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The Heterotrimeric Kinesin-2 Family Member KIF3A Directly Binds to Creatine Kinase B

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Heterotrimeric kinesin-2 is a molecular motor protein of the kinesin superfamily (KIF) that moves along a microtubule plus-end directed motor protein. It consists of three different motor subunits (KIF3A, KIF3B, and KIF3C) and a kinesin-associated protein 3 (KAP3) that form a heterotrimeric complex. Heterotrimeric kinesin-2 interacts with many different binding proteins through the cargo-binding domain of the KIF3s. The activity of heterotrimeric kinesin-2 is regulated to ensure that the cargo is directed to the right place at the right time. How this regulation occurs, however, remains in question. To identify the regulatory proteins for heterotrimeric kinesin-2, we performed yeast two-hybrid screening and found a specific interaction with creatine kinase B (CKB), which is the brain isoform of cytosolic creatine kinase enzyme. CKB bound to the cargo-binding domain of KIF3A but did not interact with the KIF3B, KIF5B, or KAP3 in the yeast two-hybrid assay. The carboxyl (C)-terminal region of CKB is essential for the interaction with KIF3A. Another protein kinase, CaMKIIa, interacted with KIF3A, but GSK3a did not interact with KIF3A in the yeast two-hybrid assay. KIF3A interacted with GST-CKB-C but not with GSK-CKB-N or GST alone. When co-expressed in HEK-293T cells, CKB co-localized with KIF3A and co-immunoprecipitated with KIF3A and KIF3B but not KIF5B. These results suggest that the CKB-KIF3A interaction may regulate the cargo transport of heterotrimeric kinesin-2 under energy-compromised conditions in cells.

Key words : Binding protein, creatine kinase, kinesin, phosphorylation

Introduction

The intracellular transport of membrane-bounded vesicles and organelles contributes for morphogenesis and functioning of the many cell type. Microtubules are polymerized with tubulin polymers, α-tubule and β-tubule at plus ends, organized in a radial array from the cell body with plus ends directed toward the cell periphery [5]. Two major microtubule-based motor proteins have been identified, kinesin and dynein. Kinesins are plus-end-directed molecular motor proteins that move along microtubule tracks [5, 18]. These proteins play the key roles in moving of various kinds cargoes, including membrane vesicles, organelles, and protein complex [5]. Kinesin-2 is the amino (N)-terminal motor domain member of kinesin superfamily protein (KIF) [6, 18]. It represents two subfamilies of heterotrimeric and homodimeric motors found in mammalian cells [6, 18]. Heterotrimeric kinesin-2 is essential for the intracellular transport of cargoes and intraflagellar transport (IFT) along cilia that is essential for the ciliary and flagellar assembly [2, 18]. Several diseases are linked to the loss of activity of heterotrimeric kinesin-2, such as Kartagener's syndrome, and cystic kidney [3, 6, 18].

KIF3, a member of the heterotrimeric kinesin-2, forms a heterotrimeric complex that consists of three different kinesin motor proteins (KIF3A, KIF3B and KIF3C), which moved

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toward the plus ends of microtubule and a kinesin associated protein 3 (KAP3), which was associated with the tail region of KIF3A and KIF3B [6, 21]. Heterotrimeric kinesin-2 is abundantly expressed in nerve tissue and ubiquitously expressed in other tissues [18, 21].

Previously studies suggested that KIF3s play an important role in the intraflagellar transport (IFT) complex and axonal transport of fodrin and polarity complex vesicles [18, 20]. In the *Caenorhabditis elegans*, tubulin is transported into the sensory cilia by KIF3s [9, 17]. KIF3s knock-out mice exhibit randomized establishment of left-right asymmetry [6]. The molecular mechanisms of this left-right asymmetry were approached microscopically by observing the ventral node, which is a ciliated organ transiently exposed on the surface of the ventral midline [6]. Thus, KIF3s plays important roles for anterograde IFT into the flagella and cilia formation in many species and in many cell types [9, 17]. Also, KIF3 has been implicated in intracellular transport of vesicles and organelles [6]. For example, in neuronal cultured cells, anti-KIF3B antibody microinjection and immunoprecipitation experiments suggested that KIF3 moves the fodrin-containing vesicles along microtubule [20]. Previous study of KIF3A using RNA interference, and live cell imaging suggested that KIF3 mediates the intracellular transport of human immunodeficiency virus (HIV)-containing compartment along microtubule in HIV-infected cells [4].

