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The cytoplasmic region of the amyloid β-protein precursor (APP) is necessary and sufficient for the enhanced fast velocity of APP transport by kinesin-1

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Amyloid β-protein precursor (APP) is transported mainly by kinesin-1 and at a higher velocity than other kinesin-1 cargos, such as Alcadein α (Alcα); this is denoted by the enhanced fast velocity (EFV). Interaction of the APP cytoplasmic region with kinesin-1, which is essential for EFV transport, is mediated by JNK-interacting protein 1 (JIP1). To determine the roles of interactions between the APP luminal region and cargo components, we monitored transport of chimeric cargo receptors, Alcα (luminal)–APP (cytoplasmic) and APP (luminal)–Alcα (cytoplasmic). Alcα-APP is transported at the EFV, whereas APP-Alcα is transported at the same velocity as wild-type Alcα. Thus, the cytoplasmic region of APP is necessary and sufficient for the EFV of APP transport by kinesin-1.

Keywords: alcadein; APP; axonal transport; calsyntenin; JIP; kinesin-1

Kinesin-1, a major anterograde transport motor in neurons, was the first molecular motor shown to move on microtubules in axons [1]. Kinesin-1 functions as a heterotetramer composed of two kinesin light chains (KLCs) and two kinesin heavy chains (KHCs) (reviewed in ref. [2]). The KHC contains the ATP-binding motor domain and attaches to the microtubule, whereas the KLC interacts with cargo receptors such as Alcadein α (Alcα)/calsyntenin-1 or adaptor proteins such as JNK-interacting protein 1 (JIP1), which mediates the interaction between KLC and cargo receptors including amyloid β-protein precursor (APP) and apolipoprotein E receptor 2 (ApoER2) [3–6]. The C-terminal region of KHC can also associate directly or indirectly with cargo and organelles such as mitochondria, thus KHC without KLC also functions as KIF5 to execute anterograde transport (reviewed in ref. [7]).

Kinesin-1 moves on microtubules in 8 nm steps with a velocity of 1.36 $\mu m \cdot s^{-1}$ *in vitro* at 35 °C [8], which is almost the same temperature used in the presented study with cells cultured at 37 °C. *In vivo*, however, some kinesin-1 cargos are transported on microtubules faster than the gliding velocity measured in *in vitro* biophysical studies [5]. Alcα associates with KLC directly, and

Abbreviations
Alcα, Alcadein α; APP, amyloid β-protein precursor; EGFP, enhanced green fluorescent protein; JIP, c-Jun NH2-terminal kinase (JNK)-interacting protein; KHC, kinesin heavy chain; KLC, kinesin light chain; TIRF, total internal reflectance fluorescence.

Alcα cargo in axons is transported by kinesin-1 at a velocity of 1.5–1.8 $\mu m \cdot s^{-1}$ [5,9], consistent with the kinesin-1 velocity calculated *in vitro* [8]. In contrast to Alcα, APP associates with KLC either directly or via JIP1 [5,10]. In neurons, in the presence of JIP1, APP predominantly associates with KLC via JIP1, and this association (APP–JIP1–KLC–KHC) generates the enhanced fast velocity (EFV). Consequently, APP cargo in axons is transported with a velocity of 2.5–3.5 $\mu m \cdot s^{-1}$, which is almost 1.5–2-fold faster than the transport of Alcα cargo [5,6]. Although we cannot rule out a possibility that JIP1 and/or KLC may recruit another motor, the APP–KLC interaction mediated by JIP1 is essential for the EFV of APP cargo transport [6], and the molecular regulation of EFV generation was recently revealed [11]. JIP1 interacts with the NPTY motif in the cytoplasmic domain of APP [12], and this interaction is responsible for connecting the APP cargo to kinesin-1. However, it remains unclear whether the interaction of the APP cytoplasmic domain with kinesin-1, mediated by JIP1, is sufficient to generate the EFV of APP cargo transport. Alternatively, a further association of the APP luminal region with one or more factors within the APP cargo vesicle may be required. Such interactions could contribute to EFV generation by inducing a conformational change of the cytoplasmic region associated with JIP1. Consistent with this possibility, the cytoplasmic region of APP can dynamically alter its conformation [13–15]. To assess whether the luminal region of APP is required to generate the EFV of APP cargo transport, we designed chimeric cargo receptors composed of the luminal domain of Alcα conjugated to the cytoplasmic domain of APP (Alcα-APP-EGFP or αA-EGFP) or the luminal domain of APP conjugated to the cytoplasmic domain of Alcα (APP-Alcα-EGFP or Aα-EGFP). We analyzed these chimeric proteins, along with APP-EGFP and Alcα-EGFP, to determine the velocity of their anterograde axonal transport.

