with ethidium bromide.

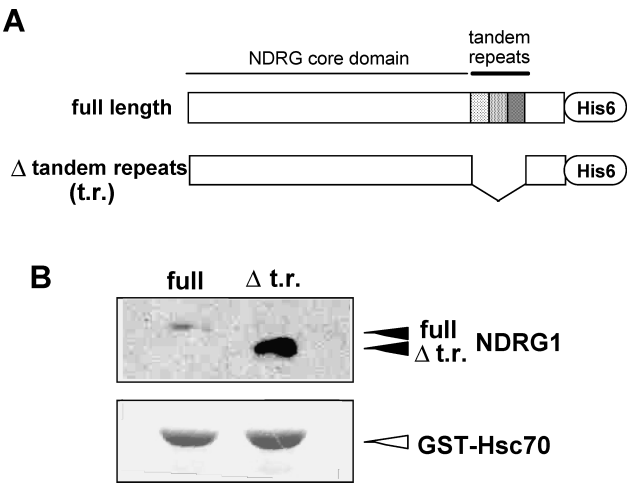

Fig. 2. Interaction of NDRG1 with Hsc70 *in Vitro*

(A) Structure of the full-length NDRG1 and its mutant in which the three hydrophilic tandem repeats are deleted. Both forms are tagged with a His_6 epitope at their C-termini. (B) The His_6-tagged full-length or truncated NDRG1 was incubated with GST-fused Hsc70 and then precipitated with gluthathione-Sepharose. The resulting pellets were subjected to immunoblotting with anti-His_6 and -HSC70 antibodies.

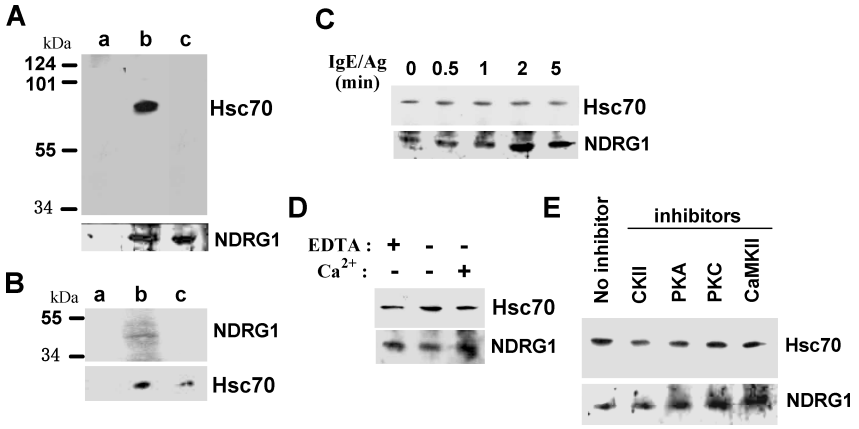

Fig. 3. Interaction of NDRG1 with Hsc70 in Mast Cells

(A) Lysates of RBL-2H3 cells were incubated with protein G-Sepharose in the presence (lanes b and c) or absence (lane a) of anti-NDRG1 antibody, and the resulting pellets were subjected to immunoblotting as follows. In the upper panel, lanes a and b were blotted with anti-Hsc70 antibody and lane c with control IgG. In the lower panel, all lanes were blotted with anti-NDRG1 antibody. (B) Replicate cell lysates were incubated with (lanes b and c) or without (lane a) of anti-Hsc70 antibody. After immunoprecipitation, the pellets were subjected to immunoblotting as follows. In the upper panel, lanes a and b were blotted with anti-NDRG1 antibody and lane c with control IgG. In the lower panel, all lanes were blotted with anti-Hsc70 antibody. (C) Lysates of RBL-2H3 cells, which were stimulated for the indicated periods with IgE/Ag, were subjected to immunoprecipitation with anti-NDRG1 antibody, followed by immunoblotting with anti-NDRG1 and -Hsc70 antibodies. (D) Lysates of RBL-2H3 cells were incubated with anti-NDRG1 antibody in the presence (+) or absence (−) of Ca^{2+} or EDTA. Then the samples were subjected to immunoprecipitation with anti-NDRG1 antibody, followed by immunoblotting with anti-NDRG1 and -Hsc70 antibodies. (E) Lysates of the cells, which were pretreated with several protein kinase inhibitors, were subjected to immunoprecipitation with anti-NDRG1 antibody, followed by immunoblotting with anti-NDRG1 and -Hsc70 antibodies.

with anti-NDRG1 antibody (Fig. 3A) and anti-Hsc70 antibody (Fig. 3B) verified that the efficiency of immunoprecipitation was equal among the different samples.

