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NIP1/XB51/NECAB3 is a potential substrate of Nek2, suggesting specific roles of Nek2 in Golgi

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Abstract

Nek2 is a mammalian protein kinase structurally homologous to *Aspergillus* NIMA. We previously observed that the Nek2 protein was localized in multiple sites within a cell in a cell cycle stage-specific manner. Such dynamic behavior of Nek2 allowed us to propose that Nek2 may be a mitotic regulator that is involved in diverse cell cycle events. To better understand the cellular processes in which Nek2 participates, we carried out yeast two-hybrid screening and isolated Nek2-Interacting Protein 1 (NIP1), which has been also named as XB51 and NECAB3. Physical interactions of Nek2 with NIP1 were confirmed. In fact, Nek2 can phosphorylate NIP1 in vivo. Immunostaining experiments revealed that NIP1 is a Golgi protein. These results propose a possible involvement of Nek2 in biological processes of the Golgi body, perhaps in relation to the inheritance of Golgi during mitosis or to cell cycle stage-specific regulation of exocytosis.

Keywords: Nek2; Cell cycle; XB51; NECAB3; NIP1; Golgi; Mitosis

Introduction

Cells in mitosis go through a highly orchestrated series of changes, such as chromosome segregation, distribution of cellular organelles, and the eventual partitioning and separation of two daughter cells. Several protein kinases are involved in this regulation. Cdc2 is a critical regulator for overcoming checkpoints that reside within the M phase. Reduction in the Cdc2 activity is also critical for the M phase exit [1]. Polo-like kinase (Plk) is a mitotic kinase that is involved in multiple processes, such as modulation of Cdc2 activity, centrosome and spindle maturation and function, chromosome segregation, anaphase-promoting complex regulation, and execution of cytokinesis [2,3]. Aurora kinases are known to play critical roles in chromosome condensation and segregation, and in completion of cytokinesis [4].

Nek2 was initially introduced as a mammalian structural homologue of *Aspergillus nimA* [5]. The importance of Nek2 in centrosomal functions has been studied extensively [6]. Gain-of-function mutations caused splitting of centrosomes, dispersal of centrosomal material, and loss of a focused microtubule-nucleating activity [7]. Specific inhibition of Nek2 function did not interfere with the early embryonic cell cycle in *Xenopus*; however, it did cause abortive cleavage of early embryos, in which bipolar spindle formation was severely impaired due to fragmentation or dispersal of the centrosomes [8,9]. Thus, it was proposed that Nek2 is specifically required for centrosome assembly and maintenance [9]. Nek2 was known to phosphorylate a specific centrosomal protein identified as a Nek2-associated protein (C-Nap1) [10]. The phosphorylation state of C-Nap1 was also influenced by protein phosphatase 1 that formed a kinase–phosphatase complex with Nek2 [11]. When the function of C-Nap1 was interfered with antibody injection, centrosome splitting was induced [12]. Based on these results, it was proposed that C-Nap1 is a key component of a dynamic, cell cycle-regulated structure that mediates centriole–centriole cohesion and is regulated by Nek2 [12].

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A few lines of evidence suggest that Nek2 is involved in other cellular functions in addition to centrosomal cycle. First, the presence of two splice variants of Nek2 was reported in *Xenopus* early embryos [13] and in cultured mammalian cells [14,15]. These variant proteins behave differently during the cell cycle, suggesting that they might be involved in distinct cellular functions. Second, we observed that Nek2 was distributed dynamically during mitosis [16,17]. Association of Nek2 with chromosomes became evident when cells entered into mitosis and their association maintained until the end of metaphase. Once cells started anaphase, Nek2 was dissociated from the condensed chromosome and redistributed throughout the cytoplasm. Distinct localization of Nek2 on the midbody of the telophase cells was evident [17]. Such dynamic behavior of Nek2 suggested that Nek2 might be involved in diverse cell cycle events.

To investigate cellular functions in which Nek2 participates, we carried out yeast two-hybrid screen with Nek2 as bait. The current study reports the association of Nek2 with a Golgi protein named NIP1. These results support the specific role of Nek2 for Golgi during the cell cycle.

Materials and methods

Yeast two-hybrid screening

The yeast two-hybrid screening was carried out as described previously [18,19]. In brief, the *pGBT9*/*Nek2* in which the full-length mouse *Nek2* open reading frame (ORF) had been linked to the GAL4 DNA-binding domain sequence was transformed into the HF7c yeast strain along with the human liver library. Positive clones were selected with the β-galactosidase activity assay among colonies survived in SD synthetic medium lacking leucine, tryptophan, and histidine. False-positive colonies were removed following a standard protocol [18]. For the identification of isolated clones, nucleotide sequences were determined.

Northern blot hybridization analysis

Northern blot hybridization analysis was carried out as described previously [20]. In brief, total RNA from cell lines and tissues was prepared by the acid guanidinium thiocyanate-phenol-chloroform method [21]. Total RNA was electrophoresed in a denaturing 0.85% agarose gel containing 2.2 M formaldehyde, transferred to a nitrocellulose membrane, and hybridized with a *NIP1* riboprobe. Ethidium bromide staining of the 18S and 28S RNAs was used to determine equal loading for each sample. For the detection of human and mouse *NIP1* transcripts, we used the 177–1915-bp fragment of the human *NIP1* cDNA and the full-length mouse *NIP1* cDNA fragment, respectively.

