

## Traffic Interchange

# Occam's Razor Slices Through the Mysteries of Neurofilament Axonal Transport: Can it Really be so Simple?

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Neurofilaments (NFs) are prominent constituents of mature axons. How the neuron establishes and maintains the NF array has been the subject of intense controversy (1). NFs also represent the most highly phosphorylated proteins within the axonal cytoskeleton, yet how phosphorylated NF are segregated within axons, and how phosphorylation contributes to NF dynamics are also not entirely clear (1). The recent demonstration that the microtubule motor protein kinesin participates in the intracellular distribution and axonal transport of NFs, and that phosphorylation regulates the association of NFs and kinesin (2,3), affords a new look at these unresolved aspects of NF dynamics.

## How do NFs Accumulate Within Axons and not Dendrites?

If one accepts that the anterograde motor kinesin either directly or indirectly mediates NF transport, then the inter-neuronal distribution of NFs may be viewed as by default arising from the inter-neuronal distribution of microtubules (MTs) (4,5). MTs within dendrites are non-uniformly oriented. That is, some MTs have their '+' end facing away from the perikaryon, while others have it facing towards the perikaryon. Accordingly, the '+'-end-directed kinesin (and its cargo) would be equally likely to move into and out of dendrites. Conversely, axonal MTs are uniformly oriented with their '+' ends toward the synapse/growth cone. Once entering an axon, kinesin (and its cargo) is committed to an anterograde procession along the length of the axon. The unique orientation of axonal MTs itself may therefore dictate how kinesin effects the accumulation of NFs within axons.

## In What Form(s) do NF Subunits Undergo Axonal Transport?

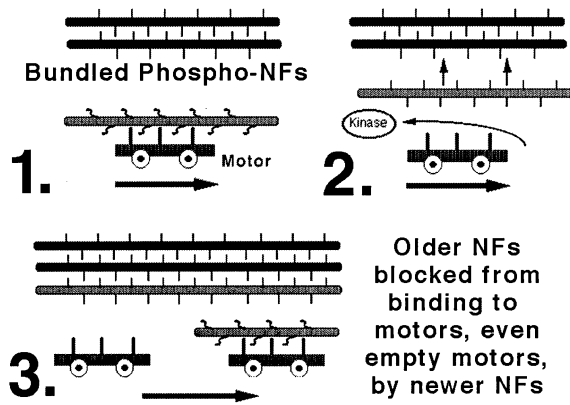
The vast majority of NF subunits are polymerised within axons

(6). However, newly-expressed Triton-soluble subunits (7), and monomeric or non-filamentous oligomeric subunits (1,8) also undergo axonal transport at rates equivalent to or exceeding those of cytoskeleton-associated NFs. Any proposed transport mechanism must encompass the ability to translocate subunits in multiple assembly states. This line of reasoning is substantiated by the intra-axonal association of kinesin with Triton-soluble as well as Triton-insoluble NF subunits (3). Nevertheless, the vast majority of subunits assemble shortly after synthesis and remain as stable polymers once assembled (6,9). Therefore, at least following maturation, a NF motor protein that does not discriminate among assembly states, would translocate the vast majority of subunits in assembled form. Neurons may simply rely on the rapid kinetics of NF assembly to guarantee that the majority of subunits are transported as filaments, and need not invoke additional selection mechanisms to preclude transport of potentially inappropriate forms.

The dynamics of establishing the NF array in growing axons may differ dramatically from those required for its maintenance following maturation. The observation of NF subunit immunoreactivity within axons *in situ* prior to the appearance of NFs themselves (10) the initial predominance of non-filamentous oligomers within growing axons in culture, and the subsequent predominance of filaments (2), suggests that axonal transport of non-filamentous subunits may facilitate establishment of the axonal cytoskeleton. Once fully established, continued transport of monomers, non-filamentous oligomers and short filaments themselves may provide for regional repair/remodelling and/or elongation of larger NFs, or instead may inadvertently be transported with no such physiological role. Hypotheses suggesting that monomers and/or small oligomers represent the major form of transported subunits seem intuitive in terms of energy conservation, since the neuron could replace portions of the cytoskeleton rather than continually translocate the entire axonal cytoskeleton (11). However, each transport 'unit' by definition requires its own motor—monomers would require one motor per subunit, and translocation of small oligomers would require one motor for a relatively small number of subunits, a situation at odds with energy conservation. Further analyses will be required to resolve these considerations.

## How Does Phosphorylation Regulate NF Axonal Transport?

Mitogen-activated protein kinase phosphorylates C-terminal side-arms of NFs (12) and, in doing so, disrupts their association with kinesin (3). Reversible phospho-dependent dissociation



**Figure 1: Working model for the influence of phosphorylation on NF transport by kinesin.** Shown are older NFs (black), and more-recently transported NFs (grey). Hypophosphorylated neurofilaments (indicated by curved sidearm extensions) are associated with the transport motor (1) pending phosphorylation (2), some of which also promote neurofilament bundling. Neurofilaments within bundles may be sterically blocked from re-associating with motors, in part by the continued transport of additional neurofilaments (3), which would induce the characteristic broadening of the transport wave.

tion and re-association could account for the saltatory transport of individual NFs and oligomeric precursors (2,13): while the average transport rate of individual NFs falls within slow axonal transport, their transport often consists of combinations of very rapid and very slow or no motion. Spending only a portion of their time associated with kinesin further allows for NFs to display a net slow transport rate while kinesin itself travels within fast axonal transport (14–16).

Potentially compounding the influence of phospho-dependent dissociation of NFs from kinesin are phospho-dependent NF–NF interactions (17,18), which may compete with NF–kinesin interactions. NF–NF complexes may be too large to be translocated effectively. In addition, we have observed that faster-moving NFs and non-filamentous oligomers that surround a slower-translocating ‘bundle’ of closely-apposed NFs (19). The latter bear a relatively higher concentration of a developmentally delayed C-terminal phospho-epitope; this same phospho-epitope is predominant on those NFs that are selectively not associated with kinesin (3). NFs within bundles may be sterically inhibited from re-association with their transport vector (Figure 1). The combination of phospho-dependent dissociation from their transport vector, coupled with phospho-dependent NF–NF interactions, could generate both the observed slowing of NFs during their continued transport along the axon, as well as the broad range of rates under which NFs undergo axonal transport (20–22).

In summary, the demonstration that kinesin is involved in the distribution of NFs has allowed us to offer simplified mechanisms as potentially underlying several aspects of NF biology. Put simply, kinesin-mediated transport in a phospho-dependent but not assembly-dependent manner via the neuron’s polarised microtubule array can account for many aspects of

NF dynamics. Further studies to determine which kinesin(s), KIFs (23) or additional motors participate in NF transport, as well as site-specific phosphorylation events that disrupt NF–kinesin interactions and foster NF–NF interactions, will extend our understanding of NF dynamics in normal and pathological conditions.

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