Supplemental materials

**Construction of expression plasmids encoding EGFP-Rab GTPase fusion proteins.**

Expression plasmids encoding EGFP-tagged Rab GTPases were prepared as described elsewhere with some modifications (1). Template cDNA was synthesized with a First Strand cDNA Synthesis Kit (GE Healthcare) using total RNA extracted from N2a-3 cells with TRIzol Reagent (Life Thechnologies). The cDNA fragments, which included the entire open reading frame of each of the following wild-type mouse genes (Rab4a, Rab5a, Rab7, Rab9, Rab11a and Rab22a), were amplified from the template cDNAs using the corresponding pair of oligonucleotide primers (Table S2) in Table S2. The PCR products were cloned into pCRII-topo with TA Cloning Kit (Invitrogen). The cDNA fragments of a GTP-binding deficient dominant-negative mutant of each of six Rab GTPases, Rab4aS22N, Rab5aS34N, Rab7T22N, Rab9S21N, Rab11aS25N and Rab22aS19N (2-7), were produced by site-directed mutagenesis by overlap extension PCR (8) using mutagenic oligonucleotide primers (Table S3). The resulting PCR products were cloned into pCR-BluntII-topo with Zero Blunt TOPO PCR Cloning Kit (Invitrogen). Thereafter, the cloned cDNA fragments encoding the wild-type Rab GTPase or a dominant-negative mutant were excised by digestion with Xho I and then cloned into the Xho I site of pEGFP-C1.

**Purification of PrP-res**

Brains of Jcl:ICR mice infected with the Obihiro or 22L prion strain (about 2 g) were used as starting materials. The brains were homogenized in citrate-buffered saline (CBS, 137 mM NaCl and 20 mM citrate, pH 6.0). Brij-96V (Fluka) was added to the brain homogenate to a
final concentration of 0.5%, and the homogenate was incubated for 30 min at 4°C. Each sample was then adjusted to 26% OptiPrep (Axis-shield) in CBS and stepwise OptiPrep gradients were prepared by overlaying 23% and 8% OptiPrep in CBS, and CBS in an ultra-centrifugation tube (Beckman). The stepwise OptiPrep gradients were centrifuged at 18,000 rpm for 2 h at 4°C using a SW32Ti rotor (Beckman). The lipid band at the 8%-23% interface was collected and mixed with 1/2 volume of 5 M NaCl, 0.15 M Tris-HCl (pH 8.0). The sample was adjusted to 26% OptiPrep and stepwise OptiPrep gradients were prepared by overlaying 23% and 8% OptiPrep (in PBS), and PBS in an ultra-centrifugation tube. The gradient was centrifuged at 18,000 rpm for 2 h at 20°C using a SW32Ti rotor. The lipid band at the 8%-23% interface was collected and adjusted to 2% sarkosyl and incubated with 25 units/ml Benzonase (Novagen) for 30 min at 37°C. Each sample was then digested with 10 μg/ml of PK for 1 h at 37°C, and the PK digestion was terminated by incubation with 100 μM Pefabloc SC (Roche) for 15 min at 4°C. Each sample was adjusted to 30 mM EDTA, and thereafter, 1/1.8 volume of 5 M NaCl solution was mixed in to the sample. The sample was overlaid onto a sucrose pad solution [1 M sucrose, 0.1 M NaCl, 0.5% Zwittergent 3-14 (Calbiochem), and 10 mM Tris-HCl (pH 7.5)] in an ultracentrifuge tube and centrifuged at 32,000 rpm for 2 h at 20°C using a SW32Ti rotor. The pellet was washed twice with 0.5% Zwittergent 3-14 in PBS followed by centrifugation at 64,000 rpm for 30 min at 20°C using a S80AT3 rotor (Hitachi). The final pellet was resuspended in PBS containing 0.5% Zwittergent 3-14.

Co-localization statistics

Quantitative co-localization analysis was performed as described elsewhere (9). The
co-localization ratio of inoculated PrP-res with each organelle marker that represents a percentage of weighted co-localization coefficient was quantified using ZEN2009 software. Briefly, the weighted co-localization coefficient was calculated using the following formula;

\[ \frac{\sum_{i} PrPres_{coloc}}{\sum_{i} PrPres_{total}} \]

where \( PrPres_{coloc} \) represents the intensity of a pixel of inoculated PrP-res signal co-localized with an organelle marker, whereas, \( PrPres_{total} \) represents the intensity of a pixel of inoculated PrP-res signal regardless of whether it co-localized with an organelle marker or not.