Antibodies

For generating polyclonal antibodies specific to NIP1, a partial coding sequence of the human *NIP1* (43–362 a.a.) was subcloned into bacterial expression vectors, such as *pET21a* (Novagen, Madison, WI, USA) or *pGEX4T-1* (Amersham Pharmacia, Buckinghamshire, UK). The fusion proteins were purified and injected into rabbits. The antisera were affinity-purified by incubation with a strip of nitrocellulose membrane blotted with the NIP1 fusion protein and elution with 100 mM glycine, pH 2.5.

Anti-FLAG and anti-BiP monoclonal antibodies were purchased from Sigma (St. Louis, MO, USA), the anti-HA monoclonal antibody was purchased from Berkeley Antibody Company (Richmond, CA, USA), and the anti-Rab6 polyclonal antibody and anti-NSF monoclonal antibody were kind gifts of Dr. S. H. Hong (Seoul National University, Seoul, Korea).

Cell transfection

The *NIP1* cDNA was inserted into the *pFLAG-CMV2* expression vector (Sigma). The FLAG tag is localized at the amino-terminal end of the NIP1 protein. BOSC and 293T cells (1×10^7 cells) were transfected with the calcium phosphate method. COS7 cells were transfected with the LipofectAMINE method (GibcoBRL, Grand Island, NY, USA).

Immunoblot analysis

The immunoblot analysis was carried out as described previously [20]. In brief, cells were lysed with 2× Laemmli sample buffer [22] and boiled for 10 min. Protein samples were resolved by 10% SDS-PAGE and electroblotted onto a nitrocellulose membrane. The membrane was blocked by soaking in Blotto (TBS, 0.1% Tween 20, 5% nonfat dried milk) for 1 h, incubated with the primary antibody diluted with Blotto for 3 h, washed three times with Blotto, incubated with the alkaline phosphatase-conjugated secondary antibody (Roche Molecular Biochemicals, Mannheim, Germany) for 45 min, and washed with TBST (TBS, 0.1% Tween 20) three times. For the detection of signals, NBT/BCIP (Promega, Madison, WI, USA) was used according to the manufacturer's recommendations. Affinity purified anti-NIP1 antibody and anti-Nek2 antibody were used in concentrations of 2.5 and 10 μg protein/ml, respectively, and the secondary antibodies were diluted to 1:5000.

GST pull-down assay

Nek2-transfected BOSC cells were lysed in RIPA buffer (150 mM NaCl, 10 mM Tris–HCl, pH 7.5, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, and 1 mM EDTA) with protease inhibitors (100 mM PMSF, 5 mg/ml chy-

mostatin, 5 mg/ml leupeptin, 5 mg/ml pepstatin A, 1 mM Na_3VO_4, and 1 mM NaF) for 30 min on ice and centrifuged at 5000 *g* for 30 min at 4°C. The supernatant was incubated with GST or GST-NIP1 proteins for 3 h at 4°C, and with 30 μl of glutathione Sepharose beads (Amersham Pharmacia) for three more hours. Beads were washed three times with RIPA buffer and analyzed by immunoblotting.

Immunoprecipitation analysis

Cells were lysed in the RIPA buffer with protease inhibitors for 30 min on ice and centrifuged at 5000 *g* for 30 min at 4°C. The supernatant was incubated with the NIP1 or Nek2 antisera (30 μl) for 3 h, and with protein A Sepharose (Amersham Pharmacia) for three more hours. The immunoprecipitates were analyzed by immunoblotting.

Immunocytochemistry analysis

COS7, NIH3T3, and K28 cells were used for immunocytochemistry analysis. Cells were rinsed with PBS, fixed in 3.5% paraformaldehyde in PBS for 10 min at room temperature, permeabilized with 0.1% Triton X-100 for 10 min, and incubated with primary antibodies in 3% BSA for 1 h. After a brief washing, the cells were incubated with secondary antibodies conjugated with FITC or TRITC (Jackson ImmunoResearch Laboratories, West Baltimore Pike West Grove, PA, USA), mounted in a mounting solution with DAPI, and observed with a fluorescence microscope (Leica, Bensheim, Germany). Affinity purified antibody against NIP1 was used in concentrations of 0.1 μg protein/section and the FITC- or TRITC-conjugated secondary antibody was diluted to 1:200.

In vitro kinase assay

The Nek2 kinase assay was carried out as described previously [20]. In brief, 293T cells were transfected with either a wild-type Nek2 (*pNek2RHA1*) or a kinase-defect Nek2 (*pNek2KHA5*; the lysine at the 37th residue was substituted to leucine) expression vector. The cells were lysed with lysis buffer (50 mM HEPES, pH 7.5, 5 mM $MnCl_2$, 10 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA, 100 mM NaCl, 5 mM KCl, 0.1% Nonidet P-40, DNase I 30 μg/ml, RNase 30 μg/ml, and heparin 1 μg/ml) and subjected to immunoprecipitation with an antibody against the HA tag. The immunoprecipitates were washed with the lysis buffer three times and with the kinase buffer (50 mM HEPES, pH 7.5, 5 mM $MnCl_2$, 5 mM NaF, 5 mM β-glycerophosphate, and 1 μg/ml heparin) once. Kinase reactions were carried out for 20 min at 30°C in the kinase buffer supplemented with 4 μM ATP, 1 mM dithiothreitol, and 10 μCi [gamma-^{32}P]ATP, in a total volume of 20 μl. The substrates were included at 0.25 mg protein/ml. The reactions were stopped by the addition of 20 μl of 2× Laemmli sample buffer and heated for 5 min at 95°C. Protein samples were resolved with 10% SDS-PAGE and electroblotted onto a nitrocellulose membrane. The membrane was exposed to X-ray film.

