Video Article

Purification of the Dendritic Filopodia-rich Fraction

Yutaka Furutani[1,2], Yoshihiro Yoshihara[3]

[1]Laboratory for Neurobiology of Synapse, RIKEN Brain Science Institute

[2]Liver Cancer Prevention Research Unit, RIKEN Center for Integrative Medical Sciences

[3]Laboratory for Systems Molecular Ethology, RIKEN Center for Brain Science

Correspondence to: Yutaka Furutani at yfurutani@riken.jp

URL: https://www.jove.com/video/59292
DOI: doi:10.3791/59292

Keywords: Neuroscience, Issue 147, dendritic filopodia, synapse, spine, telencephalin, ICAM-5, vitronectin, hippocampal neurons, low-density culture, magnetic microbeads, phagocytic cup

Date Published: 5/2/2019

Citation: Furutani, Y., Yoshihara, Y. Purification of the Dendritic Filopodia-rich Fraction. *J. Vis. Exp.* (147), e59292, doi:10.3791/59292 (2019).

Abstract

Dendritic filopodia are thin and long protrusions based on the actin filament, and they extend and retract as if searching for a target axon. When the dendritic filopodia establish contact with a target axon, they begin maturing into spines, leading to the formation of a synapse. Telencephalin (TLCN) is abundantly localized in dendritic filopodia and is gradually excluded from spines. Overexpression of TLCN in cultured hippocampal neurons induces dendritic filopodia formation. We showed that telencephalin strongly binds to an extracellular matrix molecule, vitronectin. Vitronectin-coated microbeads induced phagocytic cup formation on neuronal dendrites. In the phagocytic cup, TLCN, TLCN-binding proteins such as phosphorylated Ezrin/Radixin/Moesin (phospho-ERM), and F-actin are accumulated, which suggests that components of the phagocytic cup are similar to those of dendritic filopodia. Thus, we developed a method for purifying the phagocytic cup instead of dendritic filopodia. Magnetic polystyrene beads were coated with vitronectin, which is abundantly present in the culture medium of hippocampal neurons and which induces phagocytic cup formation on neuronal dendrites. After 24 h of incubation, the phagocytic cups were mildly solubilized with detergent and collected using a magnet separator. After washing the beads, the binding proteins were eluted and analyzed by silver staining and Western blotting. In the binding fraction, TLCN and actin were abundantly present. In addition, many proteins identified from the fraction were localized to the dendritic filopodia; thus, we named the binding fraction as the dendritic filopodia-rich fraction. This article describes details regarding the purification method for the dendritic filopodia-rich fraction.

Video Link

The video component of this article can be found at https://www.jove.com/video/59292/

Introduction

Dendritic filopodia are thought to be precursors of spines. Actin filaments in the dendritic filopodia regulate their extension and retraction[1,2,3]. After contacting with an axon, selected dendritic filopodia begin their maturation into spines, and a synapse is formed[4,5]. Components of spines have been determined from comprehensive analysis of postsynaptic density fractions[6,7], while components of dendritic filopodia remain largely unknown. It has been shown that telencephalin (TLCN), ERM, SynGAP, Ras, PI3 kinase, Akt, mTOR, polo-like kinase 2, CaMKII, syndecan-2, paralemin-1, ARF6, and EphB regulate dendritic filopodia formation[5,8,9,10,11], while a method has not been developed for the comprehensive analysis of molecules present in the dendritic filopodia.

TLCN (ICAM-5) is specifically expressed by spiny neurons in the most rostral brain segment, the telencephalon[12]. TLCN has 9 Ig-like domains in its extracellular region, a transmembrane region, and a cytoplasmic tail[13]. TLCN binds to vitronectin (VN) and LFA-1 integrin in its extracellular region, to presenilin in its transmembrane region, and to phospho-ERM and α-actinin in its cytoplasmic region[5,8,14,15,16]. TLCN binds to the actin cytoskeleton through phospho-ERM at the tips of dendritic filopodia and α-actinin in spines and dendritic shafts[8,16].

