Mechanisms of axonal transport of slow component-b

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Abstract

Proteins are transported along axons in two overall groups, fast and slow axonal transport. While membranous organelles are conveyed in the fast component (FC), cytoskeletal and cytosolic proteins are conveyed in the slow component (SC) at overall velocities that are 2 to 3 orders of magnitude slower. The SC can be further sub-divided into a group carrying mainly cytoskeletal proteins (called SCa), and another slightly faster group composed of over 200 diverse cytosolic proteins called SCb. SCb members include proteins critical in axonal growth and regeneration and are also implicated in various neurodegenerative diseases. While overall principles of vesicles moving in FC and cytoskeletal polymers moving in SCa are generally understood, transport mechanisms of cytosolic proteins in SCb have remained unclear. To address this issue, we generated a model-system to visualize axonal transport of various fluorescently labeled SCb proteins in living cultured hippocampal neurons. Using this system, we now show that the movement of individual SCb cargoes is rapid but infrequent, with pauses in transit. Quantitative analysis and direct comparison of SCb with fast transport show that though individual SCb cargoes move rapidly like FC cargoes during bursts of movement, the intermittent and infrequent nature of SCb cargoes is likely responsible for the overall slow movement of the SCb population. Furthermore, simultaneous visualization of multiple SCb proteins reveals that SCb proteins are transported in multi-protein complexes. Finally, cytoskeletal disruption studies show that SCb transport is microtubule-dependent, likely powered by kinesins and dyneins. Thus our live-cell studies have begun to provide critical insights into fundamental mechanisms of transport of SCb proteins and provide a tool to probe mechanistic disruptions in diseased states.

Fig.1: Characterization of mRFP::α-SYN (SCb protein) in axons and synapses

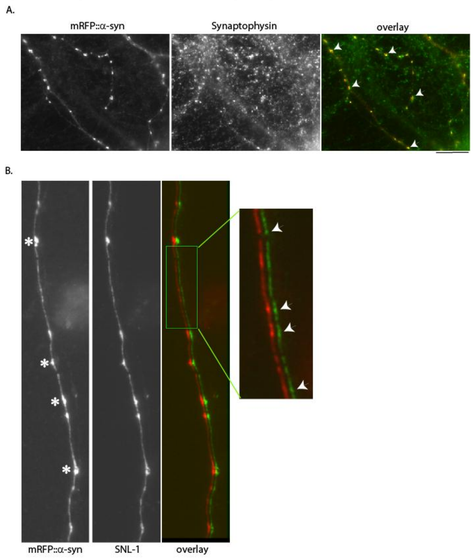

Fig. 1: **(A)** Cultured hippocampal neurons were transfected with mRFP::α-SYN (left) and subsequently immunostained with the presynaptic marker synaptophysin (middle); overlay on right. Note that the bulk of the red-tagged α-synuclein localizes to the presynaptic boutons (arrowheads). **(B)** Cultured neurons transfected with mRFP::α-SYN (human α-synuclein, left) were fixed and stained with the antibody SNL-1 (middle) that is reactive to both human (transfected) and mouse (endogenous) α-synuclein. The merged image (right) is intentionally shifted to reveal co-localization. Note extensive co-localization of the red and green signals both at synapses (asterisk) and also along naïve axons (arrowheads). This suggests that the transfected mRFP::human-α-SYN co-assembles with the endogenous α-synuclein and is a reliable of α-synuclein behavior in axons.

Fig. 2: Rapid but infrequent transport of α-synuclein particles with pauses of varying durations