In vivo labeling assay

COS7 cells were labeled by incubation for 4 h in a phosphate-free DMEM medium that had been supplemented with 10% dialyzed FBS and 400 μCi/ml of phosphoric acid. Cells were collected and lysed with lysis buffer (0.4% Igepal, 0.1% deoxycholic acid, 150 mM NaCl, 50 mM Tris–HCl, pH 8.0, 20 mM β-glycerophosphate, 20 mM NaF, and 0.3 mM $NaVO_4$). The lysates were immunoprecipitated with anti-HA or anti-FLAG antibodies, incubated with protein A Sepharose, washed three times with lysis buffer, suspended in 2× Laemmli sample buffer, heated for 10 min at 95°C, resolved by 10% SDS-PAGE, and electroblotted onto a nitrocellulose membrane. The membrane was exposed to X-ray film.

Results

Identification of NIP1

To gain insight into the cellular processes in which Nek2 participates, we carried out yeast two-hybrid screen with Nek2 as bait and isolated *Nek2-Interacting Protein 1* (*NIP1*) along with several other clones [19]. A human *NIP1* cDNA clone of 1915 bp contained a 362 amino acid-long putative ORF that started from a canonical Kozak sequence (access number AF409141). The mouse *NIP1* gene was also isolated on the basis of its homology with the human *NIP1* cDNA. Sequence analysis revealed that NIP1 is identical to XB51, which was known to interact with X11L/Mint2 [23,24], and to NECAB3, a Ca^{2+}-binding protein isolated from brain lysates [25].

Northern blot hybridization analysis was carried out to determine *NIP1* expression in several cell lines and tissues. The *NIP1* was expressed ubiquitously in human cell lines (Fig. 1). In mouse tissues, the NIP1 transcript was detected abundantly in the brain, but not in the testis and liver (Fig. 1). However, the presence of NIP1 transcripts in diverse tissues including the testis was confirmed with the reverse transcriptase-PCR method (data not shown). These results suggest that NIP1 is a ubiquitous protein, but exists more abundantly in selected tissues, such as the brain.

Interaction of NIP1 with Nek2

To determine the interaction of NIP1 with Nek2, we carried out GST pull-down assay. The bacterially expressed GST-NIP1 protein was incubated with lysates of *Nek2*-overexpressed BOSC cells and precipitated with glutathione Sepharose 4B beads. The GST antibody detected both the

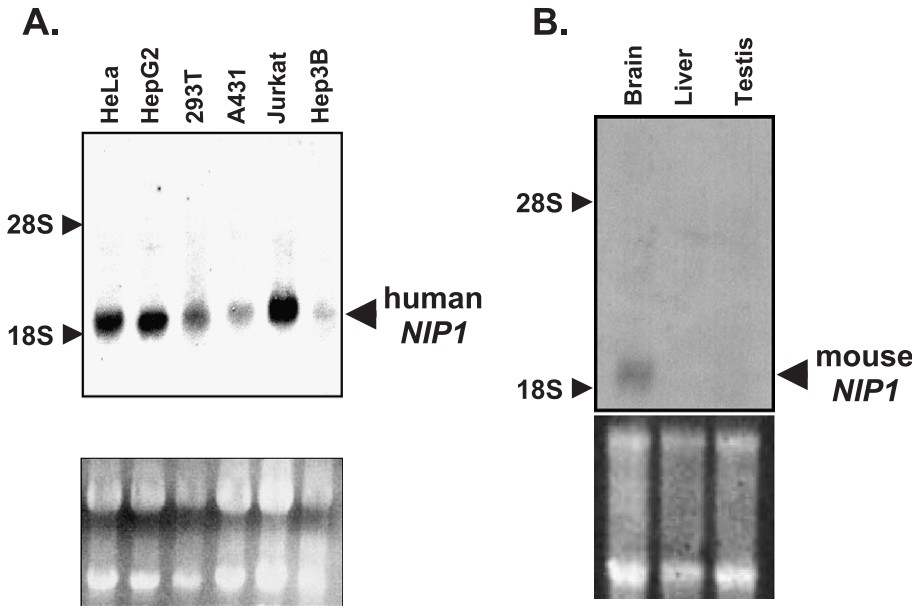

Fig. 1. Northern blot hybridization analyses of *NIP1* with human cell lines and mouse tissues. Total RNA (10 μg) (A) from several human cell lines, including HeLa, HepG2, 293T, A431, Jurkat, and Hep3B cells, and (B) from a few mouse tissues, including brain, liver, and testis, were used for the analysis. Equal loading of the RNA samples was confirmed with ethidium bromide staining of the 18S and 28S rRNA bands (lower panel). A specific human *NIP1* band of 2.0 kb was detected in all human cell lines tested while a specific mouse *NIP1* band of 2.0 kb was detected prominently in the brain.

GST and the GST-NIP1 proteins, while the NIP1 antibody detected the GST-NIP1 protein in the precipitates (Fig. 2). The 47-kDa band marked with an asterisk is likely a proteolytic fragment of the GST-NIP1 protein. In such conditions, Nek2 was coprecipitated with GST-NIP1, but not with GST, suggesting that NIP1 interacts with Nek2 specifically (Fig. 2).