We showed that overexpression of TLCN enhanced dendritic filopodia formation and induced the reversion of spines to filopodia[10]. The constitutive active form of ezrin bound to the TLCN cytoplasmic region and enhanced dendritic filopodia formation[8]. Thus, TLCN regulates dendritic filopodia formation through actin-binding proteins. Esselens et al. demonstrated that microbeads induced TLCN accumulation on cultured neurons[17]. We showed that phagocytic cup structures were formed on neuronal dendrites around VN-coated microbeads in a TLCN-dependent manner[15]. Constituents of dendritic filopodia are similar to those of the phagocytic cup. It is difficult to collect dendritic filopodia, but it is relatively easier to collect the phagocytic cup using magnetic microbeads. Thus, we developed a method to purify the phagocytic cup instead of dendritic filopodia[18]. Here, we describe the purification method for the dendritic filopodia-rich fraction.

Protocol

All methods described here have been approved by the Institutional Animal Care and Use Committee of RIKEN Wako.

1. Culture of Hippocampal Neurons

1. **Preparation of culture medium**
    1. Preparation of 200x Vitamin mix. Dissolve 100 mg of D-pantothenic acid hemicalcium salt, 100 mg of choline chloride, 100 mg of folic acid, 180 mg of i-inositol, 100 mg of niacinamide, 100 mg of pyridoxal HCl, and 100 mg of thiamine HCl in 500 mL of ultrapure water using a magnetic stirrer. The solution is not completely dissolved. Carefully mix, aliquot in 50 mL tubes and store at -20 °C.
    2. Preparation of Riboflavin solution. Dissolve 100 mg of Riboflavin in 500 mL of ultrapure water using a magnetic stirrer. The solution is not completely dissolved. Carefully mix, aliquot in 50 mL tubes and store at -20 °C.
    3. Preparation of 1 M $CaCl_2$. Dissolve 7.35 g of $CaCl_2 \cdot 2H_2O$ in 50 mL of ultrapure water using a magnetic stirrer.
    4. Preparation of Minimum essential medium (MEM). Dissolve 400 mg of KCl, 6800 mg of NaCl, 2,200 mg of $NaHCO_3$, 158 mg of $NaH_2PO_4 \cdot 2H_2O$, 7000 mg of D-glucose, and 200 mg of $MgSO_4$-$7H_2O$ in 950 mL of ultrapure water using a magnetic stirrer.
    5. Titrate 1.8 mL of 1 M $CaCl_2$ to the MEM in a drop-by-drop manner using a 1 mL pipet with a constant agitation on a magnetic stirrer. Adjust the pH of the MEM to pH 7.25 with 1 mol/L HCl.
    6. Add 5 mL of 200x Vitamin mix and 200 µL of Riboflavin solution to the MEM. Adjust the volume of the solution to 1,000 mL with ultrapure water. Filter the solution using a 0.22 µm filter system and store it at 4 °C.
    7. Preparation of 10x DNase-I stock solutions. Dissolve 100 mg of DNase-I in 12.5 mL of Hanks' Balanced Salt Solution (HBSS), filter through a 0.22 µm filter, aliquot in 1.5 mL tubes, and store the tubes at -20 °C.
    8. Preparation of cytosine β-D-arabinofuranoside (Ara-C) stock solution. Dissolve 25 mg of Ara-C in 8.93 mL of ultrapure water (final concentration of 10 mM), filter through a 0.22 µm filter, aliquot in 1.5 mL tubes and store at -20 °C
    9. Preparation of Plating medium. Mix 1 mL of MEM amino acid solution, 750 µL of 1 M HEPES, 1 mL of B27, 125 µL of 200 mM glutamine, 250 µL of penicillin/streptomycin, 2.5 mL of fetal bovine serum (FBS), and 44.375 mL of MEM in a 50 mL tube.
    10. Preparation of Stop medium. Mix 1 mL of MEM amino acid solution, 750 µL of 1 M HEPES, 5 mL of FBS (final 10%), and 43.25 mL of MEM in a 50 mL tube.

2. **Preparation of poly-L-Lysine-coated dishes**
    1. Coat 35 mm plastic cell culture dishes with 0.2 mg/mL of poly-L-lysine hydrobromide for 1 day at 25 °C.
    NOTE: Poly-L-lysine should not be used instead of poly-L-lysine hydrobromide.
    2. Wash the dishes with 2 mL of ultrapure water 3 times. Incubate the dishes with 1.5 mL of Stop medium at 25 °C until use.

