Published in final edited form as:

Traffic. 2008 November ; 9(11): 1823–1827. doi:10.1111/j.1600-0854.2008.00818.x.

No Conventional Function for the Conventional Kinesin?

Virgil Muresan and Zoia Muresan

Department of Pharmacology and Physiology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103, U.S.A.

Abstract

A paper by DeGiorgis et al (*Traffic* 2008 DOI: 10.1111/j.1600-0854.2008.00809.x) in this issue of *Traffic* reports on the identification and function of a second squid kinesin, a Kinesin-3 motor. As expected, the newly discovered motor associates with axoplasmic organelles in situ, and powers motility along microtubules of vesicles isolated from squid axoplasm. Less expected was the finding that Kinesin-3 may be the predominant motor for anterograde organelle movement in the squid axon, which challenges the so far undisputed view that this function is fulfilled by the conventional kinesin, Kinesin-1. These novel findings let us wonder what the real function of Kinesin-1 – the most abundant motor in squid axons – actually is.

Keywords

Kinesin-1; Kinesin-3; squid giant axon; axonal transport; vesicle transport; microtubule transport

It is now common knowledge that the anterograde transport of membranous cargo into axons, known as fast axonal transport, occurs primarily along microtubule tracks, and is powered by members of a large family of motor proteins, the kinesins. However, determining which kinesin transports what cargo is still work in progress. The identification of the first kinesin (1-3), done by combining biochemical purification with in vitro reconstitution of motility, was a tour de force, which was made possible by – among others – the right choice of the experimental system: the squid giant axon (4-13). This axon is large, and shows robust vesicle motility (detectable by video-enhanced differential interference contrast microscopy) that is unequalled in any other system. Most importantly, this motility can be reconstituted from purified axoplasmic organelles and soluble components.

At the time of its discovery, kinesin was viewed as *the* motor for anterograde transport of vesicles in the squid axon. Since then, kinesin homologues have been found in almost every species in which investigators looked. Moreover, we learned that several other kinesin-related proteins participate in the axonal transport of the many different types of vesicular cargo (14,15). Kinesin, occasionally termed “conventional kinesin”, because of its abundance and ubiquitous distribution, became the founding member of the kinesin superfamily. In the current nomenclature, it is known as kinesin-1 (16).

The identification of kinesin-1 cargoes – as well as cargoes for the other kinesins - came quite late, as did the identification of kinesin-cargo linkers (also called kinesin receptors) (14,15). Not surprisingly, while Kinesin-1 proved to be a major motor, transporting many different cargoes, other kinesins – including Kinesin-3 motors - also carried specific

cargoes. Yet, while all these advancements were made in species that benefited from the cloning of their genomes, few bothered to continue studying axonal transport in the squid, a species that has not had its genome sequenced¹. In the squid axon, the cargoes are still vesicles with uncharacterized content (a sort of “generic cargo”), classified at best by their size. Moreover, in the absence of genetic information, other kinesins were not identified in the squid, although their existence was occasionally documented in preliminary reports over the years. It was generally assumed that these putative kinesins are minor motors, in terms of the relative proportion of cargo vesicles transported. The view today continues to be that the robust, anterograde vesicle transport in the squid axon is powered primarily by Kinesin-1. In this context, the data reported by DeGiorgis et al. (17), suggesting that Kinesin-3 - rather than Kinesin-1 - may account for the anterograde transport of a large fraction of vesicles in squid axons come as a surprise.

Kinesin-1 May Not Be a Major Vesicle Motor in the Squid Axon

To some, the statement made by the above subtitle may sound puzzling and exciting, and to others an unrealistic, offensive, even heretical hypothesis that challenges a dogma. Yet, data have accumulated over the years, which make a re-evaluation of the role of Kinesin-1 in vesicle transport in the squid axon necessary. Here are pros and cons for such a role.

Pros: First, the procedure aimed at purifying the motor that powers anterograde motility of squid axon vesicles produced Kinesin-1 (3). In reconstitution experiments, the purified motor reproduced the characteristics of microtubule gliding and of bead motility along microtubules seen in the presence of axoplasmic supernatant. Moreover, the newly discovered Kinesin-1 showed properties expected for the fast axonal transport motor, such as nucleotide sensitivity of microtubule binding, solubility, and abundance (3,9). Second, Kinesin-1 co-purified with native vesicles isolated from the axoplasm, which moved in a plus-end-directed manner in motility assays (18,19). Third, immunocytochemical localization of Kinesin-1 in squid axoplasm revealed a pattern consistent with labeling of vesicles aligned along microtubule tracks (20). Finally, an anti-Kinesin-1 antibody inhibited organelle motility, when perfused into the squid axoplasm (20). Curiously, this antibody blocked vesicle motility in both the anterograde and retrograde directions, but the effect on retrograde motility could have been indirect.