Co-immunoprecipitation experiments were carried out to examine physical interactions between Nek2 and NIP1 proteins in cell. BOSC cells that had been transfected transiently with the HA-tagged *Nek2* construct (*pNek2RHA1*) and the FLAG-tagged *NIP1* construct (*pNIP1FLAG*) were lysed with RIPA buffer and subjected to immunoprecipitation using anti-NIP1 or anti-Nek2 polyclonal antisera. Monoclonal antibodies against the HA or FLAG epitopes were used for detection of exogenous gene products in the immunoprecipitants.

The polyclonal antibodies against Nek2 and NIP1 could immunoprecipitate the target proteins specifically, but did not cross-react with each other (Fig. 3A, lanes 7 and 8). At

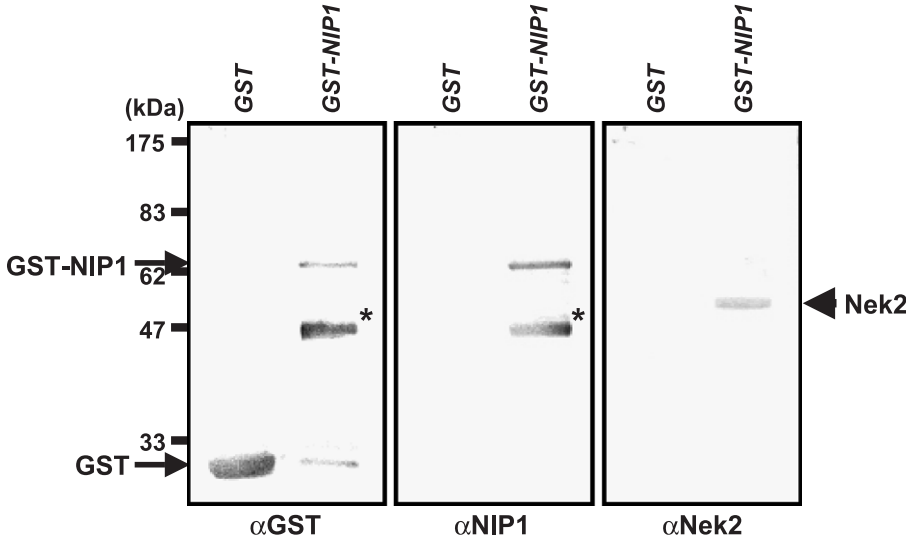

Fig. 2. Interaction of NIP1 with Nek2 in vitro. The GST pull-down assay was carried out by incubating the GST-NIP1 fusion protein with lysates of the *Nek2*-overexpressed BOSC cells. The GST protein was used as control. Precipitated proteins were analyzed with antibodies against GST, NIP1, and Nek2. Specific bands for GST-NIP1, GST, and Nek2 were marked. A band marked with asterisk is likely a proteolytic fragment of NIP1.