3. **Dissection of hippocampal neurons from mouse embryo**
    1. Tissue source of hippocampal neurons. Dissect the hippocampus from wild-type and TLCN-deficient C57BL6/J mice on the embryonic days 16-17 according to the method of Lu et al.[19].
    2. Incubate dissected hippocampi in 0.25% trypsin and 1x DNaseI in HBSS containing 15 mM HEPES, pH 7.2 for 15 min at 37 °C with agitation every 3 min. Remove the solution. Incubate the hippocampi in 10 mL of STOP medium to inactivate trypsin at 4 °C for 5 min.
    3. Move the hippocampi into 10 mL of fresh STOP medium and incubate at 4 °C for 5 min. Move the hippocampi into 10 mL of fresh STOP medium and incubate at 4 °C for another 5 min. Move the hippocampi into 900 µL of STOP medium and 100 µL of 10x DNaseI in a 15 mL tube. Dissociate the hippocampi into isolated neurons by pipetting 20 times using a 1 mL pipet.
    NOTE: The tip of the 1 mL pipet slightly touches the bottom of the 15 mL tube during dissociation of the hippocampi.
    4. Add 9 mL of plating medium, and filter through a 70 µm cell strainer into a 50 mL tube. Count the number of cells using a hemocytometer and adjust to 3.5 x 10^4 cells/mL in plating medium.
    5. Aspirate STOP medium from poly-L-lysine-coated dishes. Plate the cells on poly-L-Lysine-coated dishes at 7 x 10^4 cells/dish (2 mL/dish).
    6. After 60-64 h of incubation under 5% CO_2 at 37 °C, add 2 µL of Ara-C stock solution (final 10 µM) to the neurons, and shake the dish slowly. Keep the culture dishes in a humidified box without changing the culture medium under 5% CO_2 at 37 °C.

2. Purification of Dendritic Filopodia-rich Fraction

1. After 13 days in vitro (DIV), add magnetic polystyrene microbeads (3 x 10^6 particles/dish) to 20 dishes containing the cultured neurons. After 1 day, wash the neurons in 1 mL of PBS with agitation 3 times to remove the medium and unbound microbeads. After removing PBS, lyse the neurons with 500 µL/dish of lysis buffer (PBS containing 0.01% Triton X-100, EDTA-free protease inhibitor cocktail, and phosphatase inhibitor cocktail).
2. Collect the lysate with a cell scraper and move the lysate to low protein-binding microtubes (10 tubes). Set the tubes on a magnet separator and wait for 1 min. Collect the supernatant and use it as the unbound fraction for silver staining and Western blot analysis.
3. Transfer the beads to a new low-protein-binding microtube, set on a magnetic separator, and completely remove the supernatant. Add 500 µL of lysis buffer and wash the beads using a vortex mixer for 15 s. Set the tube on a magnet separator, wait for 1 min, remove the supernatant, and add 500 µL of lysis buffer. Repeat the washing of the beads 10 times and remove the supernatant.
4. Elute proteins bound to the beads (the bound fraction) by the addition of 50 µL of 1x SDS sample buffer (62.5 mM Tris HCl, pH 6.8, 2.5% SDS, and 10% glycerol) and boil the tube at 98 °C for 5 min. Centrifuge the tube at 860 x *g* for 10 s and set the tube on a magnetic separator for 1 min. Collect the supernatant and use it as the bound fraction.
NOTE: The protocol can be paused here.
5. Measure protein concentrations of the unbound and bound fractions by the BCA protein assay. Visualize protein solutions with bromophenol blue and adjust the concentration to 5 ng/µL for SDS-PAGE.

3. Silver Staining and Western Blot Analysis

1. Separate the bound and unbound fractions (50 ng) by SDS-PAGE using a 5-20% gradient gel. Silver-stain the gel.

2. Western blot using anti-TLCN-C (1/3,000), anti-bovine vitronectin (1/5,000), anti-actin (1/1,000), and anti-α-tubulin (1/1,000) as primary antibodies and HRP-conjugated goat anti-rabbit IgG (1/5,000) as the secondary antibody. Visualize the antigens using chemiluminescent Western Blotting Detection Reagent and a chemiluminescence imager.

