Neurofilaments are Transported Rapidly but Intermittently in Axons: Implications for Slow Axonal Transport.

**Abstract**

Proteins are transported along axons in two overall groups, fast and slow axonal transport. While membrane anchors can be 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 cytoskeletal 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 vesicle moving in FC, and cytoskeletal polymer movements in SC are generally understood, transport mechanisms of cytoskeletal 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 cargos is rapid but intermittent, with pauses in transit. Quantitative analysis and direct comparisons of SCb with fast transport show that although individual SCb cargos move rapidly like FC cargos during bursts of movement, the intermittent and infrequent nature of SCb cargos 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-component complexes. Finally, cytoskeletal disruption studies show that SCb transport is microtubule-dependent, likely powered by kinesin and dynein. Thus our live-cell studies have provided new critical insights into fundamental mechanisms of transport of SCb proteins and provide a tool to probe mechanistic disruption in disease states.

**Figure 1:** Characterization of mRFP::α-synuclein (SCb) protein in axons and synapses

**Figure 2:** Rapid but infrequent transport of α-synuclein particles with pauses of varying durations

**Figure 3:** Comparison of SCb and fast axonal transport

**Figure 4:** Effects of fast- and microtubule-disrupting agents on cultured hippocampal neurons

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

**Figure 6:** Co-transport of SCb proteins continue in latrunculin treated axons

**Figure 7:** SCb transport is microtubule-dependent, likely powered by microtubule motors

**Figure 8:** Cultured hippocampal neurons were transfected with mRFP::α-SYN and then treated with trichostatin A (TSA) to disrupt microtubules. Only a vertical movement was seen in 20% of the axons, and represents a major transport system in neurons. Controls treated with TAT-cytosine deaminase displayed uniform movements as seen in untreated neurons. Fast axonal transport was disrupted in SCb when they were treated with the microtubule-disrupting drug, coupled with the microtubule-stabilizing agent, sodium vanadate. They propose that the use of microtubule-disrupting drugs in combination with the microtubule-stabilizing agent provides a novel tool to understand the role of SCb proteins.

**Conclusions:**

Our direct observation of SCb transport show that axin cunacrines do not play a major role in SCb transport, either as a sub-distributing protein maintaining the SCb complex or as the "rail" on which SCb transport is mediated, as suggested by earlier studies. We also show that SCb transport is micromotile-dependent, likely powered by microtubule motor, similar to FC and SCa. We propose that the cytoskeletal disruption mechanisms in SCb may be a result of the instability of SCb proteins to members of the same net-culture.