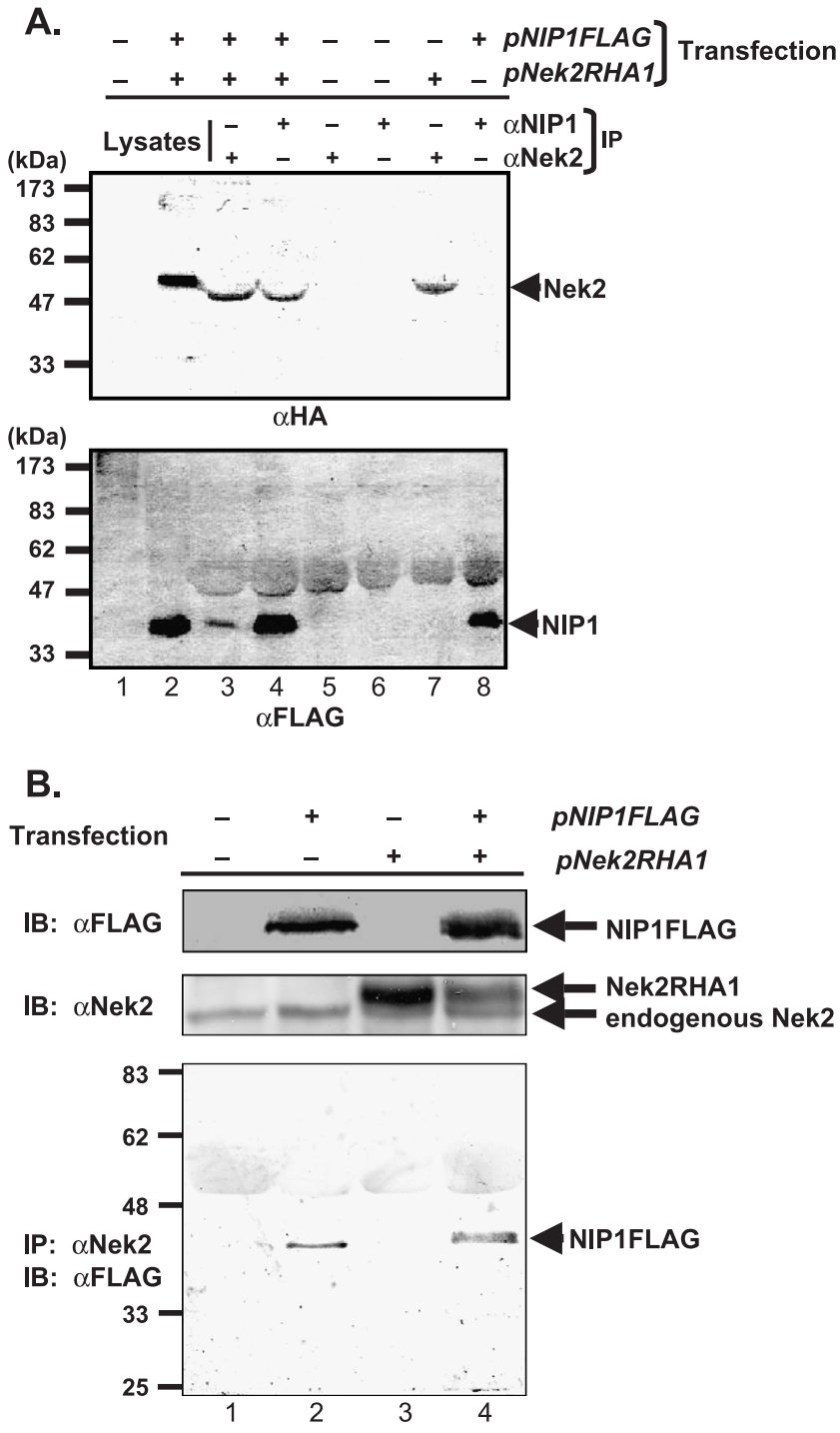

Fig. 3. Direct interaction of NIP1 and Nek2 in cell. (A) The cell lysates were obtained from BOSC cells that had been transfected transiently with the HA-tagged Nek2 (*pNek2RHA1*) or the FLAG-tagged NIP1 (*pNIP1FLAG*) constructs. The cell lysates were immunoprecipitated with either anti-NIP1 or anti-Nek2 polyclonal antisera, followed by immunoblot analysis using anti-HA or anti-FLAG antibodies. As a control, cell lysates were used directly for the immunoblot analysis. (B) Interaction of NIP1 with the endogenous Nek2 protein. *pNek2RHA1* and *pNIP1FLAG* were transfected into BOSC cells and their expression was confirmed with antibodies specific to the FLAG tag and Nek2. The endogenous Nek2 protein was also detected with the Nek2 antibody. Immunoprecipitation was carried out with the Nek2 antibody and the co-immunoprecipitated NIP1 protein was detected with the FLAG antibody.

the same time, NIP1FLAG was co-immunoprecipitated with Nek2 (Fig. 3A, lane 3), while Nek2RHA1 was co-immunoprecipitated with NIP1 (Fig. 3A, lane 4). These results indicate that Nek2 and NIP1 interact directly in the cell. Migration of the Nek2-specific band in Fig. 3A was distorted due to the IgG heavy chain proteins of almost identical migration speed with Nek2 on the SDS-PAGE gel.

We were not able to detect an endogenous NIP1 protein with the immunoblot analysis probably because the cellular NIP1 level is very low. Nevertheless, we were able to detect interaction of NIP1 with endogenous Nek2 proteins (Fig. 3B). When *NIP1FLAG*-expressed BOSC cells were immunoprecipitated with the Nek2 antibody, the NIP1FLAG was coprecipitated not only with an exogenous Nek2 protein (Fig. 3B, lane 4) but also with the endogenous Nek2 protein (Fig. 3B, lane 2). The amount of co-immunoprecipitated NIP1 protein was somewhat proportional to the amount of cellular Nek2 proteins. These results reinforce the notion that interaction of Nek2 with NIP1 occurs in a physiological condition.

Subcellular localization of NIP1

In immunoblot analyses with lysates from *pNIP1FLAG*-transfected BOSC cells, a single specific band of 42 kDa was detected both with the FLAG antibody and with the NIP1 polyclonal antibody (Fig. 4A). However, we were not able to detect an endogenous NIP1 protein in the untransfected BOSC cell as well as in other cell lines that we had tested, suggesting that the endogenous NIP1 protein is present at a very low level in cultured cells. Nevertheless, we confirmed the specificity of the NIP1 antibody that we had generated.

The COS7 cells that had been transfected with *pNIP1-FLAG* were co-immunostained with antibodies specific to the NIP1 protein and the FLAG epitope. The results showed that both antibodies stained in an identical pattern around the nuclei of the transfected cells (Fig. 4B). Similar staining patterns were also observed in other cell lines, such as HeLa, NIH3T3, and BOSC cells (data not shown).

Since the staining pattern in Fig. 4B suggests localization of NIP1 in the Golgi or ER, we decided to co-immunostain NIP1 with marker proteins for Golgi (Rab6) or ER (BiP). The results showed that localization of NIP1 overlapped with both Rab6 and BiP in some extent, but they were not exactly identical: the distribution of exogenous NIP1 looked broader than Rab6, but more restricted than BiP (Fig. 4C). However, it was clear that NIP1 was associated with ER/Golgi. Our observations are consistent with the previous report in which the exogenous XB51 protein was localized around the nucleus, possibly anchored to ER or cis-Golgi membrane components [23].