As shown in Fig. 3C, the amounts of Hsc70 coprecipitated with NDRG1 were unchanged even after IgE/Ag-dependent activation of mast cells, indicating that the interaction between NDRG1 and Hsc70 in mast cells is constitutive. In support of this finding, Ca^{2+} (or other divalent cations), an essential second messenger for mast cell activation, did not affect the binding of NDRG1 to Hsc70, since the addition of Ca^{2+} or EDTA to cell lysates did not alter the amount of Hsc70 coprecipitated with NDRG1 (Fig. 3D). Moreover, although NDRG1 is phosphorylated by protein kinase A and calcium-calmodulin kinase-II in mast cells (accompanying paper), neither inhibitors of these two kinases nor inhibitors of PKC and casein kinase-II affected the NDRG1-Hsc70 interaction (Fig. 3E).

Translocation of the NDRG1-Hsc70 Complex to the Nucleus We previously found that a fraction of NDRG1 translocates to the nucleus following mast cell activation,[13] even though NDRG1 does not have a potential nuclear localization sequence. The nuclear location of NDRG1 has also been found in other cells in accordance with cell cycle.[8,9] Hsc70 has been also reported to shuttle between the cytosol and nucleus under certain conditions.[23,24] Based on this background, we asked whether NDRG1 moves to the nucleus as a complex with Hsc70 in activated mast cells. To this end, the homogenates of RBL-2H3 cells with or without stimulation with IgE/Ag were separated into the cytosol and nuclear fractions. Appropriate fractionation was verified by immunoblotting of lamin B, which was detected only in the nuclear fraction (Fig. 4). Each sample was then subjected to immunoprecipitation with anti-NDRG1 antibody followed by immunoblotting with anti-NDRG1 and anti-Hsc70 antibodies. As shown in Fig. 4, although NDRG1 was predominantly present in the cytosol of RBL-2H3 cells throughout the experimental periods, a small but significant portion of NDRG1 was detected in the nuclear fraction after stimulation

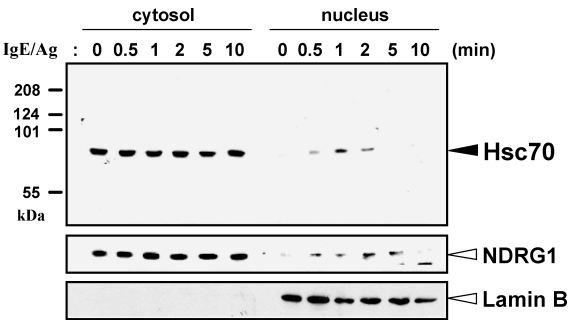

Fig. 4. Translocation of the NDRG1-Hsc70 Complex into the Nucleus

Lysates of RBL-2H3 cells, which were stimulated for the indicated periods with IgE/Ag, were separated into the cytosol and nuclear fractions. Then individual samples were subjected to immunoprecipitation with anti-NDRG1 antibody, followed by immunoblotting with anti-NDRG1 and -Hsc70 antibodies. Aliquots of the cytosol and nuclear fractions were immunoblotted with anti-lamin B antibody.

with IgE/Ag for 0.5 to 5 min. After 10 min, the nuclear NDRG1 was decreased to the basal, undetectable level. The dynamics of NDRG1 are consistent with the event observed in our previous immunocytostaining study.[13] Notably, a fraction of Hsc70 coimmunopreciptated with NDRG1 also appeared transiently in the nuclear fraction after cell activation, which was evident after 0.5 to 2 min and then disappeared by 5 min (Fig. 4). Thus, transient translocation of NDRG1 to the nucleus accompanies that of associated Hsc70, and the disappearance of Hsc70 from the nucleus precedes that of NDRG1.

DISCUSSION

In the yeast two-hybrid screening and pull-down assay, we found that NDRG1 is associated with the molecular chaperone Hsc70. The binding appears to be mediated between the NDRG core domain, which is conserved among all NDRG proteins and is devoid of the C-terminal domain containing the three tandem repeats,[1,2] and the N-terminal *ca.* 300 amino acids of Hsc70, which reside in the prey plasmid of

the yeast two-hybrid screening. Determination of the minimal regions in NDRG1 and Hsc70 required for their interaction awaits a future study using their truncated mutants. Deletion of the C-terminal tandem repeats from NDRG1 markedly enhances its binding to Hsc70 *in vitro*, indicating that the tandem repeat domain negatively regulates the NDRG1-Hsc70 interaction. Considering that molecular chaperones generally play a role in correcting the folding of denatured proteins, inadequate folding of the tandem repeat-deleted NDRG1 mutant may facilitate its interaction with Hsc70. Although direct association of the full-length NDRG1 with Hsc70 *in vitro* appears weak, they are efficiently coprecipitated from mast cells, suggesting the presence of another accessory cellular component that supports NDRG1-Hsc70 interaction.