Representative Results

In cultured hippocampal neurons, TLCN was abundantly localized to the dendritic filopodia, shaft, and soma and colocalized with F-actin (**Figure 1A, B**). When polystyrene microbeads were added to cultured hippocampal neurons, the beads were automatically coated with vitronectin (VN) derived from fetal bovine serum (FBS) in the culture medium; they were mainly bound to dendrites, and they induced the formation of phagocytic cups (**Figure 1B-E**). Phagocytic cups were based on sheet-shaped actin filaments around microbeads on dendrites. TLCN, phospho-ERM, and $PI(4,5)P_2$, which are markers for dendritic filopodia, are highly accumulated around beads (**Figure 1D**)[8,15]. Phagocytic cups were only formed on wild-type hippocampal neurons, but not on TLCN-deficient hippocampal neurons (**Figure 2A-D**). Thus, the phagocytic cup formation was crucially dependent on the presence of TLCN in dendrites.

Constituents of the dendritic filopodia appeared similar to those of phagocytic cups. Next, we purified phagocytic cups instead of dendritic filopodia. The protocol for the purification of phagocytic cups is schematically shown in **Figure 3**. Magnetic microbeads were added to wild-type cultured hippocampal neurons, which induces the formation of phagocytic cup structures. The phagocytic cup structures were lysed with a weak detergent. The microbeads were collected after the lysis using a magnet separator. After washing of the beads, their binding proteins were boiled and eluted in an SDS sample buffer.

The amount of proteins in the bound and unbound fraction was measured using the BCA protein assay kit. The same amount of proteins in the unbound and bound fractions was separated by SDS-PAGE and stained by silver staining (**Figure 4A**). The protein band patterns were almost the same for the unbound and bound fractions, but the intensities at 50 and 70 kDa in the bound fraction were lower than those in the unbound fraction. However, the band intensity was not obviously different between the unbound and bound fractions prepared from TLCN-deficient culture hippocampal neurons. To confirm the purification of phagocytic cup structures, we performed Western blotting using anti-TLCN-C, anti-bovine vitronectin, anti-actin, and anti-α-tubulin (**Figure 4B**). TLCN and VN were mainly detected in the bound fraction. Actin, ezrin, Gαq, PLCβ1, MAP-2, and spectrin were detected in both the bound and unbound fractions. Moesin, PSD-95, α-actinin, and α-tubulin were detected in the unbound fraction.