Cons: First, Kinesin-1 is loosely bound to squid axon vesicles; only minor amounts are associated with KI-extracted, axoplasmic vesicles that show robust, plus-end motility in vitro, indistinguishable from that of native vesicles (19). Second, the Kinesin-1-driven microtubule gliding and bead motility (in vitro, and when injected into axons) occur at a speed of ~ 0.5 $\mu\text{m}/\text{sec}$, which is significantly lower than the 1.5-2 $\mu\text{m}/\text{sec}$ velocity of axoplasmic vesicles, in vivo and in vitro (13,20). Third, as shown by DeGiorgis et al. (17), an antibody against Kinesin-1 had no effect on the motility of KI-extracted organelles isolated from axoplasm. Although debatable, the KI-extracted vesicles preserve the characteristics of plus-end-directed motility of native organelles (19). Finally, DeGiorgis et al. (17) now provide very convincing data of immuno-electron microscopy (EM) of axoplasmic spreads, which show the complete absence of Kinesin-1 labeling of organelles. Earlier studies (published as preliminary reports), using a different anti-Kinesin-1 antibody, also showed only marginal labeling of organelles in the squid axoplasm. Although negative immunocytochemical data may not always be conclusive (since they may be the result of epitope masking and steric hindrance), the fact that anti-Kinesin-1 antibodies recognizing

¹The squid genome project is currently underway at the Marine Biological Laboratory (Woods Hole, MA), under the direction of Joe DeGiorgis (the lead author of the DeGiorgis et al. paper) and J. Peter H. Burbach (Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht).

distinct epitopes fail to detect Kinesin-1 on organelles strongly suggests that this motor is largely absent. Importantly, in the same preparations of axoplasmic spreads, DeGiorgis et al. (17) detected Kinesin-1 predominantly on microtubules. These, and other observations made over the years, raise the possibility that Kinesin-1 may not be *the* major vesicle motor in the squid axon.

Kinesin-3 Is a Dominant Vesicle Motor in the Squid Axon

If not Kinesin-1, then what other motor may drive anterograde motility of the majority of vesicles in the squid axon? In their paper, DeGiorgis et al. (17) show compelling evidence suggesting that a newly identified squid kinesin, Kinesin-3 powers anterograde motility of a significant fraction of vesicles present in the squid axon. The authors show that unlike Kinesin-1, Kinesin-3 co-purifies with, and is tightly attached to organelles isolated from axoplasm. Tight attachment to vesicles that is resistant to extraction with the chaotropic agent, KI is a characteristic of the generic plus-end motor of a significant fraction of squid axoplasmic vesicles (19). Further, DeGiorgis et al. show that highly specific antibodies against Kinesin-3 block the plus-end motility of isolated organelles, apparently by impeding the motor function of Kinesin-3 rather than its interaction with microtubules (17). In addition, the velocity of axoplasmic organelles *in situ* and in reconstituted assays corresponds to the speed of Kinesin-3-driven motility (1-2 $\mu\text{m}/\text{sec}$) (21,22). Finally, anti-Kinesin-3 antibodies label a large fraction of squid vesicles in axoplasmic spreads (17). In carefully controlled immuno-EM experiments, the authors find that Kinesin-3 clusters at one pole of the vesicles, even when these are not attached to microtubules. This result suggests that motor recruitment to the cargo and dimerization (arguably required for processivity of motility) are microtubule independent.

Of note, the squid Kinesin-3 is highly similar to the *C. elegans* UNC104 (23) and mouse KIF1A (21) kinesins (the prototypes of the Kinesin-3 family), and contains the distinctive pleckstrin homology (PH) and forkhead-associated (FHA) domains proposed to be involved in its recruitment to membranes and regulation of microtubule binding (24,25).

Non-conventional Roles for the Conventional Kinesin

As shown above, evidence accumulated over the years raises the possibility that Kinesin-1 may not be a major motor for the fast, anterograde vesicle transport in the squid axon. Moreover, the study by DeGiorgis et al. (17) now suggests that this task is most likely carried out by Kinesin-3. Then, what could be the real function of Kinesin-1 – a function that would justify the large amounts of this motor present in the squid axon? One possibility, which is consistent with its predominant localization to microtubules (as reported by DeGiorgis et al.) (17), is that Kinesin-1 functions as a motor for slow axonal transport, carrying soluble protein complexes and cytoskeletal polymers (short microtubules and neurofilaments) along microtubules (26,27). Due to its second microtubule binding site located in its tail, Kinesin-1 could also have a structural role, by crosslinking microtubules (28,29). Along with these roles, Kinesin-1 may still participate in the transport of a fraction of the vesicles that move anterogradely in the squid axon, and of larger organelles, such as mitochondria. Also, a less conventional role for Kinesin-1 is suggested by its ability to serve as the scaffold for recruiting active signaling complexes (such as the JNK signaling cascade) to substrates for phosphorylation (30). Kinesin-1 could thus not only transport signaling complexes to the cell periphery, but also directly participate in signaling events that lead to phosphorylation of microtubule-associated targets. The light chains of Kinesin-1, with their heptad repeat and TPR domains (31-33), are typical scaffolding proteins capable of recruiting signaling complexes to microtubules, and thus facilitate the phosphorylation of microtubule associated proteins.