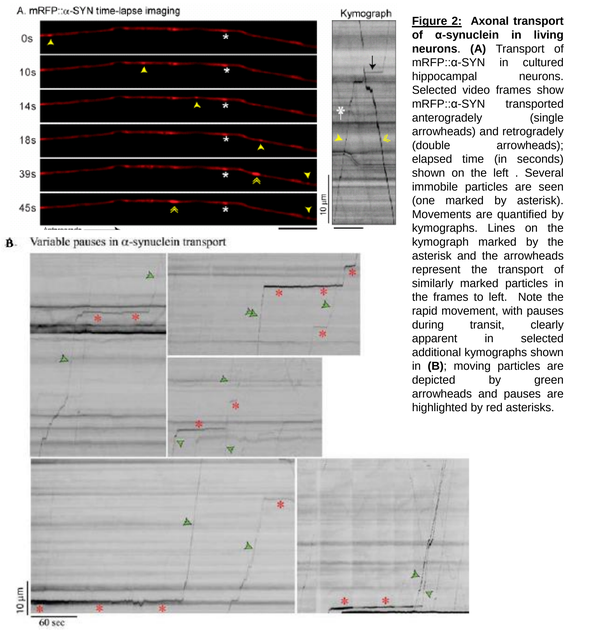

Figure 2: Axonal transport of α-synuclein in living neurons. **(A)** Transport of mRFP::α-SYN in cultured hippocampal neurons. Selected video frames show mRFP::α-SYN transported anterogradely (single arrowheads) and retrogradely (double arrowheads); elapsed time (in seconds) shown on the left . Several immobile particles are seen (one marked by asterisk). Movements are quantified by kymographs. Lines on the kymograph marked by the asterisk and the arrowheads represent the transport of similarly marked particles in the frames to left. Note the rapid movement, with pauses during transit, clearly apparent in selected additional kymographs shown in **(B)**; moving particles are depicted by green arrowheads and pauses are highlighted by red asterisks.

Fig. 3: Comparison of SCb and fast axonal transport

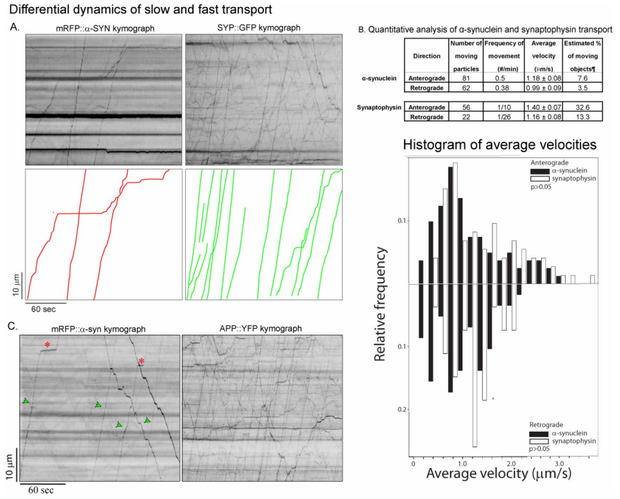

Figure 3: (A) Representative kymographs showing axonal transport of mRFP::α-SYN compared to fast-transport proteins synaptophysin::GFP::α-SYN. Note that while only a few α-synuclein particles move over ~ 5 minutes of imaging. This is quantified in (B). Note that though average velocities of moving α-synuclein and synaptophysin particles are similar, there are large differences in the frequncy of movement. (C) Comparison of α-synuclein with another fast transport marker, amyloid precursor protein (APP) tagged with YFP. Diagonal lines from bottom-left to upper right (few marked by arrowheads) represent anterograde transport; some pauses are denoted by an asterisk. Note the tremendous difference in the transport frequencies of the α-synuclein and APP and the pauses in α-synuclein transport.

Fig.4: Effects of f-actin and microtubule disrupting agents on cultured hippocampal neurons.

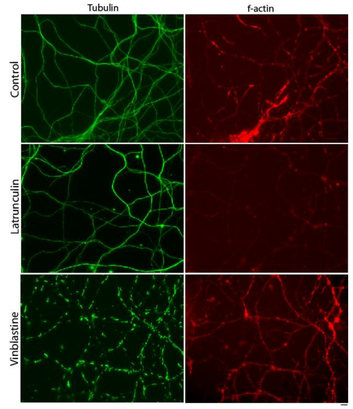

Figure 4: Cultured hippocampal neurons (DIV 10) were treated with 5 μM latrunculin for 24 hours or vinblastine for 3 hours and fixed under conditions that preserve actin filaments and polymerized tubulin. They were then stained with anti-tubulin antibody or rhodamine-phalloidin to reveal microtubules and actin filaments respectively. Note the dramatic depletion of actin filaments under these conditions and the disruption of microtubules. Scale bar for images: 10μm.

Figure 5: Quantitative analysis of α-synuclein transport in cytoskeletal disrupted axons

B. Quantitative analyses of cytoskelton depletion experiments

	Total imaging time (min)	# of moving particles	Frequency (#/min)	Average velocity (μm/s)
Control	226	137	0.62±0.37	2.37±0.9
5μM latrunculin	212	139	0.74±0.53	2.31±0.9
5μM vinblastine	46	4	0.09	N/A

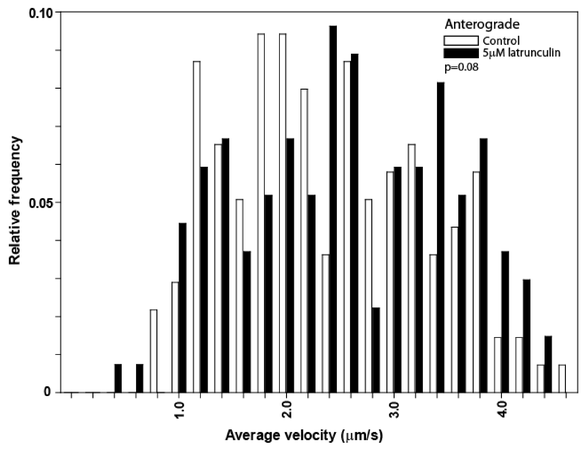

Figure 5: (Top) Quantitative analysis of all moving mRFP::α-SYN particles in control and latrunculin treated axons. All data are derived from kymograph analysis. Average velocities (when moving) are expressed as mean ± standard deviation. **(Bottom)** Frequency histograms of average anterograde velocities of moving mRFP::α-SYN particles (excluding pauses) in control and latrunculin-treated axons. Note that in both groups, α-synuclein moves rapidly at a broad range of velocities and the groups are statistically similar (p>0.05, Mann-Whitney test, two tailed).