The binding proteins of heterotrimeric kinesin-2 have been studied in detail to the specific cargo level. However, less is well studied about how heterotrimeric kinesin-2 is regulated when bound to cargo, released to cargo, and the regulation of kinesin motor activity. Understanding how heterotrimeric kinesin-2 is able to regulate the loading, unloading of their cargo, and the regulation of motor activity remain to be important question. In this study, we screened for proteins that bind with the cargo-binding domain of KIF3A, and found protein interacting with brain type creatine kinase (CKB), which is plays an important role in the cellular energy metabolism and homeostasis by catalyzing the transfer of phosphate between ATP and creatine phosphate [11]. The interaction of KIF3A and CKB suggests that CKB may be regulate the heterotrimeric kinesin-2 activity under energy-compromised conditions in cells.

Materials and Methods

Plasmid constructs

Full-length mouse glycogen synthase kinase 3a (GSK3a), and calcium-calmodulin-dependent kinase II a (CaMKIIa), and the carboxyl (C)-terminal region of KIF3B were amplified by polymerase chain reaction (PCR) from Marathon-Ready™ cDNA library (Clontech, Palo Alto, CA, USA) and cloned into pGEM T-easy vector (Promega Corp, Madison, WI, USA). Mouse KIF3A and the cargo-binding domain (aa 585-701) of KIF3A were subcloned from pCAGGS-KIF3A obtained from Prof. Kaibuchi K. Nagoya University, Nagoya, Japan [14] into the *Eco*RI and *Xho*I restriction sites of the pLexA. The resulting recombinant plasmid, pLexA-cargo-binding domain-KIF3A, was used as bait plasmid this yeast two-hybrid screening. The series of deletion mutants of mouse KIF3A were subcloned from pCAGGS-KIF3A.

Screening of KIF3A-binding proteins by yeast two-hybrid assay

The Matchmaker LexA two-hybrid system was used for screening according to the manufacturer's manual (Clontech). In brief, pLexA-cargo-binding domain-KIF3A was transformed into yeast strain EGY48 carrying the p8op-lacZ gene. Transformed cells were transformed with the mouse brain cDNA library [20] and grown on synthetic dextrose (SD) plates supplemented with glucose but with no histidine, tryptophan, or uracil (SD/-His/-Trp/-Ura). The selection of positive clones was performed on an SD/-His/-Trp/-Ura/-Leu plate containing galactose, raffinose, X-gal, and BU salts. Plasmids from positive clones were analyzed by *Eco*RI and *Xho*I restriction digestion. Unique inserts were sequenced and protein sequence analysis was performed with the BLAST algorithm at the National Center for Biotechnology Information (NCBI). Sequence-verified clones were tested again for interactions of with the bait in yeast by the retransformation.

β-Galactosidase activity in liquid cultures of yeast

The β-galactosidase activity of yeast was assayed as described previously [20]. Mid-log phase yeast cells were collected and permeabilized with 0.1% sodium dodecyl sulphate (SDS) and chloroform. An excess amount of o-nitrophenyl-β-D-galactoside (ONPG) (Sigma-Aldrich, St. Louis, MO, USA) was added to yeast lysate, and the mixture was incubated for each time at 30℃, and then the reaction was stopped by increasing pH to 11 by the addition of 1 M Na_2CO_3. The formation of the reaction product, o-nitrophenol, was determined by measuring absorbance at 420 nm

on a spectrophotometer and normalizing for the reaction time. The units of enzyme activity were calculated by the following equation: units=1,000 × [(OD_{420} - 1.75 × OD_{550})]/(reaction time × culture volume × OD_{600}) [1].

Glutathione S-transferase (GST) pull-down assays

cDNA encoding the full length, the N-terminal region, and the C-terminal region of CKB was cloned in pET41a. The recombinant GST-CKB fusion protein was expressed in bacterial strain BL21 GOLD (Stratagene, La Jolla CA, USA) after induction with 0.5 mM isopropyl thio-β-D-galactopyranoside (IPTG) for 2.5 hr. The fusion proteins were purified using glutathione-agarose beads (Sigma-Aldrich) according to the manufacturer's protocol. The mouse brain S2 fraction was incubated overnight at 4℃ with the GST fusion protein-coupled glutathione beads. The beads were pelleted by centrifugation, washed three times with the extraction buffer (1% Triton X-100 in PBS containing 10 μg/ml each aprotinin, leupeptin, and pepstatin and 1 μM phenylmethanesulfonyl fluoride), and once with PBS. The pulled-down proteins were analyzed by Western blotting with anti-KIF3A antibody [20]. The animal study was approved by the institutional review board (IRB), and the approval number was 17-12 of Inje University animal center.