Hsc70 is a member of the heat-shock protein 70 (Hsp70) molecular chaperone family. In contrast to Hsp70, which is induced and assists the refolding of denatured proteins during stress responses, Hsc70 is expressed in the cytosol relatively constitutively and plays variable roles in organelle membrane trafficking, endocytosis and exocytosis.[14,15] In a comprehensive model of membrane fusion (the so-called "the SNARE theory"), fusion of vesicles to acceptor membranes requires the action of *N*-ethylmaleimide-sensitive fusion protein (NSF), a cytosolic ATPase that binds to membrane-associated soluble NSF attachment protein (SNAP) receptors (SNAREs) *via* its cofactor SNAPs.[25,26] NSF and SNAPs break up preformed complexes of v- and t-SNAREs to accelerate the turnover of membrane fusion. Although SNAREs were originally identified in neuronal cells, it has become apparent that several SNARE proteins are present in mast cells.[27—29] Cysteine string protein (CSP), which interacts with particular SNARE proteins and thereby stabilizes the process of membrane fusion[30,31] binds to Hsc70 and activates its ATPase activity.[32] The CSP-Hsc70 complex is recruited to the vesicular membranes and promotes exocytosis. Thus, Hsc70 might play a role in bringing NDRG1 to the SNARE membrane fusion machinery.

The yeast-two-hybrid screening also identified two additional regulators of membrane fusion, PICK-1 and p47, as potential NDRG1-binding proteins, even though we have not yet confirmed whether NDRG1 can be associated with these two proteins in mammalian cells. PICK-1, which was originally identified as a protein that binds and activates PKC,[33] regulates the docking of the vesicular membrane and plasma membrane by interacting with NSF and SNAP.[18,19] p47 is functionally relevant to SNAP, acting as a cofactor for p97, an ATPase that is closely similar to NSF.[16,17] The p97–p47 interaction (as is the NSF-SNAP interaction) is required for fusion and reassembly of cellular membranes.[34,35] Considering that Hsc70, PICK-1, and p47 are converged in the same SNARE membrane fusion machinery, it is tempting to speculate that the combined interaction of NDRG1 with these proteins allows it to participate in regulatory exocytosis. Punctate distribution of NDRG1 in the cytosol of mast cells[13] may reflect its anchoring on secretory granules through interaction with these fusion-related proteins, a possibility that is worth examining.

Apart from a possible role of NDRG1 and Hsc70 in the regulation of mast cell degranulation, we found that a significant pool of the NDRG1-Hsc70 complex moves transiently from the cytosol to the nucleus after mast cell activation. Since the nuclear localization of NDRG1 occurs during cell cycle progression or cellular stress and since some epithelial tumor cell lines cease to proliferate after NDRG1 overexpression, it has been argued that NDRG1 exerts its antitumor effect in the nucleus.[8,9] Interestingly, similar events have also been observed with Hsc70.[23,24] In the nucleus, Hsc70 may participate in refolding of the tumor suppressors p53 or Rb.[36,37] These observations lead to the intriguing possibility that the shuttling of NDRG1 between the cytosol and nucleus is mediated by Hsc70 (or *vice versa*) and that these two proteins can cooperate in the nucleus.

We found in this study that NDRG1 interacts with Hsc70 in mast cells. Despite its marked imducibility, the cellular function of NDRG1 remains elusive. Clarifying the functional importance of the NDRG1/Hsc70 interaction will open new insights into the regulatory function of this unique protein in mast cells and other cell types that express both proteins.

Acknowledgments This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and from the Sankyo Foundation of Life Science.