Experimental procedures

Plasmid construction

Human cDNAs encoding APP695-EGFP, Alcα1-EGFP, and FLAG-JIP1b in vector pcDNA3.1 were described previously [5,16,17]. Chimera Aα-EGFP protein was designed by combining APP (1–648), the N-terminal fragment of APP695 truncated at amino acid position 648 (C-terminal of the transmembrane [TM] domain), with Alcα (871–971), a C-terminal fragment of Alcα that includes the cytoplasmic (Cyt) domain. To generate Aα-EGFP chimeric cDNA, template pcDNA3.1-hAlcα1-EGFP was subjected to an initial PCR in the presence of APP-TM/Alcα-Cyt primer (forward, GTCATCACCTTGGTGATGCTGCGGATCC GGGCCGCAC) and N-terminal EGFP primer (reverse, GCCCTCGCCGGACACGCTGAA) using ExTaq DNA polymerase (Takara-Bio, Inc., Kusatsu-Shiga, Japan). The PCR product was purified and used as the reverse primer for a second PCR with pcDNA3.1-APP695-EGFP as the template and pcDNA3.1 forward primer (CAGAGCTCT CTGGCTAACTAG). The product of the second PCR product was purified and digested with *Nhe*I and *Cla*I, and then inserted into pcDNA3.1 with a C-terminal EGFP sequence to generate pcDNA3.1-APP/Alcα-EGFP (Aα-EGFP) chimeric plasmid.

Chimeric αA-EGFP protein was designed by combining Alcα1 (1–870), an N-terminal fragment of Alcα1 truncated at amino acid position 870 (C-terminal of the TM domain), with APP (649–695), a C-terminal fragment of APP695 that includes the cytoplasmic (Cyt) domain. To generate αA-EGFP chimeric cDNA, pcDNA3.1-APP-EGFP was used as the template for PCR in the presence of Alcα-TM/APP-Cyt primer (forward, GATTATCCTGGGGGTATTTAAG AAGAAACAGTACACA) and N-terminal EGFP primer (reverse) as described above. In the second PCR, template pcDNA3.1-Alcα1-EGFP was subjected to thermal cycles in the presence of the first PCR product with Platinum Pfx DNA polymerase (Invitrogen/Thermo Fisher Scientific, Carlsbad, CA, USA). The template plasmid included in the product mixture was digested with *Dpn*I, and the product was transformed into *E coli* to generate pcDNA3.1-Alcα/APP-EGFP (αA-EGFP) chimeric plasmid.

Antibodies, coimmunoprecipitation assay, and immunoblot analysis

Mouse N2a neuroblastoma cells were transfected with plasmid using Lipofectamine 2000 (Invitrogen/Thermo Fisher Scientific) or Polyethylenimine Max (Polysciences, Inc. Warrington, PA, USA), and cultured for 24 h. The cells were lysed in HBS-T (20 mM HEPES pH 7.6, 150 mM NaCl, 0.5% [v/v] Triton X-100) containing protease inhibitor cocktail (25 $\mu g \cdot mL^{-1}$ chymostatin, 25 $\mu g \cdot mL^{-1}$ leupeptin, and 25 $\mu g \cdot mL^{-1}$ pepstatin). Cell lysates were subject to immunoprecipitation with anti-FLAG antibody (M2, Sigma-Aldrich, St. Louis, MO, USA) plus Protein G–Sepharose 4B (GE Healthcare Bio-Sciences, Little Chalfont, UK). Immunoprecipitates and cell lysates were separated by electrophoresis on a 9% (w/v) polyacrylamide Tris-glycine gel, transferred onto a nitrocellulose membrane, incubated with the indicated antibodies and HRP-linked anti-mouse or -rabbit IgG antibodies, and detected by ECL (GE Healthcare Bio-Sciences).

Anti-α-tubulin 10G10 (Wako Pure Chemicals, Ltd., Osaka, Japan), anti-GFP (598; Medical & Biological Laboratories, Nagoya, Japan), and anti-FLAG M2 (Sigma-Aldrich) antibodies were purchased from the indicated suppliers. Rabbit polyclonal anti-APP cytoplasmic antibody and anti-Alcα cytoplasmic region antibody UT195 were

described previously [18,19]. HRP-linked sheep anti-mouse IgG and anti-rabbit IgG antibodies were purchased from GE Healthcare Bio-Sciences.

Total internal reflectance fluorescence (TIRF) microscopy analysis

CAD cells (mouse CNS catecholaminergic cell line) were cultured in an eight-well cover-glass chamber coated with poly L-lysine and transfected with the indicated plasmid using Lipofectamine 2000. After 6 h of culture, cells were subjected to differentiation for 16 h [20]. Mouse primary cultured cortical neurons were prepared from embryonic day 15.5–16 C57BL/6 strain with a modified version of the methods previously described [21]. The neurons were spread and cultured at 5×10^4 cells/cm^2 in Neurobasal Medium (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA) containing 30% (v/v) Nerve-Cell Culture Medium (DS Pharma Biomedical, Osaka Japan), 2% (v/v) B-27 Supplement (Invitrogen/Thermo Fisher