Cell culture and transfection

Human embryonic kidney (HEK)-293T [American Type Culture Collection (ATCC) CRL-3216] cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin at 37℃ in a humidified 5% CO_2 incubator. Transient transfections were performed using the $CaPO_4$ precipitation method [15].

Immunocytochemistry

For immunocytochemistry analysis, HEK-293T cells grown on poly-D-lysine-coated coverslips were transfected with KIF3A and EGFP-CKB constructs. Twenty-four hours after transfection, cells were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde in PBS for 5 min, and permeabilized with 0.2% Triton X-100 in PBS for 10 min. After blocking with 5% normal goat serum in PBS for 30 min, cells were incubated overnight at 4℃ with anti-KIF3A antibody [20] diluted 1:500 in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20. After washing 3 times with PBS, cells were incubated for 40 min with Dylight 594-conjugated goat anti-rabbit IgG antibody (Jackson ImmunoResearch Labs, West Grove, PA, USA) diluted 1:800. After washing 3 times with PBS, the cells were mounted with Fluoromount (DAKOKorea, Seoul, Korea). Fluorescence images were acquired on Zeiss LSM510 META confocal laser scanning microscope (Carl Zeiss, Oberkochem, Germany).

Co-immunoprecipitation and immunoblot analysis

HEK-293T cells were transfected with myc-KIF3A and FLAG-CKB constructs. Cells were rinsed with ice-cold PBS twice and lysed with ice-cold lysis buffer [PBS containing 0.5% NP-40 and 1x protease inhibitor cocktail set V (Calbiochem, San Diego, CA, USA)] by gentle rotation for 30 min. Lysates were centrifuged at 16,000× *g* for 10 min at 4℃. The supernatant was incubated with anti-FLAG M2 agarose beads (Sigma-Aldrich) for 2 hr at 4℃ with constant shaking. The beads were collected by centrifugation at 2,000x *g* for 30 sec and washed 5 times with ice-cold PBS containing 0.5% NP-40. The washed beads were resuspended with Laemmli's loading buffer and the proteins were eluted and denatured by boiling for 5 min. The proteins were processed for 10% SDS-PAGE and immunoblot analysis with antibodies against KIF3A, KIF3B, KIF5B, myc, and FLAG epitope as described elsewhere by Nakajima et al. [15].

Immunocytochemistry

HEK-293T cells grown on poly-D-lysine-coated coverslips were transfected with enhanced green fluorescent protein (EGFP)-CKB, and myc-KIF3A constructs. Twenty-four hours after transfection, cells were washed with PBS, fixed with 4% paraformaldehyde in PBS for 5 min, and permeabilized with 0.2% Triton X-100 (Sigma-Aldrich) in PBS for 10 min. After blocking with 5% normal goat serum in PBS for 30 min, cells were incubated overnight at 4℃ with anti-KIF3A antibody [Nakajima] in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20 (Sigma-Aldrich). After washing three times with PBS, cells were incubated for 40 min with Dylight 594-conjugated goat anti-rabbit IgG antibody (1:800, cat. no. 111-516-046; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). After washing three times with PBS, the cells were mounted with Fluoromount (DAKO, Santa Clara, CA, USA). Fluorescence images were acquired on a Zeiss LSM510 META confocal laser-scanning microscope (Carl Zeiss Inc).

Results

Identification of KIF3A interacting proteins by yeast two-hybrid assay

Heterotrimeric kinesin-2 consists of two identical motor subunits (KIF3A, KIF3B, and KIF3C) whose an N-terminal motor domains use energy from ATP and are linked by a C-terminal globular tail domain that associates with KAP3 and binds to various proteins or cargoes [6, 21]. To identify the specific interacting proteins and regulating proteins of heterotrimeric kinesin-2, we used the C-terminal cargo-binding domain (aa 585-701) of KIF3A fused to the DNA-binding domain of pLexA as a bait and isolated positive clones from a mouse brain cDNA library (Fig. 1A). From 1×10^7 colonies screened, we obtained eight positive clones. The positive clones turned out to possess CKB cDNA fragments (Fig. 1C). All positive clones overlapped at the open reading frame (ORF) of CKB and possessed cDNA fragments corresponding to the C-terminal region of CKB (Fig. 1C).