REFERENCES

1) Okuda T., Kondoh H., *Biochem. Biophys. Res. Commun.*, **266**, 208—215 (1999).
2) Zhou R. H., Kokame K., Tsukamoto Y., Yutani C., Kato H., Miyata T., *Genomics*, **73**, 86—97 (2001).
3) Zhou D., Salnikow K., Costa M., *Cancer Res.*, **58**, 2182—2189 (1998).
4) Lin T. M., Chang C., *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 4988—4993 (1997).
5) Kokame K., Kato H., Miyata T., *J. Biol. Chem.*, **271**, 29659—29665 (1996).
6) Shimono A., Okuda T., Kondoh H., *Mech. Development*, **83**, 39—52 (1999).
7) Kalaydjieva L., Gresham D., Gooding R., Heather L., Baas F., de Jonge R., Blechschmidt K., Angelicheva D., Chandler D., Worsley P., Rosenthal A., King R. H., Thomas P. K., *Am. J. Hum. Genet.*, **67**, 47—58 (2000).
8) Kurdistani S. K., Arizti P., Reimer C. L., Sugrue M. M., Aaronson S. A., Lee S. W., *Cancer Res.*, **58**, 4439—4444 (1998).
9) Guan R. J., Ford H. L., Fu Y., Li Y., Shaw L. M., Pardee A. B., *Cancer Res.*, **60**, 749—755 (2000).
10) Galli S. J., Tsai M., Lantz C. S., "Signal Transduction in Mast Cells and Basophils," ed. by Razin E., Rivera J., Springer-Verlag, New York, 1999, pp. 11—30.
11) Kitamura Y., Morii E., Jippo T., "Signal Transduction in Mast Cells and Basophils," ed. by Razin E., Rivera J., Springer-Verlag, New York, 1999, pp. 31—38.
12) Ogasawara T., Murakami M., Suzuki-Nishimura T., Uchida M. K., Kudo I., *J. Immunol.*, **158**, 393—404 (1997).
13) Taketomi Y., Sugiki T., Saito T., Ishii S., Hisada M., Suzuki-Nishimura T., Uchida M. K., Moon T. C., Chang H. W., Natori Y., Miyazawa S., Kikuchi-Yanoshita R., Murakami M., Kudo I., *Biochem. Biophys. Res. Commun.*, **306**, 339—346 (2003).
14) Zinsmaier K. E., Bronk P., *Biochem. Pharmacol.*, **62**, 1—11 (2001).
15) Braun J. E., Wilbanks S. M., Scheller R. H., *J. Biol. Chem.*, **271**, 25989—25993 (1996).
16) Pecheur E. I., Martin I., Maier O., Bakowsky U., Ruysschaert J. M., Hoekstra D., *Biochemistry*, **41**, 9813—9823 (2002).
17) Yuan X., Shaw A., Zhang X., Kondo H., Lally J., Freemont P. S., Matthews S., *J. Mol. Biol.*, **311**, 255—263 (2001).
18) Hanley J. G., Khatri L., Hanson P. I., Ziff E. B., *Neuron*, **34**, 53—67 (2002).

19) Iwakura Y., Nagano T., Kawamura M., Horikawa H., Ibaraki K., Takei N., Nawa H., *J. Biol. Chem.*, **276**, 40025—40032 (2001).
20) Matalon R., Michals-Matalon K., *Adv. Pediatr.*, **46**, 493—506 (1999).
21) Surendran S., Michals-Matalon K., Quast M. J., Tyring S. K., Wei J., Ezell E. L., Matalon R., *Mol. Genet. Metab.*, **80**, 74—80 (2003).
22) Lachat P., Shaw P., Gebhard S., van Belzen N., Chaubert P., Bosman F. T., *Histochem. Cell. Biol.*, **118**, 399—408 (2002).
23) Martinez J., Georgoff I., Martinez J., Levine A. J., *Genes Dev.*, **5**, 151—159 (1991).
24) Zeise E., Kuhl N., Kunz J., Rensing L., *Cell Stress Chaperones*, **3**, 94—99 (1998).
25) Sollner T. H., *Mol. Membr. Biol.*, **20**, 209—220 (2003).
26) Sollner T. H., *Dev. Cell*, **2**, 377—378 (2002).
27) Pombo I., Martin-Verdeaux S., Iannascoli B., Le Mao J., Deriano L., Rivera J., Blank U., *J. Biol. Chem.*, **276**, 42893—42900 (2001).
28) Vaidyanathan V. V., Puri N., Roche P. A., *J. Biol. Chem.*, **276**, 25101—25106 (2001).
29) Guo Z., Turner C., Castle D., *Cell*, **94**, 537—548 (1998).
30) Pupier S., Leveque C., Marqueze B., Kataoka M., Takahashi M., Seagar M. J., *J. Neurosci.*, **17**, 2722—2727 (1997).
31) Dawson-Scully K., Bronk P., Atwood H. L., Zinsmaier K. E., *J. Neurosci.*, **20**, 6039—6047 (2000).
32) Braun J. E., Wilbanks S. M., Scheller R. H., *J. Biol. Chem.*, **271**, 25989—25993 (1996).
33) Staudinger J., Zhou J., Burgess R., Elledge S. J., Olson E. N., *J. Cell Biol.*, **128**, 263—271 (1995).
34) Otter-Nilsson M., Hendriks R., Pecheur-Huet E. I., Hoekstra D., Nilsson T., *EMBO J.*, **18**, 2074—2083 (1999).
35) Patel S. K., Indig F. E., Olivieri N., Levine N. D., Latterich M., *Cell*, **92**, 611—620 (1998).
36) King F. W., Wawrzynow A., Hohfeld J., Zylicz M., *EMBO J.*, **20**, 6297—6305 (2001).
37) Nihei T., Sato N., Takahashi S., Ishikawa M., Sagae S., Kudo R., Kikuchi K., Inoue A., *Cancer Lett.*, **73**, 181—189 (1993).