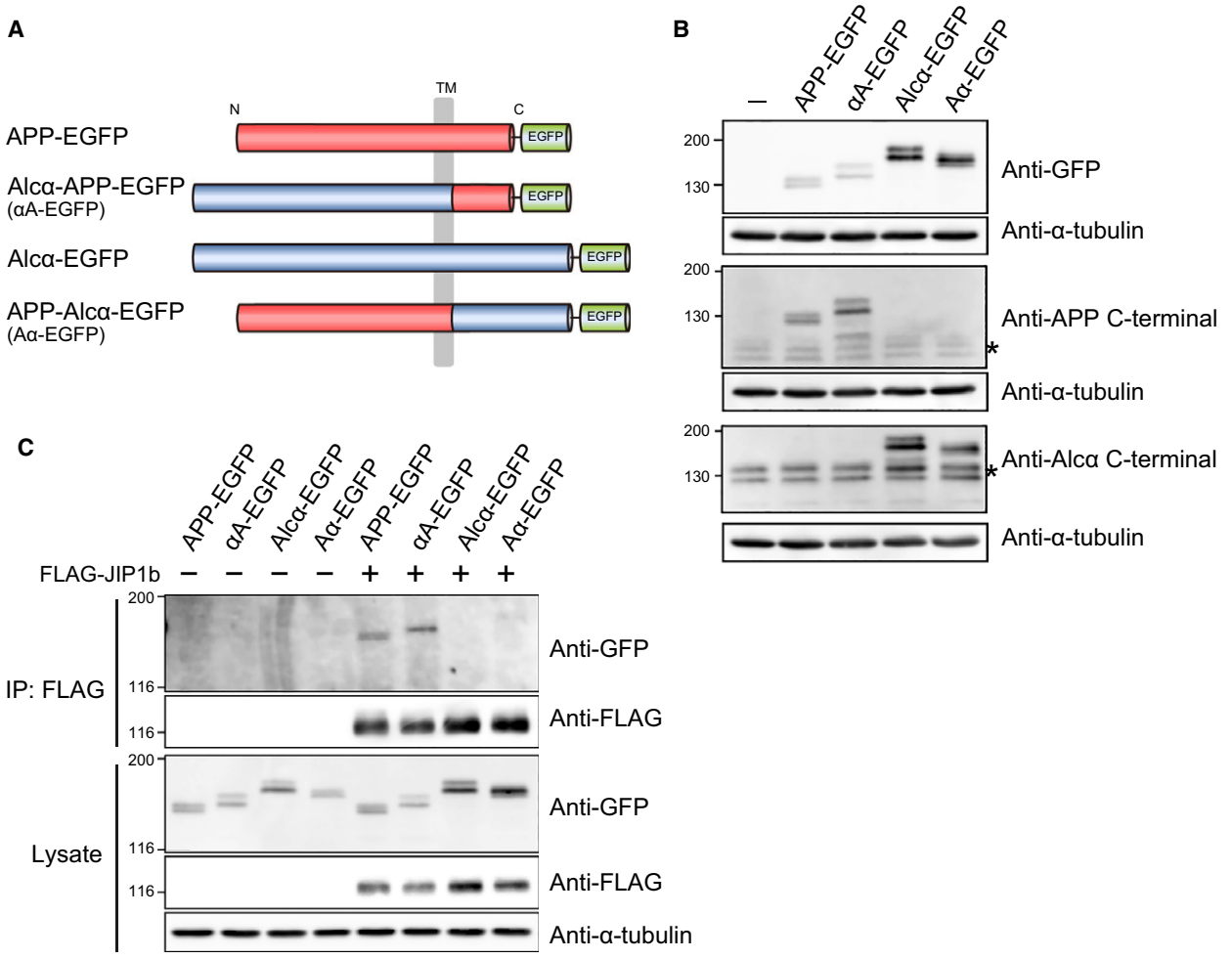

Fig. 1. Construction, expression, and JIP1b interaction of Alcα-APP (αA) and APP-Alcα (Aα) chimeric proteins. (A) Schematic structure of cargo proteins used in this study. Structures of chimeric and wild-type cargo receptors C-terminally fused to EGFP are shown. Alcα-APP (αA) consists of Alcα (1–870), the luminal plus transmembrane region, and APP (649–695), the cytoplasmic region. APP-Alcα (Aα) consists of APP (1–648), the luminal plus transmembrane region, and Alcα (871–971), the cytoplasmic region. TM, transmembrane region; EGFP, enhanced green fluorescence protein. (B) Expression of cargo proteins in cells. Plasmids pcDNA3.1-Alcα-APP(αA)-EGFP and pcDNA3.1-APP-Alcα(Aα)-EGFP were transiently transfected into N2a cells along with pcDNA3.1-hAPP695-EGFP and pcDNA3.1-hAlcα1-EGFP. Cell lysates were subjected to immunoblot analysis with anti-GFP (upper), anti-APP cytoplasmic (middle), or anti-Alcα cytoplasmic (lower) antibodies, along with anti-α-tubulin antibody. Asterisks indicate endogenous APP and Alcα proteins. (C) Interaction of JIP1b with cargo receptors. Chimeric and wild-type cargo proteins were expressed in cells with or without FLAG-JIP1b, and their interactions were examined by coimmunoprecipitation assay with anti-FLAG antibody. Proteins were detected by immunoblotting with anti-GFP (upper in IP and Lysate), anti-FLAG (lower in IP and middle in Lysate), and anti-α-tubulin (lower in Lysate) antibodies. Cotransfection of pcDNA3-FLAG-JIP1b (+) or pcDNA3 vector alone (-) is indicated. Numbers indicate molecular size markers (in kDa).

Scientific), 4 mM Glutamax, 5% (v/v) heat-inactivated horse serum, and antibiotics (Invitrogen/Thermo Fisher Scientific). After 5 days of culture, the neurons were transfected with the indicated plasmids using the calcium phosphate method, cultured for 8 h, and analyzed by total internal reflectance fluorescence (TIRF) microscopy.