Detection of the endogenous NIP1 protein was not easy, probably because extremely small amounts of the

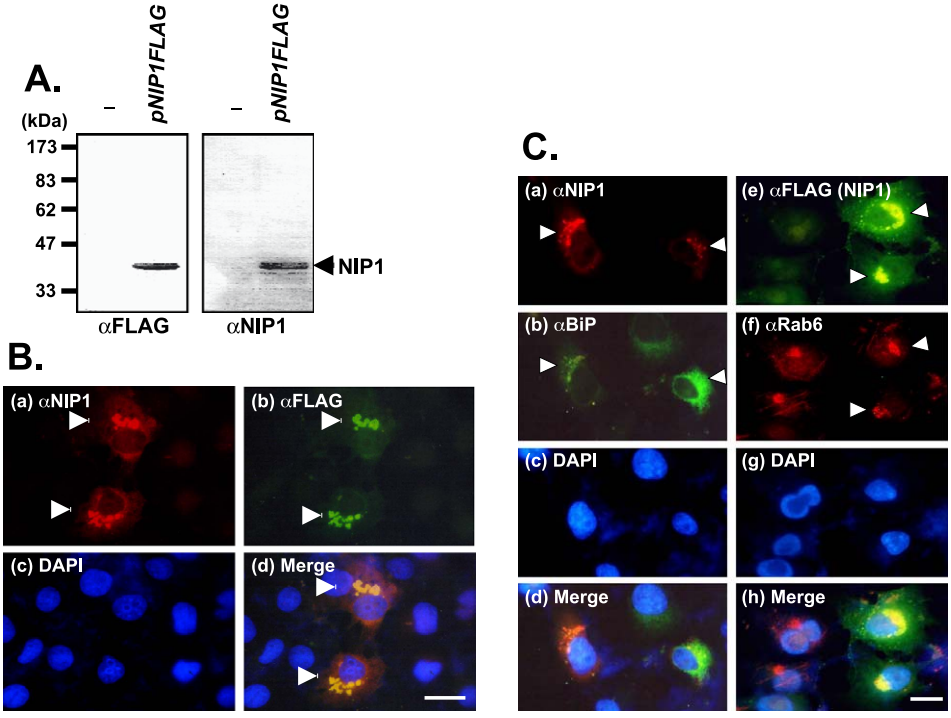

Fig. 4. Detection of the NIP1 protein. (A) Immunoblot analysis was carried out with lysates (10-μg protein) of the BOSC cells transfected with *pNIP1FLAG*. Both the FLAG and NIP1 antibodies were used to detect a specific band of 42 kDa. (B) COS7 cells that had been transfected transiently with *pNIP1FLAG* were co-immunostained with antibodies against the NIP1 (a) and FLAG epitope (b). The nucleus was stained with DAPI (c). Panel d is a merged image. The arrows indicate specific signals of the antibodies. (C) Subcellular localization of exogenous NIP1 in the Golgi. COS7 cells that had been transfected transiently with *pNIP1FLAG* were analyzed immunocytochemically. The subcellular localization of NIP1, which was immunostained with antibodies against NIP1 (a) or FLAG epitope (e), was compared with BiP, an ER marker protein (b), and Rab6, a Golgi marker protein (f). The nucleus was stained with DAPI (c, g). Panels d and h are merged images. Arrows indicate localization of NIP and the ER/Golgi marker proteins. Scale bar = 25 μm.

NIP1 protein exist in cultured cells. However, we were able to detect specific signals of NIP1 in the immunostaining experiments. The NIP1 antibody specifically stained the Golgi body, which had been confirmed with co-immunostaining with the antibody specific to NSF, a Golgi marker (Fig. 5A). No specific signals were observed when the NIP1 fusion proteins were added to the staining mixture or when the primary antibody was omitted (data not shown).

Golgi localization of the NIP1 protein was confirmed by treatment of a pharmacological reagent that is known to affect the Golgi structure (Fig. 5B). It was known that brefeldin A induces extensive retrograde transport of Golgi components to the ER mediated by growth of Golgi tubules, leading to a complete loss of the Golgi structure [26]. As a result, the Golgi structure of brefeldin A-treated cells looked scattered throughout the cell. Subcellular localization of the endogenous NIP1 in the K28 Leydig cell line also looked scattered in brefeldin A-treated cells (Fig. 5B). These results support Golgi localization of NIP1.

Phosphorylation of NIP1 by Nek2

We carried out in vitro kinase assay of Nek2 with NIP1 as a substrate. As reported previously, the wild-type Nek2

