Microtubule Transport in the Axon: Re-thinking a Potential Role for the Actin Cytoskeleton

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Microtubules are transported down the axon as short pieces by molecular motor proteins. One popular idea is that these microtubules are transported by forces generated against the actin cytoskeleton. The motor for such transport is thought to be cytoplasmic dynein. Here, the authors review this model and discuss recent studies that sought to test it. These studies suggest that the model is valid but incomplete. Microtubule transport is bidirectional and can utilize either actin filaments or longer microtubules as a substrate in the anterograde direction but only longer microtubules in the retrograde direction. Cytoplasmic dynein is one participating motor but not the only one. The authors speculate that the category of anterograde microtubule transport that involves actin filaments may have specialized functions. The relevant forces that transport short microtubules may also be crucial for the manner by which the longer immobile microtubules interact with actin filaments during events such as axonal retraction and growth cone turning. NEUROSCIENTIST 12(2):1–12, 2006. DOI: 10.1177/1073858405283428

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The cytoskeleton is composed of polymeric filaments that provide architectural support and also participate in motile events within the cell and of the cell. The two chief cytoskeletal elements are microtubules and actin filaments, which are composed of tubulin and actin subunits, respectively. Both are dynamic, both are polar, and both interact with a variety of cellular structures and molecules including molecular motors. One of the most vibrant topics of contemporary cell biology has been the means by which microtubules and actin filaments interact with one another during critical events such as cell division, cell migration, and the outgrowth of cellular processes (Schmit and Lambert 1988; Tanaka and Kirschner 1991; Letourneau 1996; Yarm and others 2001; Rodriguez and others 2003; Kodama and others 2004; Ryan and others 2005). A number of different molecules have been reported to interact with both microtubules and actin filaments and hence could serve as linkers for their interactions (Henriquez and others 1995; Houseweart and Cleveland 1999; Brunner 2002; Lee and Gottlieb 2002; D’Addario and others 2003; Lin and others 2005). In addition, it appears that small molecule regulators of the assembly dynamics of the two filament systems are interrelated, such that the dynamics of one type of filament can affect the dynamics of the other (Lin and Forscher 1993; Dent and others 2003; Roger and others 2004; Noritake and others 2005; Watanabe and others 2005). A robust literature is rapidly developing on microtubule-actin interactions in neuronal cells (Rochlin and others 1999; Dent and Kalil 2001; Salmon and others 2002; Schaefer and others 2002; Lee and Van Vactor 2003; Richman and Halpain 2004; Gordon-Weeks 2004; Gupton and others 2002; Zhou and Cohan 2004), which is not surprising given that neurons extend elongated axons with exaggerated requirements for motile events. Given the great lengths that axons can achieve, it is necessary that cytoskeletal elements are actively transported from the major site of protein synthesis in the cell body down the length of the axon (Baas and Buster 2004). Our laboratory has been focused over the years on the mechanisms by which microtubules are transported down the axon, and recently, we performed studies to investigate a potential role for actin filaments in the complex movements of microtubules within growing axons. The purpose of the present article is to summarize our results, evaluate them in a more global context, and offer speculations regarding their functional implications.

The First Evidence Suggesting a Role of Actin in the Axonal Transport of Microtubules