Cargo transport in living neuronal cells in an incubation chamber supplied with 5% CO_2 at 37 °C was observed on

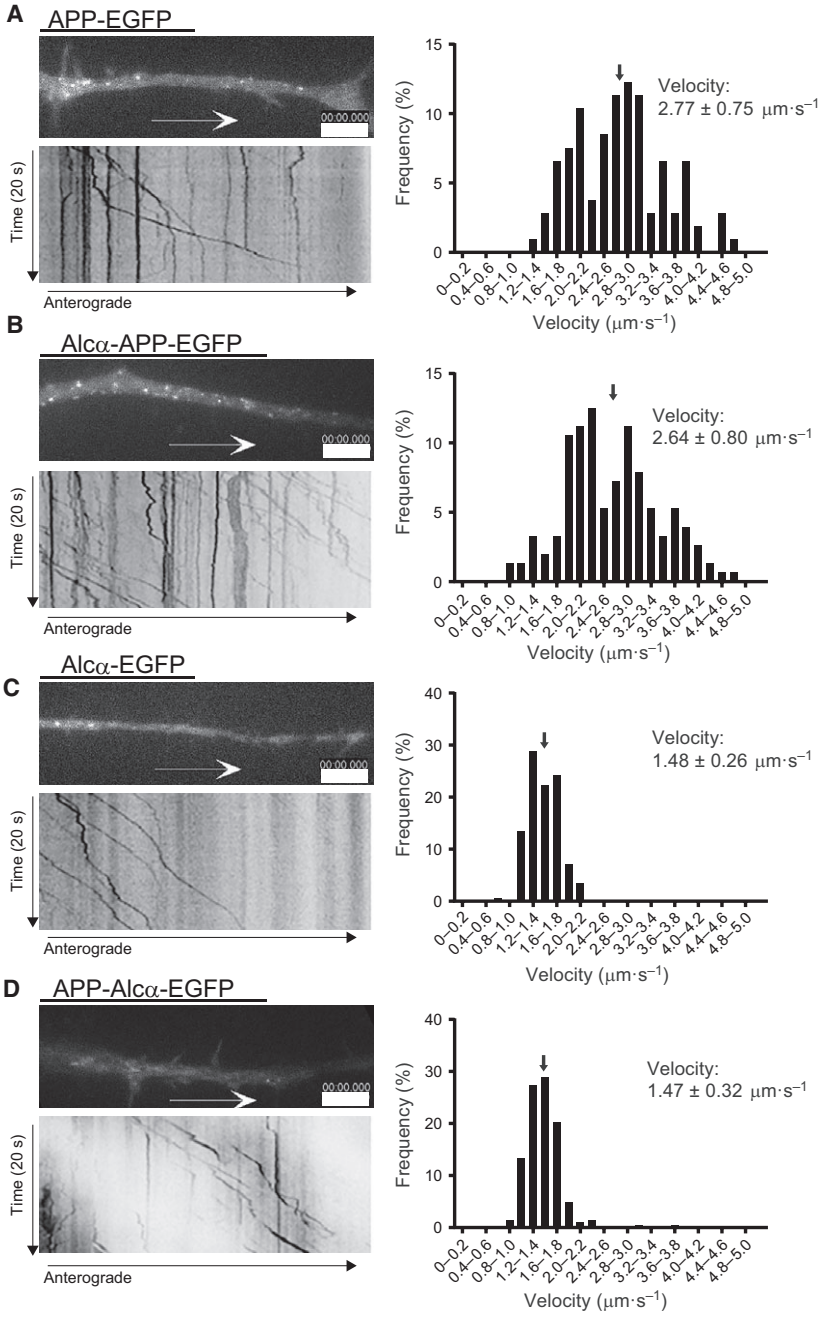

Fig. 2. Anterograde transport velocity of chimeric proteins in CAD cells. (A) APP, (B) Alcα-APP (αA), (C) Alcα, and (D) APP-Alcα (Aα) cargo velocities. Anterograde transport velocity of C-terminally EGFP-tagged cargo receptors was analyzed in differentiating CAD cells. Movies (Supplementary Movies 1A–D) are shown in left panels with kymographs to depict all vesicle movement. Arrows indicate the direction of anterograde transport. Scale bar, 5 μm. The cumulative frequencies of velocities of anterograde transport of respective cargos are shown in the right panels (A, $n = 106$; B, $n = 152$; C, $n = 170$; D, $n = 201$). Data are expressed as percentages, and position of average velocity is indicated with arrows.

a TIRF microscopy system (C1; Nikon, Tokyo Japan) equipped with a CCD camera (Cascade 650; Photometrics Co., Tucson, AZ, USA). The velocity of anterograde transport was analyzed quantitatively as described previously [5,6]; figures show average velocity ($\mu m \cdot s^{-1}$, represented as a histogram) and standard deviation (±SD). Kymographs of moving cargos were assembled using the application KymoMaker [22]; open access tool at www.pharm.hokudai.ac.jp/shinkei/Kymomaker.html].