KIF3A is composed of an N-terminal motor domain, a coiled-coil rod domain, and a C-terminal globular tail domain that binds the KAP3 and various cargoes [21]. Previously study identified that the C-terminal tail region of KIF3A has three phosphorylation sites, S689, T694, and S698 [7]. Interestingly, these three phosphorylation sites were located on the cargo-binding domain of KIF3A. The

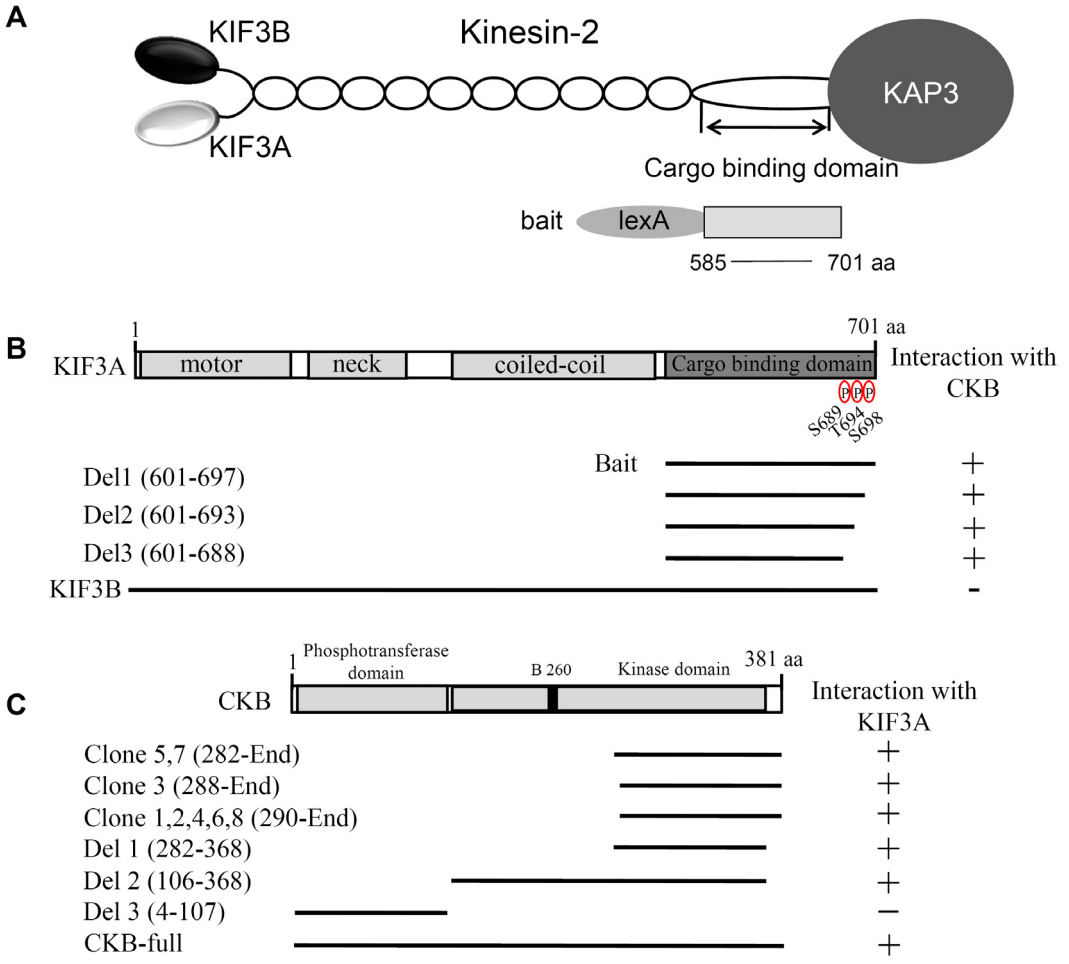

Fig. 1. Identification of the proteins interacted with KIF3A by yeast two-hybrid screening. (A) Schematic diagram of heterotrimeric kinesin 2. The cargo-binding domain of KIF3A used for the yeast two-hybrid screen. (B) CKB binding region in KIF3A. KIF3A has the motor domain and cargo binding domain, indicated in gray. The truncated forms of KIF3A were assayed in the yeast two-hybrid assay for interaction with CKB. (C) KIF3A binding region in CKB. The positive clones isolated from the yeast two-hybrid screening possesses the cDNA for CKB. CKB has phosphotransferase domain and kinase domain, indicated in gray. The truncated forms of CKB were assayed in the yeast two-hybrid assay for interaction with KIF3A. +, interaction; -, no interaction; KIF3A, kinesin superfamily protein 3A; KIF3B, kinesin superfamily protein 3B; CKB, creatine kinase; aa, amino acids.