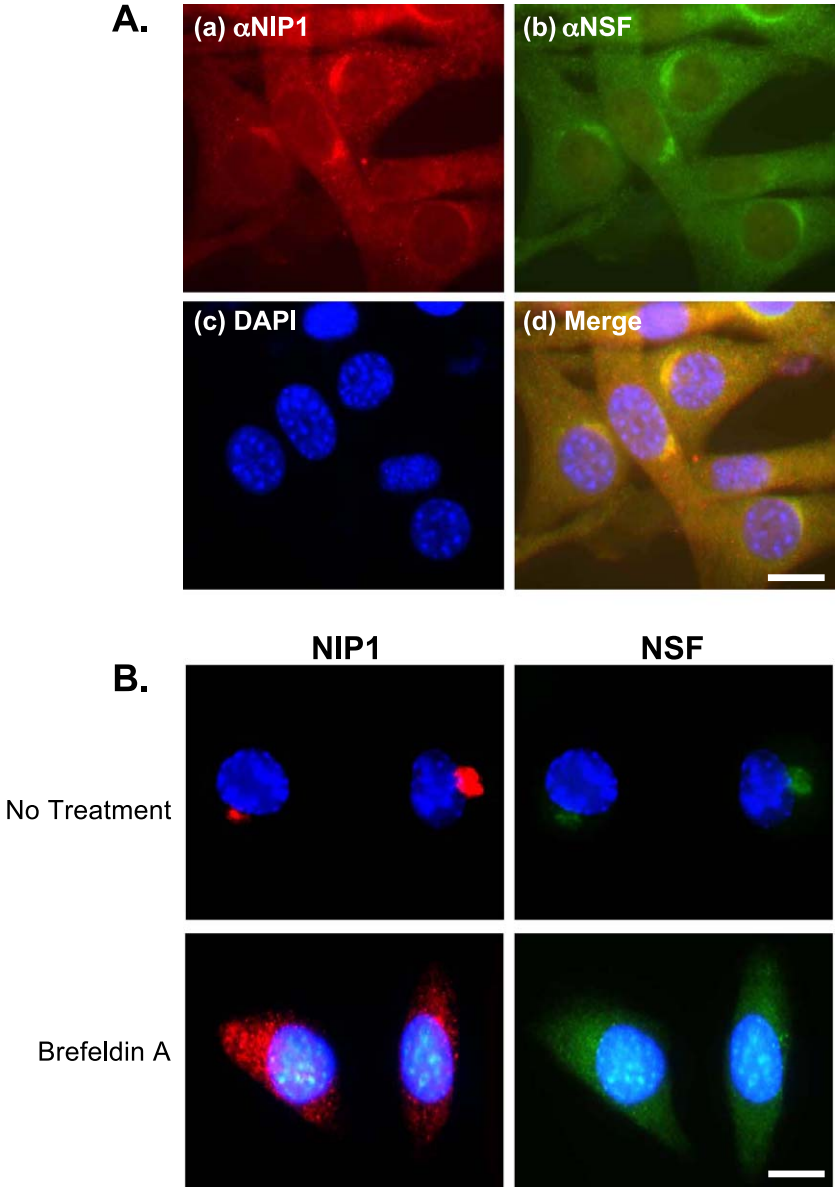

Fig. 5. Golgi localization of the endogenous NIP1 protein. (A) NIH3T3 cells were co-immunoprecipitated with antibodies against NIP1 (a) and NSF, a Golgi marker (b). The nucleus was stained with DAPI (c). Panel d is a merged image. (B) K28 Leydig cell line was treated with brefeldin A (5 μg/ml), a Golgi disruptor, for 1 h and immunostained with antibodies specific to NIP1 and NSF. The nucleus was stained with DAPI. Scale bar = 25 μm.

(Nek2RHA1) was able to phosphorylate casein as well as Nek2 itself, but not BSA or GST (Fig. 6). In such conditions, Nek2 phosphorylated the GST-NIP1 protein specifically. The kinase-defect Nek2 (Nek2KHA5) did not phosphorylate any substrate at all, as expected.

To confirm phosphorylation of NIP1 by Nek2, we carried out in vivo labeling experiments (Fig. 7). COS7 cells transfected with *pNIP1FLAG* along with wild type or mutant Nek2 constructs (*pNek2RHA1* or *pNek2KHA5*) were cultured in the presence of radioactive ortho-phosphate. When the NIP1FLAG proteins were immunoprecipitated with the FLAG antibody, they were strongly labeled with radioactive phosphate in the presence of wild type Nek2 (Nek2RHA1), but not of kinase-defect Nek2 (Nek2KHA5). At the same time, we detected the autophosphorylated Nek2RHA1 which has been co-immunoprecipitated with NIP1FLAG. Autophosphorylation of Nek2 was confirmed with autoradiogram of the immunoprecipitants with the HA antibody. At this time, phosphorylation of the co-immunoprecipitated NIP1-FLAG was also detected. These results indicate that Nek2 not only interacts with NIP1 but also phosphorylates it in vivo.

It should be mentioned that the labeling intensity of NIP1 by exogenous Nek2 appeared somewhat higher than expected (Fig. 7), considering that the exogenous Nek2 was by just several fold more abundant than the endogenous Nek2 in a cell (Fig. 3B). One possibility might be that a relatively large mount of exogenous Nek2 proteins in a transfected cell would be free of interaction with a limited amount of available protein phosphatase 1 which was known to form a complex with Nek2 and to play an opposite role against kinase activity of Nek2 [11]. As a result, the phosphate residues attached to NIP1 by the exogenous Nek2 would have less chance to be removed than those by the endogenous Nek2–phosphatase complex.

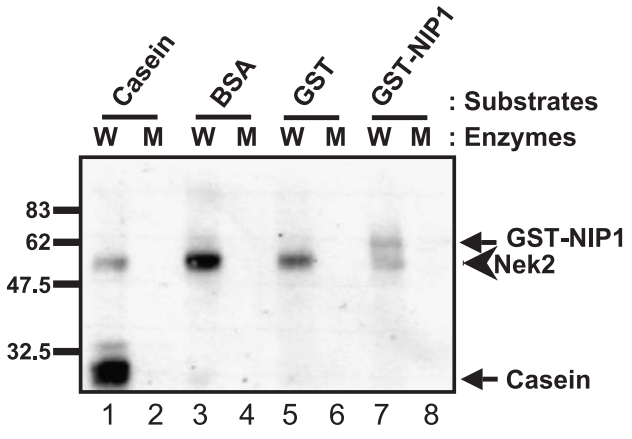

Fig. 6. In vitro kinase assay. Lysates were prepared from 293T cells transfected with *pNek2RHA1* (wild type, W) or *pNek2KHA5* (kinase-defect mutant, M) and subjected to immunoprecipitation with the HA antibody. In vitro kinase assay was carried out with casein, BSA, GST, and GST-NIP1 as substrates. Phosphorylated casein and GST-NIP1 were marked with arrows while autophosphorylated Nek2 was marked with an arrowhead.