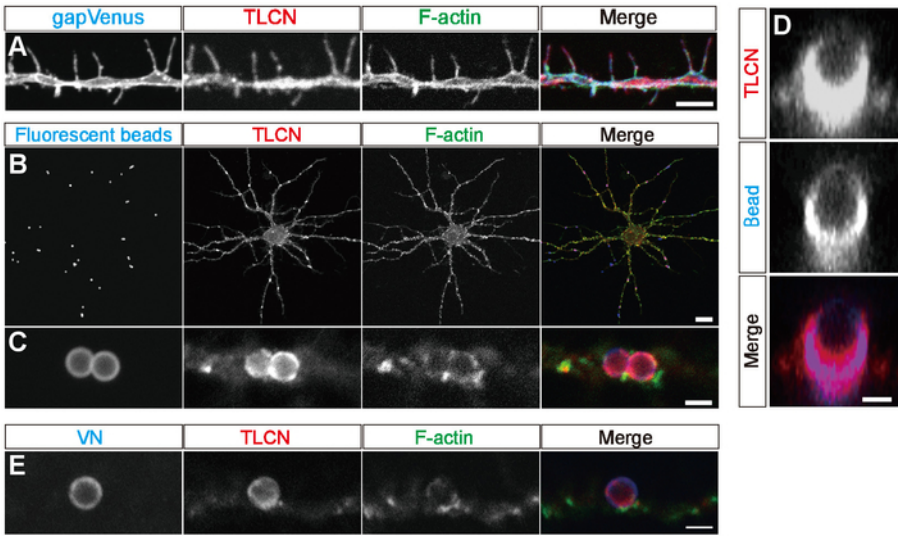

Figure 1: Localization of TLCN and F-actin in dendritic filopodia and phagocytic cups. (**A**) Immunofluorescence staining of dendrites of a cultured hippocampal neuron expressing gapVenus with anti-GFP antibody (blue in a merged image), anti-TLCN antibody (red in a merged image), and phalloidin (green in a merged image). TLCN and F-actin are abundantly observed in dendritic filopodia. (**B**, **C**) Formation of phagocytic cup structures on neuronal dendrites. Fluorescent microbeads (blue in merged images of B, C) added to cultured hippocampal neurons strongly adhere onto dendrites and induce the accumulation of TLCN (red in merged images of B, C) and F-actin (green in merged images of B, C). (**D**) A lateral view of a dendritic phagocytic cup reconstructed from confocal images reveals TLCN (red in merged images of D) surrounding the fluorescent bead (blue in merged images of D). (**E**) A dendritic phagocytic cup induced by a magnetic microbead attached onto a neuronal dendrite and immunostained with anti-VN antibody (blue in merged images of E), anti-TLCN antibody (red in merged images of E), and phalloidin (green in merged images of E). Scale bars = 1 μm in (D); 2 μm in (A), (C), and (E); and 20 μm in (B). This figure has been modified from a previous study[18]. Please click here to view a larger version of this figure.

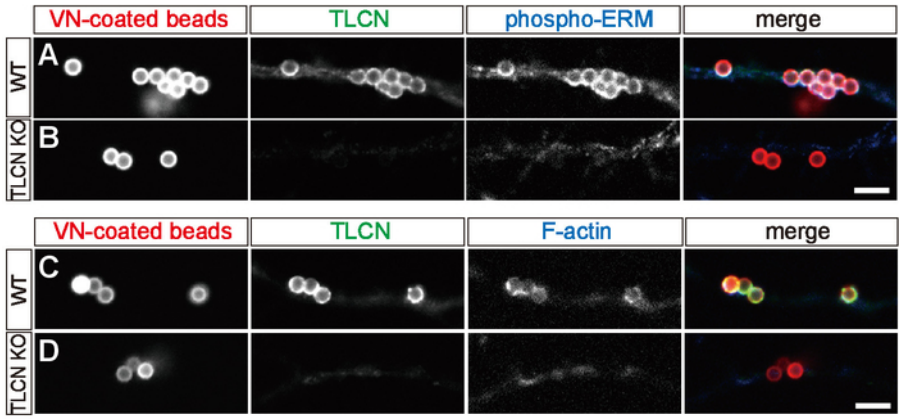

Figure 2: TLCN-dependent formation of phagocytic cup-like structure. (**A**-**D**) Triple fluorescence images of wild-type (WT; A, C) and TLCN-deficient (KO; C, D) neurons treated with VN-coated fluorescent beads (red in merged images of A, B, C, D) and labeled with anti-TLCN antibody (green in merged images of A, B, C, D) and anti-phospho-ERM antibody (blue in merged images of A, B) or Alexa488-phalloidin (blue in merged images of C, D). Scale bars = 5 μm. This figure has been modified from a previous study[15]. Please click here to view a larger version of this figure.

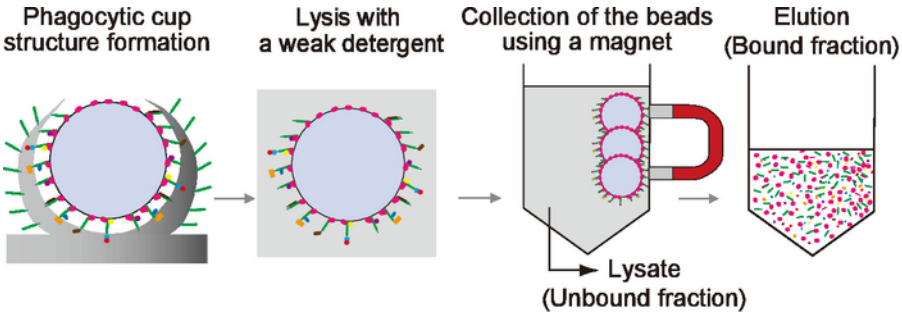

Figure 3: A schematic diagram illustrating the purification procedure of the dendritic filopodia-rich fraction. Magnetic microbeads were added onto cultured hippocampal neurons to induce the formation of dendritic phagocytic cups. After 1 day of incubation, the neurons were solubilized with lysis buffer containing 0.01% Triton X-100. The beads were separated from the unbound fraction using a magnetic separator. After washing, the bound proteins were eluted with SDS sample buffer and used as the bound fraction. Red: VN, green: TLCN, other colors: bound proteins. This figure has been modified from a previous study[18]. Please click here to view a larger version of this figure.