phosphorylation of KIF3A facilitates cargo loading and intracellular transport in cells [7]. Next, we examined the effects of the C-terminal phosphorylation sites of KIF3A on binding with CKB and KIF3A. We constructed several deletion mutants of the C-terminal phosphorylation sites and tested the interaction with CKB and KIF3A. As shown in Fig. 1B, the deletion mutants of the C-terminal phosphorylation sites of KIF3A interact with CKB. This data suggests that the phosphorylation sites of KIF3A did not affect the interaction of KIF3A with CKB. CKB was discovered as one of three cytosolic isoforms of creatine kinase (CK) that is predominantly expressed in the brain [11]. It is a multi-domain protein composed of the phosphotransferase domain, and the kinase domain [11]. To identify the domain of CKB required for the interaction with KIF3A, a series of deletion mutants of CKB was constructed and analyzed their interactions with KIF3A using the yeast two-hybrid assay. Only the C-terminal region of CKB interacted with KIF3A in the yeast two-hybrid assay (Fig. 1C). This experiment demonstrated that the minimal binding domain was located in the C-terminal region of CKB. Next, we investigated whether CKB interacts with KIF3B, KIF5B, or KAP3. CKB did not interact with KIF3B, KIF5B, and KAP3 (Fig. 2A). This data suggest that CKB specifically interacts with KIF3A. Both CaMKIIa and GSK3a are known to be important protein kinases in the intracellular cargo transport [5, 7]. Next, we investigated whether KIF3A interacts with these protein kinases. As shown in Fig. 2B, KIF3A interact with CKB and CaMKIIa. However, KIF3A did not interact with GSK3a. To quantify the binding affinity of CKB to KIF3A, the full length of CKB, or the C-terminal region of KIF3A, KIF3B, or KIF5B expression plasmids were transformed to yeast and the β-galactosidase activity was measured in liquid cultures. The interaction of CKB with KIF3A yielded approximately 402 units of β-galactosidase activity (Fig. 2C). Together, these results show that CKB specifically interacts with KIF3A.

To confirm the KIF3A and CKB interaction at the protein level, the interaction between KIF3A and full length CKB, CKB-N-terminal domain, or CKB-C-terminal domain was assayed using a GST pull-down experiments. Recombinant GST-CKB-full, GST-CKB-N-terminal domain, or GST-CKB-C-terminal domain fusion proteins were expressed in *E. coli*.