Fig. 6: Co-transport of SCb proteins continue in latrunculin treated axons

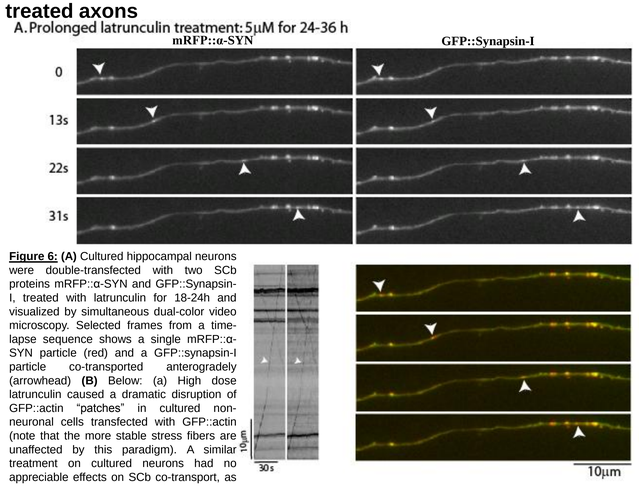

Figure 6: **(A)** Cultured hippocampal neurons were double-transfected with two SCb proteins mRFP::α-SYN and GFP::Synapsin-I, treated with latrunculin for 18-24h and visualized by simultaneous dual-color video microscopy. Selected frames from a time-lapse sequence shows a single mRFP::α-SYN particle (red) and a GFP::synapsin-I particle co-transported anterogradely (arrowhead) **(B)** Below: (a) High dose latrunculin caused a dramatic disruption of GFP::actin "patches" in cultured non-neuronal cells transfected with GFP::actin (note that the more stable stress fibers are unaffected by this paradigm). A similar treatment on cultured neurons had no appreciable effects on SCb co-transport, as shown by the representative dual-cam kymographs in (b).

Fig. 7: SCb transport is microtubule dependent, likely powered by microtubule motors

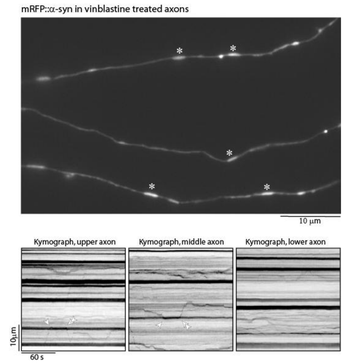

Figure 7: Cultured hippocampal neurons were transfected with mRFP::α-SYN and then treated with vinblastine for 3 hours to disrupt microtubules. Only 4 vectorial movements were seen in 2780s of imaging (1 movement every 695s imaging time). A representative image from one such time-lapse is shown on left. Elongated clusters of mRFP::α-SYN were seen along the axons that probably represent stalled α-synuclein cargoes (asterisks). The kymographs from the axon (below) show the absence of sustained vectorial transport. Note that merging/splitting of the mRFP::α-SYN clusters continue in these axons (arrowheads in kymographs) leading to short-range movements. Such merging/splitting behaviors of vesicular structures is generally attributed to SNARE mechanisms and is probably unrelated to axonal transport. The persistence of such merging/splitting behaviors also suggest that physiological processes continue in these drug treated axons.

Conclusions :

Our direct observations of SCb transport show that actin filaments do not play a major role in SCb transport, either as a scaffolding protein maintaining the SCb complex or as the "rails" on which SCb transport is mediated, as suggested by earlier studies. We also show that SCb transport is microtubule dependent, likely powered by microtubule motor, similar to FC and SCa. We propose that the co-transport of multiple SCb proteins may be a result of the intrinsic affinities of SCb proteins to members of the same rate-class.

Key References:

1. Neurofilaments are Transported Rapidly but Intermittently in Axons: Implications for Slow Axonal Transport. Roy et al. **Journal of Neuroscience** Sept 2000; 20(18):6849-6861.
2. Axonal transport defects as a common theme in neurodegenerative diseases. Roy et al. **Acta Neuropathologica** Jan 2005; 109(1):5-13.
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