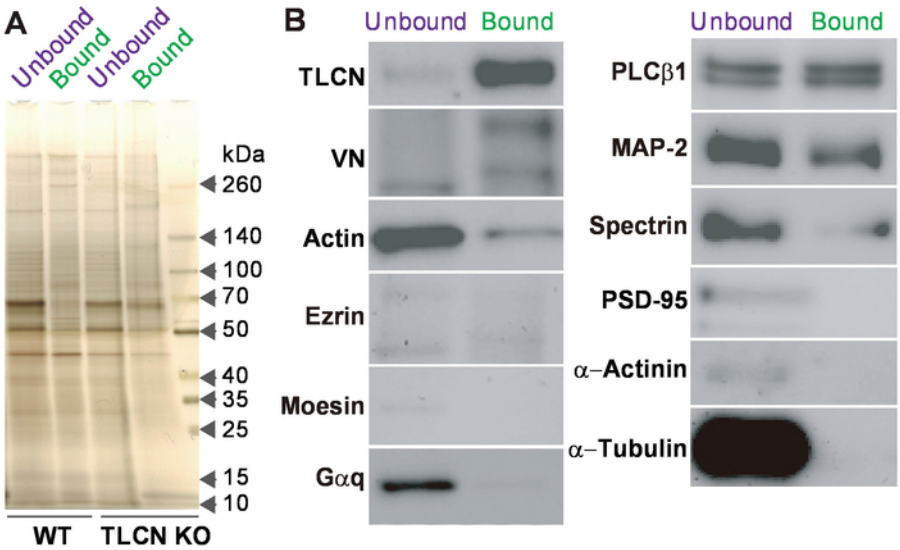

Figure 4: Confirmation of phagocytic cup fraction. (**A**) Silver staining of proteins in the unbound and bound fractions of the microbeads. The same amount (50 ng) of proteins in the unbound and bound fractions purified from wild-type (WT) and TLCN-deficient (TLCN KO) hippocampal neurons were separated by SDS-PAGE and visualized with silver staining. (**B**) Western blot analysis of the unbound and bound fractions. The same amount (50 ng) of proteins were separated by SDS-PAGE and subjected to Western blot analysis using anti-TLCN, anti-VN, anti-actin, anti-ezrin, anti-moesin, anti-Gαq, anti-PLCβ1, anti-MAP-2, anti-spectrin, anti-PSD-95, anti-α-actinin, and anti-α-tubulin antibodies. Note that TLCN, VN, actin, ezrin, PLCβ1, MAP-2, and spectrin are observed in the dendritic filopodia-rich fraction. This figure has been modified from a previous study[18]. Please click here to view a larger version of this figure.

Discussion

We developed a purification method for the dendritic filopodia-rich fraction using affinity between the cell adhesion molecule TLCN and the extracellular matrix protein vitronectin. Compared to PSD fraction, it could be possible to identify the synaptic proteins acting on the immature synapse from the dendritic filopodia-rich fraction. Thus, the constituents of the dendritic filopodia-rich fraction are different from those of the PSD fraction by 74%. Different from PSD fraction, we used cultured hippocampal neurons to actively form phagocytic cups, and the cells need to be alive. To form the phagocytic cup, we used the interaction between TLCN and vitronectin. TLCN expression is limited in the telencephalon. Thus, we cannot use cultured neurons derived from the cerebellar. However, if we coat the beads with different proteins, this activity-dependent purification method could be applied to cerebellar neurons. For example, when the N-terminal domain of glutamate receptor delta2-coated microbeads are applied to cerebellar granule cells, presynaptic neurexin and cbln1 were identified from the binding proteins. Therefore, this activity-dependent method could be used, if the coating proteins are changed. Since the access of vitronectin-coated microbeads is limited to the brain slice and brain tissue, phagocytic cups are not formed efficiently. Thus, future tasks include developing the purification method of the dendritic filopodia-rich fraction from brain slice and tissue.

In this protocol, low-density culture condition is used for hippocampal neurons, and the growth of glial cells are inhibited by the addition of Ara-C[10,20,21]. Hippocampal neurons are cultured in MEM prepared by ourselves, but not in neurobasal medium, which is widely used for the culture of hippocampal neurons. Hippocampal neurons often die by 14 DIV when cultured on neurobasal medium, which indicates that the neurobasal medium is not suitable for our low-density culture condition. In addition, an important aspect of low-density culture is coating the dish with poly-L-lysine hydrobromide, which cannot be replaced with poly-L-lysine.