For a microtubule to be transported in a certain direction, forces must be generated between the microtubule and a structure with greater resistance to movement in the other direction, sometimes termed the “substrate.” Our interest in actin as a potential substrate for microtubule transport was prompted by studies performed about a decade ago by the Pfister laboratory on the molecular motor known as cytoplasmic dynein (Dillman, Dabney, and Pfister 1995; Houseweart and Cleveland 1999; Brunner 2002; Lee and Gottlieb 2002; D’Addario and others 2003; Lin and others 2005). A robust literature is rapidly developing on microtubule-actin interactions in neuronal cells (Rochlin and others 1999; Dent and Kalil 2001; Salmon and others 2002; Schaefer and others 2002; Lee and Van Vactor 2003; Richman and Halpain 2004; Gordon-Weeks 2004; Gupton and others 2002; Zhou and Cohan 2004), which is not surprising given that neurons extend elongated axons with exaggerated requirements for motile events. Given the great lengths that axons can achieve, it is necessary that cytoskeletal elements are actively transported from the major site of protein synthesis in the cell body down the length of the axon (Baas and Buster 2004). Our laboratory has been focused over the years on the mechanisms by which microtubules are transported down the axon, and recently, we performed studies to investigate a potential role for actin filaments in the complex movements of microtubules within growing axons. The purpose of the present article is to summarize our results, evaluate them in a more global context, and offer speculations regarding their functional implications.
This multifunctional molecular motor had been suggested as a strong candidate for the motor that transports microtubules down the axon because it has the appropriate properties to transport microtubules with their plus ends leading against a stationary substrate (Vallee and Bloom 1991). Cytoplasmic dynein moves toward the minus end of the microtubule and hence moves small vesicular cargo toward its minus end. However, if the cargo domain of the dynein molecule interfaces with an immovable structure (or a structure with greater resistance to movement in the opposite direction), then the microtubule itself will move with its plus end leading (Fig. 1A). In the axon, microtubules have a uniform polarity orientation, with plus ends directed away from the cell body (Heidemann and others 1981), which is precisely the arrangement that would result if cytoplasmic dynein were the motor responsible for the movement of the microtubules into the axon from the cell body (Ahmad and others 1998). The notable finding from the Pfister laboratory was that most of the cytoplasmic dynein moves anterogradely down the axon at the same rate as the actin cytoskeleton, suggesting that the actin cytoskeleton may be the substrate against which cytoplasmic dynein pushes in order to transport microtubules.

In this model, the actin cytoskeleton moves down the axon in the same direction as the microtubule (anterogradely, i.e., from the cell body toward the axonal tip). The cargo domain of the dynein molecule associates with the actin cytoskeleton via the dynactin complex, leaving its motor domain available for interaction with microtubules. These interactions are only intermittent, however, such that microtubules are sometimes in motion and sometimes not, which explains why the average rate of microtubule transport is typically slower than the average rate of actin transport (Pfister 1999). We have found this model appealing because microtubules often align with actin bundles during axonal development (Fig. 2) and because it is consistent with the manner by which dynein generates forces between microtubules and the cortical actin meshwork in other types of cells. For example, dynein generates forces between astral microtubules and cortical actin during anaphase of mitosis to assist in driving the half-spindles apart (Baas 1999). In addition, a recent study on Dictyostelium suggests that the comet-like motility of interphase microtubule arrays is due to forces generated between centrosomal microtubules and the cortical actin via cytoplasmic dynein (Brito and others 2005). Similarly, dynein is thought to generate forces between microtubules and actin filaments during cortical rotation in Xenopus eggs (Marrari and others 2003). Although neurons are certainly specialized cells, it makes sense that they would conserve and utilize fundamental cytoskeletal mechanisms such as those...
displayed during mitosis and in these types of relatively simple interphase cells.

Visualization of Microtubule Transport in Living Neurons

Pfister’s studies were performed using the classic radio-label paradigm for studying axonal transport rates in the nerves of animals. Therefore, the paradigm did not lend itself to functional tests of the model. In more recent years, cultured neurons have been used to make important strides in our understanding of microtubule transport in axons, both because they are better suited to functional studies and because they can be used for direct visualization of the moving microtubules. A profound advance came in 2002 when the Brown laboratory discovered that the concerted transport of microtubules within axons is limited to rather short microtubules, only a few micrometers in length (Wang and Brown 2002). These studies involved the introduction of fluorescent tubulin into the neurons followed by photobleaching a roughly 30 µm long region of the axon through which microtubules could be visualized moving. It was found that these short microtubules move at fast rates, asynchronously, and in both directions within axons. Until this observation was made, microtubule transport was considered “slow” because the rate calculated from the radiolabel studies was on the order of a millimeter per day. The work of Wang and Brown (2002) establishes that individual microtubules move many times faster than this average rate and suggests that the average slow rate is due to the fact that most microtubules, at any given time, are not in motion. During axonal growth, about two thirds of the microtubule movements are anterograde, whereas one third are retrograde in direction. The bidirectionality of the movements presumably contributes to the plasticity of the axon as it develops and may or may not occur in axons that have stopped growing. The fact that only short microtubules are in transit suggests that longer microtubules are probably too encumbered by drag imposed on them by cross-links with each other and with other structures to move in such a rapid and concerted fashion. These findings accentuate the critical importance of microtubule severing proteins in rendering a microtubule sufficiently short that it can be transported (Baas and others 2005).