Results

Expression of chimera cargo receptors and interaction with JIP1b

Schematic structures of chimeric or wild-type APP and Alcα proteins C-terminally fused to EGFP are shown in Fig. 1A. Expression of wild-type APP-EGFP and Alcα-EGFP and chimeric αA-EGFP and Aα-EGFP proteins was confirmed in N2a cells (Fig. 1B). Cell lysates were analyzed by immunoblotting with anti-GFP (upper), anti-APP cytoplasmic region (middle), and anti-Alcα cytoplasmic region (lower) antibodies, along with a blot of anti-α-tubulin antibody. Both αA-EGFP and Aα-EGFP chimeric proteins were detected along with APP-EGFP and Alcα-EGFP. αA-EGFP was expressed at almost the same level as APP-EGFP, and Aα-EGFP was expressed at a similar level to Alcα-EGFP. Moreover, αA-EGFP chimeric protein existed in the glycosylated forms, as reflected by the doublet bands on the blot, representing the high-mannose and slowly migrating complex *N*-glycosylated forms of the Alcα luminal region as described previously [5]. APP-EGFP also showed the slowly migrating mature (*N*- and *O*-glycosylated) and the faster migrating immature (*N*-glycosylated) forms. This observation suggests that αA-EGFP chimera protein passed through the conventional protein secretory pathway, as did wild-type or endogenous proteins. The identities of these chimeric proteins were confirmed by immunoblotting with antibodies raised against the APP and Alcα cytoplasmic regions (Fig. 1B, middle and lower panels; asterisks show endogenous APP and Alcα proteins).

The cytoplasmic domain of APP interacts with JIP1, and this interaction plays an essential role in generating the EFV by mediating the connection with kinesin-1. Therefore, we next investigated whether the αA-EGFP chimera could associate with JIP1b, a variant of JIP1 that binds APP more strongly than another variant, JIP1a [12]. Cells coexpressing chimeric or wild-type EGFP fusion proteins with FLAG-JIP1b were lysed and subjected to coimmunoprecipitation with anti-FLAG antibody, and the immunoprecipitates and lysates were analyzed by immunoblotting with anti-FLAG and anti-EGFP antibodies (Fig. 1C). Chimeric αA-EGFP was recovered with FLAG-JIP1b, as was APP-EGFP, indicating that αA-EGFP associated with JIP1b. Because Alcα does not bind JIP1b [5], Aα-EGFP did not associate with JIP1b as did not Alcα-EGFP. In this study, only one form of doublet APP-EGFP and αA-EGFP proteins was likely to associate strongly with JIP1b, which may suggest that mature form of cargo receptor may interact with JIP1 to connect with kinesin-1 in late secretory pathway following Golgi exit. These results clearly show that the chimeric EGFP fusion proteins, αA-EGFP and Aα-EGFP, behave similarly to wild-type APP-EGFP and Alcα-EGFP in the cell. We next analyzed axonal transport of both chimeric cargos in neuronal cells and cultured mouse neurons.

Anterograde transport of chimera cargos

Next, αA-EGFP and Aα-EGFP were expressed in differentiating CAD cells along with APP-EGFP and Alcα-EGFP. Anterograde axonal transport of chimeric and wild-type cargos was analyzed by TIRF microscopy, and their transport velocities in an incubation chamber supplied with 5% CO2 at 37 °C were calculated (Fig. 2). αA-EGFP was transported at an average velocity of 2.64 ± 0.80 $\mu m \cdot s^{-1}$ (panel B), almost identical to the average velocity of APP-EGFP (2.77 ± 0.75 $\mu m \cdot s^{-1}$, panel A). This observation indicates that both αA and APP cargos achieved the EFV

Table 1. Summary of statistical analysis for transport velocity of cargos. Results of Figures 2 (CAD cells) and 3 (primary cultured neurons) are summarized with statistical analysis among the transport velocity of cargos. Statistical analysis was performed using one-way ANOVA, followed by Dunn's multiple comparisons test. N.S., not significant.

Comparison	Cell types	Significance	Figures
APP to αA	CAD	N.S.	2A to 2B
	Neuron	N.S.	3A to 3B
APP to Alcα	CAD	$P < 0.0001$	2A to 2C
	Neuron	$P < 0.0001$	3A to 3C
APP to Aα	CAD	$P < 0.0001$	2A to 2D
	Neuron	$P < 0.0001$	3A to 3D
αA to Alcα	CAD	$P < 0.0001$	2B to 2C
	Neuron	$P < 0.0001$	3B to 3C
αA to Aα	CAD	$P < 0.0001$	2B to 2D
	Neuron	$P < 0.0001$	3B to 3D
Alcα to Aα	CAD	N.S.	2C to 2D
	Neuron	N.S.	3C to 3D