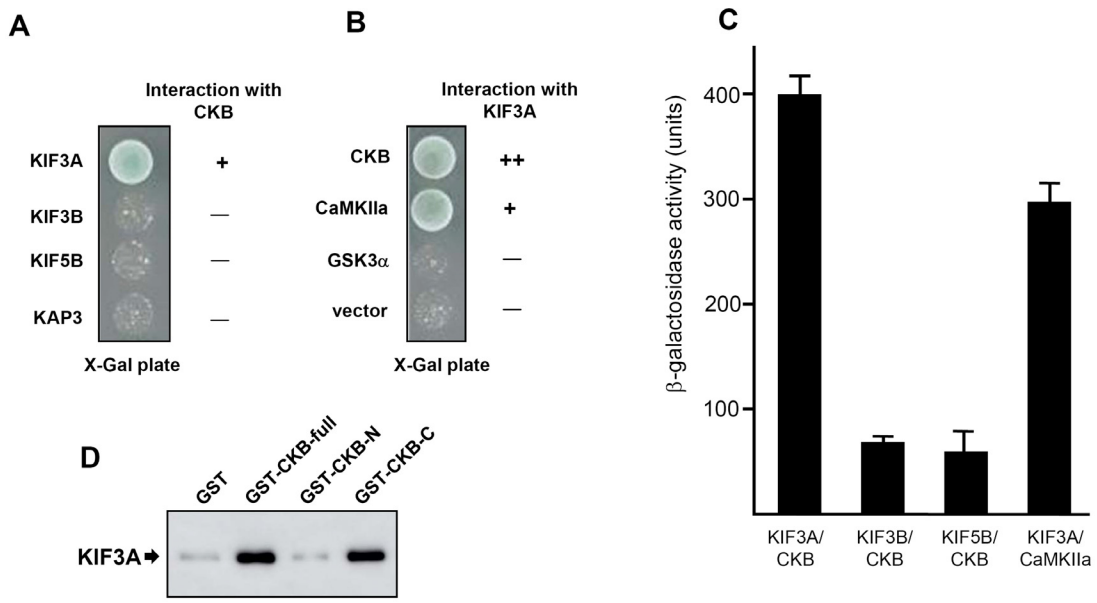

Fig. 2. Interaction of KIF3A or KIFs with CKB. (A) The tail regions of each KIF and the full length KAP3 were tested for the interaction with CKB in the yeast two-hybrid system. CKB specifically interacted with KIF3A, but not with KIF3B, KIF5B, and KAP3. (B) CKB, CaMKIIa, or GSK3a were tested for the interaction with KIF3A in the yeast two-hybrid system. KIF3A interacted with CKB, and CaMKIIa, but not with GSK3a. CaMKIIa served as a positive control for interaction. (C) The strength of interactions between CKB and KIFs was examined quantitatively using β-galactosidase activity in the yeast two-hybrid reporter assay. Values are presented as the mean ± standard deviation. (D) Direct binding of KIF3A to CKB in a GST pull-down assay using purified GST-CKB, GST-CKB-N, and GST-CKB-C. +, interaction; -, no interaction; KIF3A, kinesin superfamily protein 3A; KIF3B, kinesin superfamily protein 3B; KIF5B, kinesin superfamily proteins 5B; CKB, creatine kinase; KAP3, kinesin superfamily-associated protein 3; GST, glutathione S-transferase; CaMKIIa, Calcium-calmodulin-dependent kinase II a.

The purified GST fusion proteins are allowed to interact with mouse brain lysates. Immunoblotting analyses revealed that KIF3A interacted with GST-CKB-full length, and GST-CKB-C-terminal domain but not with GST, and GST-CKB-N-terminal domain (Fig. 3A). This result indicates that CKB associates with KIF3A at protein level.

Heterotrimeric kinesin-2 is associated with CKB in cells

Heterotrimeric kinesin-2 is composed of a KIF3A/KIF3B heterodimer and KAP3, forming a heterotrimeric complex [6, 21]. To address the question whether KIF3A mediates the interaction of CKB and heterotrimeric kinesin-2, we performed co-immunoprecipitation from HEK-293T cells that were transfected with myc-KIF3A and FLAG-CKB. Anti-FLAG antibody precipitated KIF3A and endogenous KIF3B; however, KIF5B (a KHC of kinesin-1) did not (Fig. 4A). Also, anti-myc antibody precipitated CKB and endogenous KIF3B, but not KIF5B (Fig. 4B). These results indicate that CKB interacts with KIF3A bound to KIF3B. CKB localized not only to the cytosol but also to the nucleus [14]. In order to address whether KIF3A and CKB co-localize in cells, KIF3A was co-expressed with EGFP-CKB in HEK-293T cells. KIF3A and CKB extensively overlapped at some subcellular region in cells (Fig. 4C, allow). Taken together, these results indicate that CKB is a binding partner of heterotrimeric kinesin-2 through the binding with KIF3A.

Discussion

In this study, we show that KIF3A interacts with CKB. Using the cargo-binding domain of KIF3A as bait, we identified CKB in a yeast two-hybrid assay. The C-terminal region of CKB interacted with the cargo-binding domain of KIF3A. Furthermore, using a co-immunoprecipitation, we showed that heterotrimeric kinesin-2 can be co-precipitated with CKB. Taking all of these results together, we hereby suggest that heterotrimeric kinesin-2 is a new binding partner for CKB and CKB may be regulate the cargo transport of heterotrimeric kinesin-2.