In a related article, the Popov laboratory demonstrated even higher resolution and more robust examples of these movements, particularly in more splayed regions of the axon (Ma and others 2004). These authors reported that the movements occur chiefly along other microtubules, raising the possibility that the actin-based hypothesis is invalid or incomplete. Interestingly, Popov’s group concluded that the short moving structures are not microtubules at all but rather some novel tubulin-containing organelle. This conclusion was based in part on the fact that the movement occurred along long microtubules rather than actin and in part because the moving structures appeared to bend as they moved. We have argued that this logic is weak, given that microtubules are known to move against other microtubules during mitosis and based upon a fairly rich literature showing examples of short microtubules bending (Hasaka and others 2004). Moreover, after roughly two decades of live-cell imaging of tubulin in a variety of cell types, no such non-microtubule tubulin-containing organelle has been identified (Sammak and Borisy 1988). With due respect to these authors and their beautiful work, we would conclude that the moving structures are, as originally concluded by the Brown laboratory, short microtubules.

These live-cell observations raise issues regarding the potential role of actin filaments as the substrate for microtubule transport. The observations that microtubules move bidirectionally and that at least some move along longer microtubules suggest the need to rethink the actin-based model proposed by Pfister. Bidirectional

Fig. 2. Microtubules align with actin filament bundles during early axogenesis. Shown are images of neurons fixed and stained for fluorescence visualization of actin filaments (A) and microtubules (B) during early axonal outgrowth. Large lamellae developed in which microtubules and actin bundles formed along the axes of presumed future axons. Microtubules and actin bundles were seen to colocalize in some cases (see arrows in A and B). Bar, 10 µm. Reprinted with permission from the Journal of Neuroscience (Hasaka and others 2004).
movement of microtubules would either call for other motors in addition to cytoplasmic dynein or a scenario by which short microtubules sometimes act as simple cargo, moving toward the minus ends of longer microtubules as would small vesicles. So, the question becomes whether short microtubules move exclusively along long microtubules, or whether the actin cytoskeleton remains a viable option for a potential substrate in light of these observations. To investigate, we recently performed two studies, one aimed at determining the effects on microtubule transport of depleting actin filaments from cultured neurons (Hasaka and others 2004) and the other on the effects of depleting cytoplasmic dynein (He and others 2005).

Depletion of Actin Diminishes Anterograde but Not Retrograde Microtubule Transport

Using essentially the same live-cell regime of the Brown and Popov laboratories, we ascertained the effects of pharmacologically depleting actin filaments on the movement of GFP-tubulin-containing microtubules within the axons of cultured neurons (Hasaka and others 2004) (Fig. 3). As a result of such treatment, the frequency of anterograde microtubule movements was halved, whereas the frequency of retrograde movements was unaffected (Fig. 3B). This result is consistent with the conclusion that retrogradely moving microtubules ride exclusively along longer microtubules, while anterogradely moving microtubules can ride along either actin filaments or longer microtubules. The mean change in the velocity of the movements was increased slightly after actin depletion, raising the possibility that actin-based mechanisms might generate opposing forces on the microtubules (Fig. 3D). Another possibility, perhaps more likely, is that removal of filamentous actin might make the cytoplasm less viscous and hence generally less resistant to cytoplasmic movements.