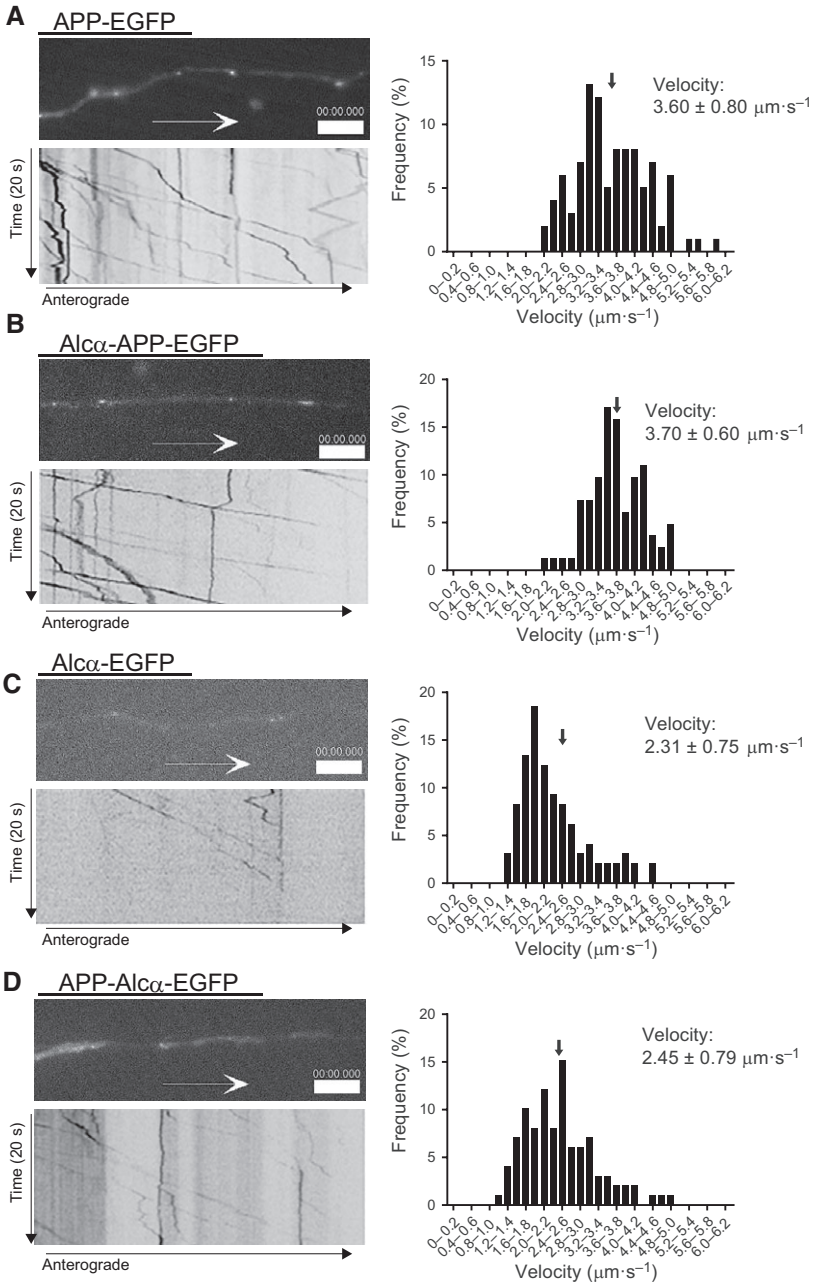

Fig. 3. Anterograde transport velocity of chimeric cargo receptors in neurons. (A) APP, (B) Alcα-APP (αA), (C) Alcα, and (D) APP-Alcα (Aα) cargo velocities. Anterograde transport velocity of C-terminally EGFP-tagged cargo receptors was analyzed in axons of primary cultured mouse neurons. Movies (Supplementary Movies 2A–D) are shown in the left panels, with kymographs to depict all vesicle movement. Arrows indicate the direction of anterograde transport. Scale bar, 5 μm. The cumulative frequencies of velocities of anterograde transport of the respective cargos are shown in the right panels (A, $n = 99$; B, $n = 82$; C, $n = 97$; D, $n = 99$). Data are expressed as percentages, and position of average velocity is indicated with arrows.

in anterograde transport. In contrast to the αA cargo, Aα-EGFP was transported at an average velocity of 1.47 ± 0.32 μm·s^{-1} (panel D), consistent with the average velocity of Alcα-EGFP (1.48 ± 0.26 μm·s^{-1}; panel C). This velocity is comparable to that of kinesin-1 moving on microtubules, calculated *in vitro* at 35 °C, and indicates the absence of the EFV. Together, these results clearly show that chimeric cargo containing the APP cytoplasmic region is transported at the EFV, whereas a cargo containing Alcα

cytoplasmic region is transported at the conventional velocity of kinesin-1. Statistical significance among the transport velocity of respective cargos was summarized (Table 1, CAD).

Next, we confirmed these findings using mouse primary cultured neurons (Fig. 3). The αA-EGFP was transported at an average velocity of 3.70 ± 0.60 μm·s^{-1} (panel B), similar to the average velocity of APP-EGFP (3.60 ± 0.80 μm·s^{-1}; panel A). Aα-EGFP was transported at an average velocity of 2.45 ± 0.79 μm·s^{-1} (panel D), comparable to the velocity of Alcα-EGFP (2.31 ± 0.75 μm·s^{-1}; panel C). Again, we observed that cargo receptors containing APP cytoplasmic region were transported at the EFV regardless of their luminal structures, and the statistical significance among the transport velocity of respective cargos was summarized (Table 1, Neuron).

In other words, the velocity of chimeric cargo receptors was determined by the properties of their cytoplasmic region, in particular whether it can or cannot associate with JIP1. In primary cultured neurons, the average velocity of all cargo receptors was faster (~ 1.5-fold) than in differentiating CAD cells. In our experience, this tendency toward higher velocity is often observed in well-differentiated neurons, but we do not currently know the exact reason. Taken together, our findings show that the APP cytoplasmic region is necessary and sufficient for the EFV of APP cargo large transport by kinesin-1 in neurons, whereas the luminal region of APP is not required for the EFV.