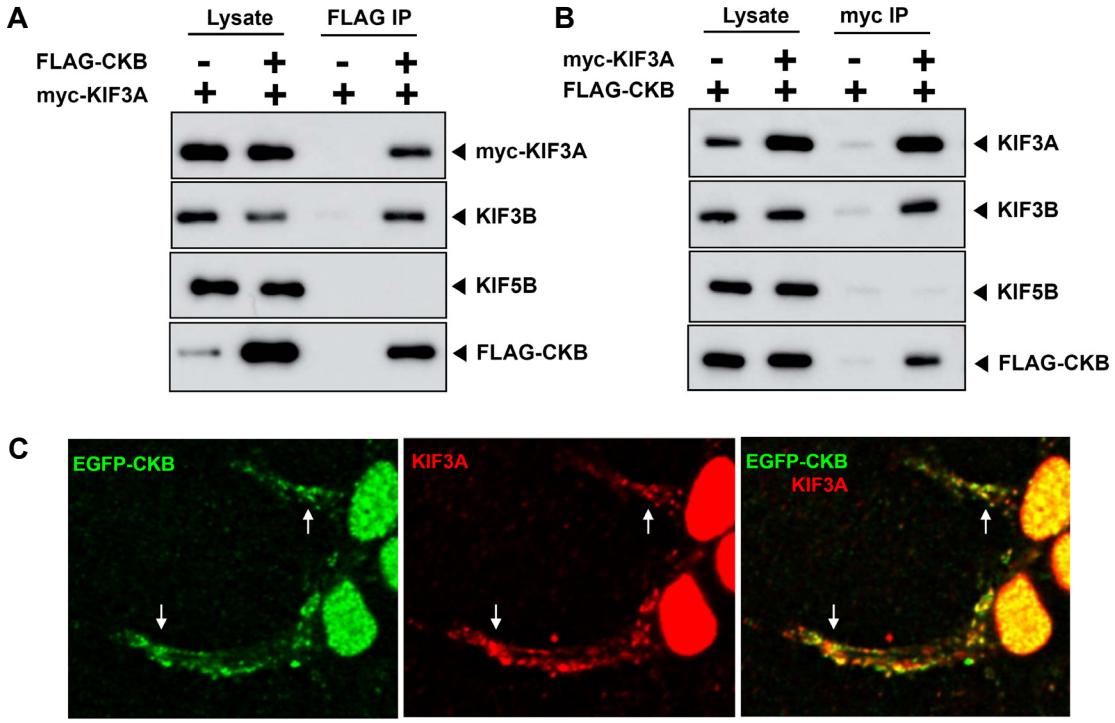

Fig. 3. Co-immunoprecipitation and co-localization of KIF3A and CKB in mammalian cells. (A and B) HEK-293T cells were transiently transfected with FLAG-CKB and myc-KIF3A plasmids as indicated. Cell lysates were immunoprecipitated with (A) monoclonal anti-FLAG antibody or (B) anti-myc antibody. Precipitates were immunoblotted with anti-KIF3A, KIF3B, KIF5B and FLAG antibodies. CKB specifically co-precipitated KIF3A and KIF3B, but not with KIF5B. (C) HEK-293T cells were transiently transfected with EGFP-CKB plasmids. Twenty-four hours after transfection, cells were subjected to immunofluorescence with anti-KIF3A antibody. KIF3A and CKB were observed in the same subcellular region in cells. KIF, kinesin superfamily proteins; CKB, creatine kinase; EGFP, enhanced green fluorescent protein; IP, immunoprecipitation.

CK catalyzes the reversible phosphoryl transfer between ATP and creatine [18]. CKB is a major enzyme of cellular energy metabolism in nonmuscle cells. In mammalian, two major cytosolic CK isoforms are found: muscle-type (CKM), and brain-type [11]. Two isoforms have a high conserved domain structure and share 77%-88% amino acid sequence identity [11]. KIF3A interacted with CKB and CKM in the yeast two-hybrid assay (data not shown). KIF3A and KIF3B has a high conserved motor domain, stalk domain, and the C-terminal cargo-binding domain [21]. However, KIF3A C-terminal region that mediate the interaction with various binding proteins or cargoes has low similarity to KIF3B [7, 21]. We found that CKB interacts only with the C-terminal region of KIF3A. Previous study has reported that phosphorylation of the C-terminal tail region of KIF3A modulates the cargo binding [7]. The phosphorylation of KIF3A on the S689, T694, and S698 stimulates binding of N-cadherin whereas phosphorylation of KIF17 disrupts the binding of Mint1 to the tail domain of KIF17 [5, 7]. We found that three phosphorylation sites of KIF3A do not affect CKB binding affinity.

