Rab10 as a novel regulator of the sorting of TrkB to the retrograde axonal transport route

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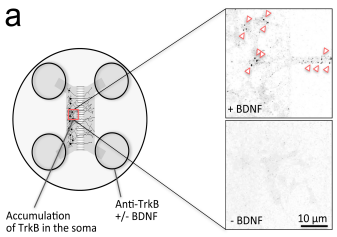
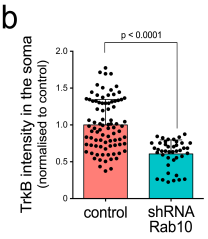
Abstract

Neurotrophic signalling from axon terminals is propagated retrogradely by organelles called signalling endosomes. At their arrival to the soma, these organelles have diverse destinations and regulate several neuronal functions, including gene expression, synaptic maturation and dendritic branching. The diversity of regulatory mechanisms controlling their transport and specific targeting is, nevertheless, only partially understood. A main determinant of the fate of these organelles and their transport is the association with different GTPases of the Rab family, including Rab5 and Rab7. By using microfluidic chambers, we found that Rab10 knockdown leads to a significant decrease of TrkB retrograde transport from axon terminals to cell bodies. Although TrkB receptors are not directly transported in Rab10 organelles, they appear to transit through this compartment, suggesting that Rab10 defines a transition domain regulating the sorting of TrkB receptors from early endosomes to retrograde axonal transport.

1. Rab10 is required for retrograde accumulation of TrkB receptors

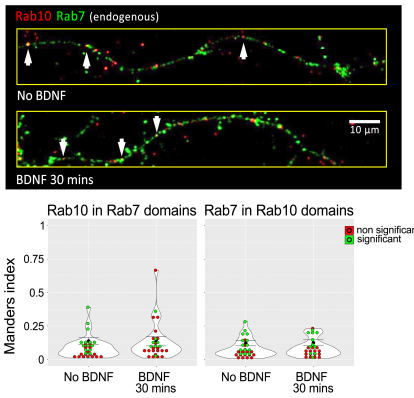
a. Retrograde accumulation assay was performed using hippocampal neurons cultured in microfluidic chambers (left) by adding an antibody against the extracellular domain of TrkB to the axonal compartment. After 2.5 hours with BDNF, accumulation of the antibody in cell bodies is revealed in the somatic compartment by using labeled secondary antibodies (pink arrows).





b. To test whether Rab10 was required for retrograde transport of TrkB, 7-10 DIV neurons were transduced with a lentivirus carrying a doxycycline-inducible shRNA for Rab10 and then treated or not with doxycycline for 18 hours. Neurons expressing the shRNA Rab10 accumulated significantly less TrkB.

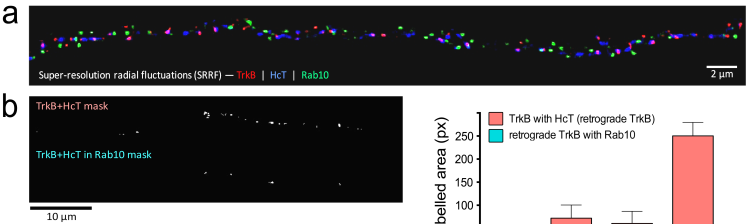
2. Rab10 and Rab7 define different vesicular domains with little overlap in axons



a. Rab7-positive endosomes transport TrkB along the axon. To test whether Rab10 defined a specific population of these carriers, we co-stained endogenous Rab10 (red) and Rab7 (green) in axons from neurons that have been completely depleted of BDNF by using a blocking antibody (upper image) or treated with BDNF 50 ng/mL for 30 min (lower image). LIttle overlapping signal was observed (white arrow heads).

b. Co-localisation was quantified by using Manders index and validated by confined displacement algorithm (CDA, p< 0.01 is indicated with green dots). Little co-localisation was found for Rab7 and Rab10 in axons with or without BDNF.