Discussion

Alcα directly binds to the KLC of kinesin-1 via WD motifs of its cytoplasmic region, and WD motif composed of 10 amino acids can activate kinesin-1 [9], indicating that the cytoplasmic region of Alcα is necessary and sufficient for Alcα cargo transport by kinesin-1. Furthermore, the interaction of the WD motifs of Alcα with KLC is regulated by multiple-site phosphorylation in an acidic region located between two WD motifs in the cytoplasmic region [19]. Moreover, phosphorylation of the Alcα cytoplasmic region is required for the proper formation of Alcα cargos by kinesin-1 [19]. These observations strongly support the functional importance of the cytoplasmic regions of cargo receptor molecules in determining the properties of vesicular cargos.

The cytoplasmic region of APP is short (45 amino acids), but includes the NPTY motif to which JIP1

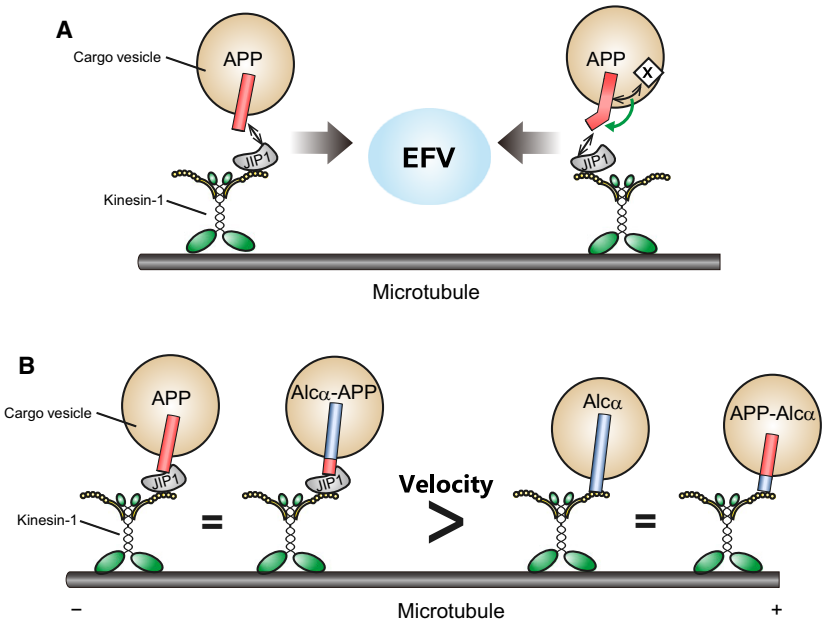

Fig. 4. The cytoplasmic region of APP is necessary and sufficient for generating the EFV of APP transport by kinesin-1. (A) Possible roles of the luminal region of APP. We considered two possibilities that the luminal region of APP contributes (right) or does not contribute (left) to the function of the cytoplasmic region. It remains unclear whether the interaction of some cargo molecule (x in square) with the luminal region of APP is required for generation of the EFV, mediated by the JIP1 interaction. (B) Alcα-APP (αA) cargo is transported by kinesin-1 at the EFV. The chimeric Alcα-APP (αA) cargo including the APP cytoplasmic domain is transported at the velocity of APP cargo, indicating that the cytoplasmic region of APP is necessary and sufficient for EFV transport by kinesin-1.

binds. We revealed that the JIP1 association, which mediates the connection between KLC and APP, is essential for the EFV of APP cargo transport [6]. Furthermore, our recent analysis revealed the regulatory mechanism in EFV of APP cargo transport [11]. However, the contribution of the APP luminal region remained unclear for the EFV of APP cargo transport. We consider two possibilities: first, that the interaction between the cytoplasmic region of APP and JIP1 is sufficient to achieve EFV transport of APP cargos by kinesin-1; and second, that a further interaction between the luminal region of APP and an unidentified factor is required to achieve the EFV. In current study, it is difficult to exclude a possibility that JIP1 and/or KLC may associate with a different type of molecular motor which can move faster than kinesin-1. Nevertheless, if the second possibility is true, such interaction within the luminal region may alter the conformation of the cytoplasmic region, which may in turn be required to generate the EFV (Fig. 4A). The results obtained with the chimeric cargo proteins designed for this study clearly show that the cytoplasmic region of APP is necessary and sufficient for the EFV (Fig. 4B). Our conclusion brings us benefits to perform *in vitro* study, to reveal molecular mechanism how does kinesin-1 transport APP cargo at faster velocity, such as a study using beads attached the short cytoplasmic region of APP. The results should provide clues regarding why various cargos are transported at different velocities by the same molecular motor *in vivo*.

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Author contributions

MT, KC, and YS carried out all the experiments. YS and YO prepared antibodies and plasmids. TN, SH, HT, and TS participated in the study design and data analysis. YS, YO, AK, SU, and MK participated in TIRF microscopy analysis. TS conceived the study and is the primary author of the manuscript. All authors read and approved the final manuscript.