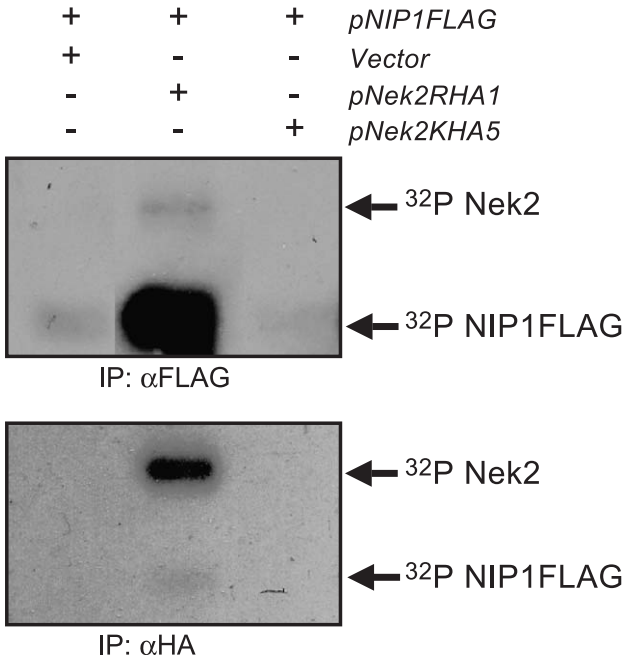

Fig. 7. In vivo labeling of NIP1. COS7 cells transfected with *pNIP1FLAG* along with vector *pNek2RHA1* (wild type Nek2) or *pNek2KHA5* (kinase-defect Nek2) were cultured in the presence of $^{32}PO_4^{2-}$ in the medium. The cell lysates were immunoprecipitated with the FLAG and HA antibodies for exogenous NIP1 and Nek2 proteins, respectively. The immunoprecipitants were subjected to SDS-PAGE followed by autoradiography.

Obviously, this hypothesis should be tested thoroughly in the future.

Discussion

The present study reports that Nek2, a mitotic regulator, interacts with NIP1, a Golgi protein. It appears that NIP1 is a substrate of Nek2. The importance of Nek2 on the centrosome has been studied most extensively [6]. Involvement of Nek2 on meiotic chromosomal condensation [27] and segregation [28] has also been suggested. We believe that the current study expanded biological functions of Nek2 to the Golgi body. Such diverse roles of Nek2 have been predicted from a previous report in which Nek2 localization was not limited to centrosome, but to other subcellular organelles too [17].

NIP1 is identical to XB51, which was isolated as an interacting protein of X11L/Mint2 [26]. X11L was known to interact with the cytoplasmic domain of amyloid precursor protein and to reduce β-amyloid secretion in cultured cell conditions [29]. X11L was also named as Mint2, which is a neuronal adaptor protein that binds to Munc18, a protein essential for synaptic vesicle exocytosis [30]. When *XB51* was coexpressed, it blocked X11L functions in controlling the generation of β-amyloid in a noncompetitive mechanism, suggesting involvement of XB51 in protein secretion pathways [23]. In fact, we observed that protein secretion

was dramatically reduced when *NIP1* was overexpressed in a cell (unpublished data).

NIP1 was also known as NECAB3, one of the neuronal Ca^{2+}-binding proteins [25]. The NECAB family, which consists of three members so far, shares structural characteristics with a single EF-hand domain for Ca^{2+} binding at the N terminal end [25]. The general functions of EF-hand Ca^{2+}-binding proteins have not been fully understood yet, but are suggested as Ca^{2+}-dependent activators of target proteins or as Ca^{2+}-buffers [25,31]. In addition, Ca^{2+} binding to EF-hand domain might regulate activities of adjacent domains within the same protein. Therefore, it is possible that biological activities of NIP1 are controlled by intracellular Ca^{2+} as well as by phosphorylation.

The biological significance of Nek2 interaction with NIP1 remains to be studied. One possibility might be the involvement of Nek2 in Golgi in heritance. In fact, it was known that protein phosphorylation is involved directly or indirectly in the fragmentation and dispersal of the Golgi membrane at the onset of mitosis. A few kinases such as Cdc2 [32], mitogen-activated protein kinase kinase 1 [32,33], and Plk [34,35] have been implicated in mitosis-specific Golgi fragmentation, and Nek2 could be a new member. Another possibility might be the involvement of Nek2 in cell cycle stage-specific regulation of exocytosis. It was proposed that vesicular transport is reduced in cells that enter mitosis [36] and protein phosphorylation is involved in the mitotic arrest of exocytic and endocytic activities [37]. We currently test both hypotheses by investigating specific changes in the NIP1 protein functions induced by Nek2.

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