What would the interaction between KIF3A and CKB mean? We are able to suggest one possibility is that CKB may be role an adaptor protein that links heterotrimeric kinesin-2 and cargo. In many cases, various cargoes interact with adaptor proteins/scaffolding proteins that mediate the attachment to kinesins [5, 15]. CKB interacts with various proteins, some of which positively or negatively regulate cytoskeletal remodeling. Predominant actin isoforms α-actin and β-actin, interacted with CKB and partial colocalized with CKB in cells [13, 18]. Earlier studies showed that CKB directly interacts with sodium-calcium exchanger (NCX) [22]. Also, CKB interacted with the cytosolic tail of the protease-activated receptor (PAR)-1 and positively regulated PAR-1-mediated signal transduction in astrocytes [13]. Thus, CKB may serve as an adaptor protein that links heterotrimeric kinesin-2 and cytoskeletal proteins, PAR-1, and/or NCX.

Another possibility is that CKB may be regulate heterotrimeric kinesin-2 activity by changing the ATP concentration in the intracellular transport. Intracellular transport that deploy kinesin to move the subcellular cargoes by using energy released from ATP hydrolysis play critical roles in cells [5]. Heterotrimeric kinesin-2 uses the energy of ATP hydrolysis to generate force and movement along microtubules [6, 19]. CKB is known to be important for the maintenance of cellular energy homeostasis [10, 12]. It catalyzes the reversible transfer of a phosphate group from phosphocreatine to ADP to yield ATP and creatine [10, 14]. In earlier study, CKB has been shown to have a physical interaction with the ATP-sensitive K^+ channels and regulates K^+ channels activity by controlling the ATP/ADP ratio near these channels. Moreover, under energy-compromised conditions CKB translocation from the cytosol to the channels localization sites [8, 18]. Previously study confirms that the C-terminal tail region of KIF3A and CKB have phosphorylation sites [7, 16]. These sites were phosphorylated by PKC, and CaMKIIa [7, 12]. The phosphorylation of KIF3A facilitates the cargo transport by heterotrimeric kinesin-2 [7]. It is possible that heterotrimeric kinesin-2 interacts with CKB under control conditions, and under energy-compromised conditions CKB is phosphorylated by PKC or other protein kinase, which further induces the maintaining heterotrimeric kinesin-2 activity. In this study, a direct interaction between KIF3A and CKB is able to provide evidence to support a novel mechanism for the regulation of heterotrimeric kinesin-2 activity in intracellular transport.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : Heterotrimeric kinesin-2의 KIF3A와 creatine kinase B의 결합

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Kinesin은 세포의 중심부에서 세포막쪽으로 미세소관을 따라 이동하며, heterotrimeric kinesin-2는 kinesin superfamily (KIF)의 한 종류로 미세소관의 plus방향으로 이동하는 분자 모터 단백질이다. Heterotrimeric kinesin-2는 모터 활성을 가지는 3종류(KIF3A, KIF3B와 KIF3C)와 kinesin-associated protein 3 (KAP3)이 결합한 형태로 KIF3s의 운반체 결합 영역을 통하여 다양한 결합 단백질과 결합한다. 그러나, 다양한 운반체를 수송하는 heterotrimeric kinesin-2를 조절하는 조절단백질에 대하여서는 아직 밝혀지지 않았다. 본 연구에서는 heterotrimeric kinesin-2를 조절하는 조절단백질을 단백질을 분리하기 위하여 KIF3A의 운반체 결합 영역과 결합하는 단백질을 효모 two-hybrid system을 사용하여 탐색한 결과, 뇌에 특이적으로 발현하는 세포질 크레아틴 키나아제(CKB)를 분리하였다. CKB의 C-말단은 KIF3A의 운반체 결합 영역과 결합하지만, KIF3B, KIF5B와 KAP3과는 결합하지 않았다. 다른 단백질 키나아제인 CaMKIIa는 KIF3A와 결합하지만 GSK3a는 KIF3A와 결합하지 않았다. 또한 KIF3A은 GST-CKB-C와는 결합하지만 GST-CKB-C와 GST와는 결합하지 않았다. HEK-293T세포에 CKB와 KIF3A을 동시에 발현시켰을 때 두 단백질은 세포 내에서 같은 부위에 존재하며, CKB 혹은 KIF3A을 면역침강한 결과 KIF3A뿐만 아니라 KIF3B와도 같이 침강함을 확인하였다. 이러한 결과들은 CKB-KIF3A 결합은 세포내에서 에너지가 부족되는 조건에서 heterotrimeric kinesin-2의 운반체 수송을 조절할 가능성을 시사한다.