References

1 Vale RD, Reese T and Sheetz MP (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* **42**, 39–50.
2 Verhey KJ and Hammond JW (2009) Traffic control: regulation of kinesin motors. *Nat Rev Mol Cell Biol* **10**, 765–777.
3 Verhey KJ, Meyer D, Deehan R, Blenis J, Schnapp BJ, Rapoport TA and Margolis B (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J Cell Biol* **152**, 959–970.
4 Konecna A, Frischnech R, Kinter J, Ludwig A, Steuble M, Meskenaite V, Indermuhle M, Engrl M, Cen C, Mateos JM *et al.* (2006) Calsyntenin-1 docks vesicular cargo to kinesin-1. *Mol Biol Cell* **17**, 3651–3663.
5 Araki Y, Kawano T, Taru H, Saito Y, Wada S, Miyamoto K, Kobayashi H, Ishikawa HO, Oshsugi Y, Yamamoto T *et al.* (2007) The novel cargo Alcadein induces vesicle association of kinesin-1 motor components and activates axonal transport. *EMBO J* **26**, 1475–1486.
6 Chiba K, Araseki M, Nozawa K, Furukori K, Araki Y, Matsushima T, Nakaya T, Hata S, Saito Y, Uchida S *et al.* (2014) Quantitative analysis of APP axonal transport in neurons – Role of JIP1 in APP anterograde transport – Mol. *Biol Cell* **25**, 3569–3580.
7 Hirokawa N, Noda Y, Tanaka Y and Niwa S (2009) Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol* **10**, 682–696.
8 Kawaguchi K and Ishiwata S (2000) Temperature dependent of force, velocity, and processivity of single kinesin molecules. *Biochem Biophys Res Commun* **272**, 895–899.
9 Kawano T, Araseki M, Araki Y, Kinjo M, Yamamoto T and Suzuki T (2012) A small peptide sequence is sufficient for intiating kinesin-1 activation through part of TPR region of KLC1. *Traffic* **13**, 834–848.
10 Kamal A, Stokin GB, Yang Z, Xia CH and Goldstein LS (2000) Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron* **28**, 449–459.
11 Chiba K, Chien K-y, Sobu Y, Hata S, Kato S, Nakaya T, Okada Y, Nairn AC, Kinjo M, Taru H *et al.* (2017) Phosphorylation of KLC1 modifies interaction with JIP1 and abolishes the enhanced fast velocity of APP transport by kinesin-1. *Mol Biol Cell* **28**, 3857–3869.
12 Taru H, Iijim AK, Hase M, Kirino Y, Yagi Y and Suzuki T (2002) Interaction of Alzheimer's β-amyloid precursor family proteins with scaffold proteins of the JNK signaling cascade. *J Biol Chem* **277**, 20070–20078.

13 Ramelot TA and Nicholson LK (2001) Phosphorylation-induced structural changes in the amyloid precursor protein cytoplasmic tail detected by NMR. *J Mol Biol* **307**, 871–884.
14 Ando K, Iijima K, Elliott JI, Kirino Y and Suzuki T (2001) Phosphorylation-dependent regulation of the interaction of amyloid precursor protein with Fe65 affects the production of β-amyloid. *J Biol Chem* **276**, 40353–40361.
15 Matsushima T, Saito Y, Eliott JI, Iijima-Ando K, Nishimura M, Kimura N, Hata S, Yamamoto T, Nakaya T and Suzuki T (2012) Membrane-microdomain localization of amyloid β-precursor protein (APP) C-terminal fragments is regulated by phosphorylation of the cytoplasmic Thr668 residue. *J Biol Chem* **287**, 19715–19724.
16 Araki Y, Tomita S, Yamaguchi H, Miyagi N, Sumioka A, Kirino Y and Suzuki T (2003) Novel cadherin-related membrane proteins, Alcadeins, enhance the X11-like protein mediated stabilization of amyloid β-protein precursor metabolism. *J Biol Chem* **278**, 49448–49458.
17 Taru H and Suzuki T (2004) Facilitation of stress-induced phosphorylation of β-amyloid precursor protein family members by X11-like/Mint2 protein. *J Biol Chem* **279**, 21628–21636.
18 Oishi M, Nairn AC, Czernik AJ, Lim GS, Isohara T, Gandy SE, Greengard P and Suzuki T (1997) The cytoplasmic domain of the Alzheimer's β-amyloid precursor protein is phosphorylated at Thr654, Ser655 and Thr668 in adult rat brain and cultured cells. *Mol Med* **3**, 111–123.
19 Sobu Y, Furukori K, Chiba K, Nairn AC, Kinjyo M, Hata S and Suzuki T (2017) Phosphorylation of multiple sites within an acidic region of Alcadein α is required for kinesin-1 association and Golgi exit of Alcadein α cargo. *Mol Biol Cell* **28**, 3844–3856.
20 Qi Y, Wang JK, McMillan M and Chikaraishi DM (1997) Characterization of a CNS cell line, CAD, in which morphological differentiation is initiated by serum deprivation. *J Neurosci* **17**, 1217–1225.
21 Bartlett WP and Banker GA (1984) An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture. I. Cells which develop without intercellular contacts. *J Neurosci* **4**, 1944–1953.
22 Chiba K, Shimada Y, Kinjo M, Suzuki T and Uchida S (2014) Simple and direct assembly of kymographs from movies using KYMOMAKER. *Traffic* **15**, 1–11.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Movies. S1A–D correspond to Fig. S2A–D, and Movies S2A–D correspond to Fig. S3A–D.