To obtain sufficient amount of proteins from the dendritic filopodia-rich fraction, the numbers of hippocampal neurons and magnetic microbeads are important factors. For immunostaining dendritic filopodia, hippocampal neurons were plated on a 35 mm dish at 5.6 x× 10^4 cells/dish, while the neurons were plated at 7.0 x 10^4 cells/dish for purification of the dendritic filopodia-rich fraction. At 14 DIV, almost no hippocampal neurons overlapped at 5.6 x 10^4 cells/dish for immunostaining, while many of hippocampal neurons partly overlapped with the other neurons at 7.0 x 10^4 cells/dish for purification of the dendritic filopodia-rich fraction. However, dendritic filopodia were abundantly present at both densities of hippocampal neurons. High-density cultures of hippocampal neurons often mature faster than low-density cultured hippocampal neurons; thus, adjusting the density of hippocampal neurons to low-density culture is indispensable to obtain the dendritic filopodia-rich fraction. Microbeads were added at 1 x 10^6 microbeads/dish for immunostaining and at 3 x 10^6 microbeads/dish for purification of the dendritic filopodia-rich fraction. For purification of the fraction, once a sufficient amount of beads was added, the unbound beads were washed out from hippocampal neurons.

In this protocol, microbeads were automatically coated with VN, which is present in the culture medium. However, microbeads can be coated with recombinant VN and added to hippocampal neurons. Because VN is a very sticky protein, VN-coated microbeads easily form aggregates. Thus, VN-coated microbeads are sonicated and pipetted to dissociate from each other just before addition to hippocampal neurons.

We previously showed that VN-coated microbeads induces phosphor-ERM, PI(4,5)P_2, and F-actin together with TLCN accumulation[15]. When the dendritic filopodia-rich fraction was analyzed by Western blotting, phospho-ERM was not detected by anti-phospho-ERM polyclonal antibody and was slightly detected by anti-ezrin and anti-moesin monoclonal antibodies. It appears that the sensitivity of the monoclonal antibodies was higher than the anti-phospho-ERM polyclonal antibody. In addition, ERM proteins were localized to almost all regions of the hippocampal neurons, but phospho-ERM proteins were only localized to the tip of the dendritic filopodia and the phagocytic cup. It is considered that the amount of phospho-ERM binding to TLCN is limited compared to the amount of nonphosphorylated ERM. Thus, detection of phospho-ERM in the dendritic filopodia-rich fraction appears to be difficult.

To detach the microbeads from the cell membrane, we used a weak detergent, 0.01% Triton X-100. TLCN is linked to actin filament through phospho-ERM in the dendritic filopodia and phagocytic cup structures. To purify proteins indirectly linked to TLCN through the actin filament, we used a weak detergent in this protocol. However, the concentration of Triton X-100 could be changed to a higher concentration depending on the purpose of the experiment.

Esselens et al. has shown that the phagocytic uptake of microbeads induced TLCN, PIP_2, and F-actin accumulation in cultured hippocampal neurons[17]. According to our analysis via confocal microscopy after 24 h of incubation with microbeads, the microbeads were not completely taken up into the cytoplasm. TLCN and PIP_2, which are localized to the cell membrane, were localized around beads, especially on the bottom of the microbeads. In addition, 319 proteins identified from the dendritic filopodia-rich fraction were analyzed using the KEGG pathway analysis. Phagocytosis and autophagy pathways were not detected, but cytoskeleton organization, exocytosis, actin-filament-based process, and microtubule-based process were significantly enriched in the fraction[18].

The molecular mechanism of dendritic filopodia formation remains largely unknown. Analysis of the phagocytic cup structure could help understand the molecular constituents and dynamic functions of dendritic filopodia. It would be interesting to analyze the dendritic filopodia-rich fraction prepared from mouse models of neurodevelopmental and neuropsychiatric disorders.

Disclosures

The authors have nothing to disclose.

Acknowledgments

We thank Shigeo Okabe and Hitomi Matsuno for the low-density culture of hippocampal neurons, Masayoshi Mishina for TLCN-deficient mice, Sachiko Mitsui and Momoko Shiozaki for technical assistance, and members of the Yoshihara laboratory for helpful discussions. This work was

supported by JSPS KAKENHI Grant Nos. JP20700307, JP22700354, and JP24500392 and MEXT KAKENHI Grant Nos. JP23123525 to YF and JP20022046, JP18H04683, and JP18H05146 to YY.