Because the inoculated PrP-res was labeled with Alexa Fluor 555, signals acquired from Alexa Fluor 555 represent the inoculated PrP-res. Signals obtained from indirect immuno-staining with mAb132 followed by Alexa Fluor 488-conjugated secondary antibodies included signals both from inoculated PrP-res and newly generated PrP\(^{sc}\). Therefore, in the case of PrP\(^{sc}\)-specific staining using mAb 132, signals of newly generated PrP\(^{sc}\) were determined by subtracting the intensity of inoculated PrP-res signal from the intensity of the signal from PrP\(^{sc}\) in the same pixels according to the following formula:

\[ \text{newPrP}^{sc}_{i} = PrP^{sc}_{i} - PrPres_{i} \]

where \( \text{newPrP}^{sc}_{i} \), \( PrP^{sc}_{i} \), and \( PrPres_{i} \) represent intensities of pixels of newly generated PrP\(^{sc}\) signals, PrP\(^{sc}\) signals by Alexa Fluor 488 and inoculated PrP-res signals by Alexa Fluor 555, respectively.

The co-localization ratio of newly generated PrP\(^{sc}\) with an organelle marker represented a percentage of the weighted co-localization coefficient that was calculated according to the following formula;

\[ \frac{\sum_{i} \text{newPrP}^{sc}_{coloc}}{\sum_{i} \text{newPrP}^{sc}_{total}} \]
where $\text{newPrP}^{Sc}_{i,\text{coloc}}$ represents the intensity of a pixel of newly generated PrP$^{Sc}$ signal co-localized with an organelle marker, whereas, $\text{newPrP}^{Sc}_{i,\text{total}}$ represents the intensity of a pixel of newly generated PrP$^{Sc}$ signal regardless of whether it co-localized with an organelle marker or not.
(a) Cell surface PrP^C
/EGFP
/DAPI

EGFP-wtRab4a  EGFP-DNRab4a  EGFP-wtRab5a  EGFP-DNRab5a  EGFP-wtRab7  EGFP-DNRab7
EGFP-wtRab9  EGFP-DNRab9  EGFP-wtRab11a  EGFP-DNRab11a  EGFP-wtRab22a  EGFP-DNRab22a

(b) Relative level of PrP^C (%)

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Supplementary Figure 1. Influence of overexpression of Rab GTPases on expression of PrP<sub>C</sub>.

(a) Expression of PrP<sub>C</sub> on cell surface. To label cell surface PrP<sub>C</sub>, living N2a-3 cells were incubated with mAb 44B1 for 15 min beginning 24 h after transfection of the expression plasmid encoding EGFP-tagged wild-type Rab GTPase or dominant-negative Rab GTPase mutant. Cells transfected with an expression plasmid encoding EGFP were used as the control. The cells were fixed and then counterstained with DAPI. Merged images of cell surface PrP<sub>C</sub> (red), EGFP (green), and DAPI (blue) are shown. Scale bars: 5 μm. (b) Immunoblotting. N2a-3 cells were processed for immunoblotting 24 h after transfection to monitor PrP<sub>C</sub>, EGFP, and β-actin. The graph on the right shows the levels of PrP<sub>C</sub> relative to control cells expressing EGFP. Means of 2 independent experiments with variation are shown.

There was no difference in the staining of cell surface PrP<sub>C</sub> among cells that overexpressed EGFP, EGFP-tagged wild-type Rab GTPase, or EGFP-tagged dominant-negative Rab GTPase mutant (a). Expression of any EGFP-tagged wild-type GTPase or dominant-negative GTPase, except for the dominant-negative mutant of Rab9 and Rab22a mutants, were reproducible based on immunoblotting (b). The PrP<sub>C</sub> levels were not markedly affected by overexpression of any of the EGFP-Rab GTPase fusion proteins. Although a slight decrease in the PrP<sub>C</sub> level was observed with the overexpression of wild-type Rab5a, Rab11a, or the dominant-negative Rab4a mutant, these tagged proteins did not influence the de novo generation of PrP<sub>Sc</sub> (Fig. 7).

Supplementary Video 1. Time-lapse image of Af555-22L-PrP-res in N2a-3 cells.

N2a-3 cells were incubated with Af555-22L-PrP-res for 6 h at 37°C and then subjected to
live-cell time-lapse imaging. The video image shows merged signals of Af555-22L-PrP-res (red) with DIC. The video film was sped up 20×.

**Supplementary references**


7. **Weigert R, Yeung AC, Li J, Donaldson JG.** 2004. Rab22a regulates the recycling of


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### Table S2. Oligonucleotide primers used for the amplification of cDNA of Rab GTPases by PCR.

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Underlines indicate the mutated nucleotides for substituting amino acids.
Table S4.  Number of foci used for co-localization statistics in Figure 3c.

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Table S5. Number of foci used for co-localization statistics in Figure 6.

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