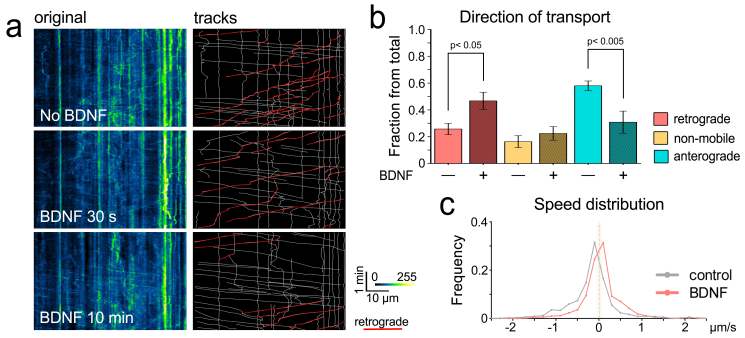
3. Axonal Rab10 organelles do not transport retrograde TrkB



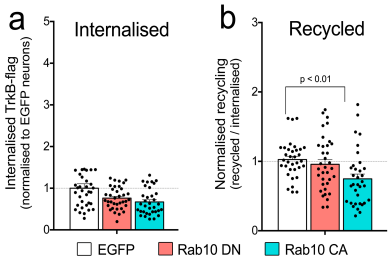
a. To observe to what extent retrogradely transported TrkB overlap with Rab10, we co-internalised anti-TrkB and the non-toxic fragment of tetanus toxin (HcT, blue) as a retrograde endosomal tracer. After 90 min with BDNF, super-resolution microscopy reveals several overlapping TrkB/HcT and little co-localisation with Rab10-positive organelles.
b. Quantitative analysis revealed that at 10, 30 and 60 min after internalisation, the amount of retrograde TrkB overlapping with Rab10 remains constant, suggesting that TrkB transits through a Rab10 compartment.

4. BDNF change the direction bias of axonal Rab10 organelles

The effect of BDNF on axonal transport of Rab10 organelles was analysed by performing live-cell imaging before and after the addition of 50 ng/mL BDNF.
a. Kymographs of the same axonal segment are shown in the absence of BDNF (upper panel), 30 s (middle) and 10 min (bottom) after BDNF addition. Traced tracks were used to analyse direction of transport (**b**), speed distribution (**c**) and pausing frequency (not shown) of the Rab10 organelles, comparing no BDNF and 10 min after BDNF conditions.



5. Rab10 activity is not required for internalisation nor recycling of TrkB receptor in the axon.

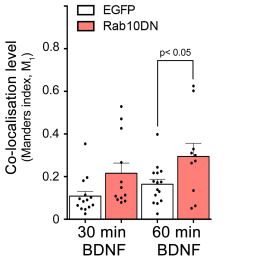


a. Neurons were transfected with TrkB-FLAG and Rab10 constitutively active (CA) or dominant negative (DN) mutants, or EGFP as a control. After 30 min starvation, surface TrkB was stained with anti-FLAG antibodies at 4ºC and then incubated 30 min with BDNF at 37ºC to induce internalisation.
b. After internalisation, were washed to remove remaining antibody from the cell surface, and then the receptors that recycled back to the plasma membrane were chased by using secondary antibodies.

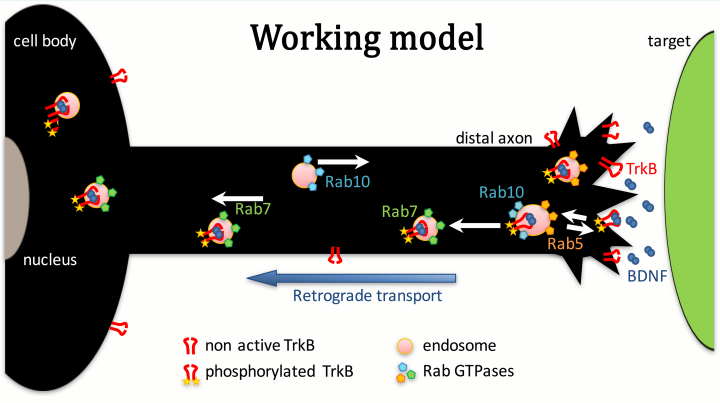
4. Rab10 activity mediate the exit of TrkB from early endosomes

Neurons were transfected with TrkB-FLAG and EGFP or Rab10DN. Internalisation was induced for 30 or 60 min as in 5, and after fixation neurons were stained for endogenous Rab5.

Expression of Rab10DN led to significantly higher co-localisation of internalised TrkB and Rab5 in the axon, suggesting that blocking the activity of Rab10 increase retention of TrkB receptor in early endosomes



Working model



Our results suggest that BDNF signalling leads to **anterograde mobilisation of Rab10 in axons**. Since **internalised TrkB requires Rab10 for its sorting to retrograde transport**, we hypothesised that a Rab10-positive sub-compartment is required to segregate a population of receptors from the early endosome that will be sorted to retrograde signalling endosomes instead of recycling back to the plasma membrane.

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