References

1. Fiala, J. C., Feinberg, M., Popov, V., & Harris, K. M. Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *Journal of Neuroscience.* **18** (21), 8900-8911 (1998).
2. Portera-Cailliau, C., Pan, D. T., & Yuste, R. Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia. *Journal of Neuroscience.* **23** (18), 7129-7142 (2003).
3. Ziv, N. E., & Smith, S. J. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. *Neuron.* **17** (1), 91-102 (1996).
4. Lohmann, C., & Bonhoeffer, T. A role for local calcium signaling in rapid synaptic partner selection by dendritic filopodia. *Neuron.* **59** (2), 253-260 (2008).
5. Yoshihara, Y., De Roo, M., & Muller, D. Dendritic spine formation and stabilization. *Current Opinion in Neurobiology.* **19** (2), 146-153 (2009).
6. Bayes, A. et al. Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PLoS One.* **7** (10), e46683 (2012).
7. Bayes, A. et al. Characterization of the proteome, diseases and evolution of the human postsynaptic density. *Nature Neuroscience.* **14** (1), 19-21 (2011).
8. Furutani, Y. et al. Interaction between telencephalin and ERM family proteins mediates dendritic filopodia formation. *Journal of Neuroscience.* **27** (33), 8866-8876 (2007).
9. Mao, Y. T. et al. Filopodia Conduct Target Selection in Cortical Neurons Using Differences in Signal Kinetics of a Single Kinase. *Neuron.* **98** (4), 767-782 e768 (2018).
10. Matsuno, H. et al. Telencephalin slows spine maturation. *Journal of Neuroscience.* **26** (6), 1776-1786 (2006).
11. Raemaekers, T. et al. ARF6-mediated endosomal transport of Telencephalin affects dendritic filopodia-to-spine maturation. *The EMBO Journal.* **31** (15), 3252-3269 (2012).
12. Mori, K., Fujita, S. C., Watanabe, Y., Obata, K., & Hayaishi, O. Telencephalon-specific antigen identified by monoclonal antibody. *Proceedings of the National Academy of Sciences of the United States of America.* **84** (11), 3921-3925 (1987).
13. Yoshihara, Y., & Mori, K. Telencephalin: a neuronal area code molecule? *Neuroscience Research.* **21** (2), 119-124 (1994).
14. Annaert, W. G. et al. Interaction with telencephalin and the amyloid precursor protein predicts a ring structure for presenilins. *Neuron.* **32** (4), 579-589 (2001).
15. Furutani, Y. et al. Vitronectin induces phosphorylation of ezrin/radixin/moesin actin-binding proteins through binding to its novel neuronal receptor telencephalin. *Journal of Biological Chemistry.* **287** (46), 39041-39049 (2012).
16. Nyman-Huttunen, H., Tian, L., Ning, L., & Gahmberg, C. G. alpha-Actinin-dependent cytoskeletal anchorage is important for ICAM-5-mediated neuritic outgrowth. *Journal of Cell Biology.* **119** (Pt 15), 3057-3066 (2006).
17. Esselens, C. et al. Presenilin 1 mediates the turnover of telencephalin in hippocampal neurons via an autophagic degradative pathway. *Journal of Cell Biology.* **166** (7), 1041-1054 (2004).
18. Furutani, Y., & Yoshihara, Y. Proteomic Analysis of Dendritic Filopodia-Rich Fraction Isolated by Telencephalin and Vitronectin Interaction. *Frontiers in Synaptic Neuroscience.* **10** 27 (2018).
19. Lu, Z., Piechowicz, M., & Qiu, S. A Simplified Method for Ultra-Low Density, Long-Term Primary Hippocampal Neuron Culture. *Journal of Visualized Experiments.* (109) (2016).
20. Okabe, S., Miwa, A., & Okado, H. Alternative splicing of the C-terminal domain regulates cell surface expression of the NMDA receptor NR1 subunit. *The Journal of Neuroscience.* **19** (18), 7781-7792 (1999).
21. Okabe, S., Vicario-Abejon, C., Segal, M., & McKay, R. D. Survival and synaptogenesis of hippocampal neurons without NMDA receptor function in culture. *European Journal of Neuroscience.* **10** (6), 2192-2198 (1998).