How can the new results of DeGiorgis et al. (17) be reconciled with previous studies supporting a major role for Kinesin-1 in anterograde vesicle transport in the squid axon? Why do antibodies to Kinesin-1 block vesicle motility when perfused in squid axons, and not in motility assays using isolated vesicles? Could it be that the procedures used by DeGiorgis et al. (17) selected for a subpopulation of vesicles that is not transported by Kinesin-1? Is it possible that the loosely associated Kinesin-1 was removed during vesicle preparation (34)? If so, the function-blocking antibodies to Kinesin-1 and to Kinesin-3 probably targeted different vesicle populations. Yet, these factors would not explain the results of immuno-EM in axoplasmic spreads of DeGiorgis et al. (17), which showed a complete lack of Kinesin-1 labeling of vesicles [a result that contradicts earlier immunofluorescence localization studies (20)]. Although a clear answer to these questions cannot be obtained without further experimentation, the apparent contradiction between these two lines of evidence could be explained by a situation in which Kinesin-1 still powers the motility of a significant fraction of vesicles without being associated with the vesicles. In such a model, rather than moving vesicles along microtubules, Kinesin-1 would power transport of short microtubules, with vesicles stably attached to them via specialized, non-motor anchoring proteins (Fig. 1). A conceptually similar model, where short cytoskeletal polymers carry associated cytosolic proteins was previously proposed for the slow axonal transport (35,36). If so, blocking the function of Kinesin-1 with specific antibodies would inhibit vesicle movement in the axoplasm by inhibiting Kinesin-1-driven microtubule motility (with associated vesicles), but would have no effect on isolated vesicles, since they carry no Kinesin-1. Transport of vesicles piggybacking on short microtubules is often seen in motility assays done with homogenized squid axoplasm, where gliding of microtubules loaded with static vesicles is frequently detected (Supplemental Movie 1). Future studies will determine whether this model accurately describes – at least in part - the participation of Kinesin-1 in vesicular transport in vivo.

Another question that remains to be answered is whether the new findings of DeGiorgis et al. (17) may also have relevance for systems other than the squid axon. Numerous studies have shown that vesicular transport powered by Kinesin-1 – in the traditional sense, with the motor attached to the vesicle – is used to carry a large variety of cargoes in both invertebrate and vertebrate (including mammalian) neurons (37). This does not exclude the possibility that kinesin-1 also performs other, less conventional functions, such as direct participation in signaling, or transport of vesicle-laden microtubules. There is ample evidence that short microtubules are transported in mammalian neurons, although this transport may involve cytoplasmic dynein as well (38,39) (see Fig. 1C). The large caliber of the giant axon may explain why a Kinesin-1-driven transport of vesicle-laden microtubules prevails in the squid, and may be less extensive in the much thinner mammalian axons. For now, the study of DeGiorgis et al. (17) has opened the way for a re-evaluation of the role of Kinesin-1 in axonal transport, in the squid giant axon, and other systems as well. It is certainly true that, with Kinesin-1, everything is on the move, including its own function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health grant 7R01GM068596 and March of Dimes Birth Defects Foundation Grant 1-FY04-240.

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Figure 1. Vesicle transport without vesicle motility

A model for anterograde and retrograde transport of cargo vesicles by Kinesin-1-driven gliding of short microtubules over long, stationary microtubules. Vesicles are stably attached to the moving microtubule via non-motor, anchoring proteins (not drawn). For anterograde transport, the vesicle-laden, short microtubule is itself a cargo for Kinesin-1, attached to Kinesin-1's tail (A). For retrograde transport, Kinesin-1 motors are stably attached (via their tail) to long, stationary microtubules, and the vesicle-laden, short microtubule is pushed in the retrograde direction (B). Note that a function-blocking anti-Kinesin-1 antibody would inhibit vesicle transport in both directions. A similar model, where short microtubules are transported anterogradely through the action of cytoplasmic dynein (rather than Kinesin-1), stably attached to a quasi-stationary actin-spectrin meshwork, was previously proposed to explain axonal transport of short microtubules (38); such a model may also account for anterograde transport of vesicle-laden microtubules (C). Arrows show direction of movement of the vesicle-laden microtubules.