Of course the possibility remained that the anterograde movements of microtubules were diminished not because actin filaments are a substrate but for some secondary reason related to the removal of an important element of the cytoplasm. Therefore, we performed additional studies to ensure that microtubules can move on actin in the absence of long microtubules. For this, we used antimicrotubule drugs to deplete or shorten the longer microtubules, such that virtually all of the microtubules in the neuron were only a few micrometers in length (Fig. 4). Under these conditions, the outward transport of microtubules from the cell body into the axon and down its length still occurred unabated (Ahmad and Baas 1995). However, when actin filaments were depleted under these experimental conditions, the outward movement of microtubules was abolished completely (Hasaka and others 2004) (Fig. 4E–F), confirming the conclusion that microtubules can move anterogradely using the actin cytoskeleton as a substrate if no long microtubules are available to fulfill this role. Interestingly, we observed, using another imaging technique, that when actin filaments were removed, the tendency of microtubules to track along other microtubules was notably increased (see Fig. 5 and its legend for details). The one region of the axon that was unable to support organized microtubule behaviors in the absence of actin filaments was the peripheral zone of the growth cone; this makes sense because this zone is deficient in a preexisting microtubule scaffolding that could potentially act as alternative tracks when the actin bundles are removed.

These results raise several questions regarding the role of actin filaments in the anterograde movement of microtubules. Can the microtubules simply choose either option, with no real difference in the functional outcome? Or are there different categories of microtubule transport with divergent functional roles to play, depending on whether the substrate is actin or other microtubules? What factors decide whether a microtubule will ride on another microtubule or on the actin cytoskeleton? Are the same or different motors involved in each type of microtubule transport?

Depletion of Cytoplasmic Dynein Almost Mimics the Effects of Actin Depletion

In pursuit of these issues, we next examined the effects on microtubule transport in axons of depleting cytoplasmic dynein (He and others 2005) (Fig. 6). Specifically, we depleted dynein heavy chain, which is the motor domain of the molecule, using siRNA. Interestingly, the results on the frequency of microtubule movements were essentially the same as observed with actin depletion. There was no diminution in the retrograde movements, whereas the anterograde movements were halved in their frequency (Fig. 6B). Dissimilar to the results with the actin depletion, however, the remaining microtubule movements were slightly slower than controls as opposed to slightly faster (Fig. 6D–G). Although we cannot dismiss the possibility that the small amount of remaining dynein heavy chain might have been enough to fuel some of the remaining movements, the most reasonable conclusion from these studies is that the retrograde microtubule movements use a different motor, presumably a kinesin family member, and so too do half the anterograde microtubule movements. Roughly half of the anterograde microtubule movements, on the other hand, would appear to be dynein dependent. Of course, the possibility exists that the actual proportion of the microtubule movements that utilize actin and/or dynein is different in control axons but shifts when the actin or dynein is depleted.

It is unknown at this time whether there is one distinct category of microtubule movements that is both dynein dependent and actin dependent and another distinct class of movements that is neither, or whether some of the dynein-dependent movements occur along longer microtubules and some of the actin-dependent microtubule movements utilize a different motor. Ongoing studies are aimed at resolving this issue. We have speculated that the movements that are both actin dependent and dynein dependent may carry out very specific functions in the neuron, given the growing literature on the impor-
tance of microtubule-actin interactions. For example, a kinesin-related protein may be capable of transporting enough short microtubules down the axon to sustain its growth, but the dynein/actin-dependent microtubule movements may be essential for more specific tasks that the axon must perform. Indeed, microtubule transport at the growth cone may be far more dependent upon actin-based mechanisms. In light of this, we are currently chal-

Fig. 3. Effects of actin depletion on microtubule transport in the axon. A, Time-lapse images reveal a microtubule moving in the anterograde direction through the photobleached region. Red arrows mark the leading and trailing ends of the microtubule. B, Analysis of microtubule transport events demonstrated that the frequency (events/min) of anterograde transport was significantly reduced in actin-depleted axons (*P < 0.001, χ²), whereas the frequency of retrograde transport was not significantly affected. C, Histogram of microtubule lengths displays no significant difference between control and latrunculin-treated neurons. D, Histogram depicting the mean velocity distributions of both anterograde and retrograde microtubule transport in control and latrunculin-treated neurons. A significant increase in mean velocity was detected in both anterograde and retrograde microtubule movements (P < 0.005 and P < 0.001, respectively; two-tailed t test). E, Histogram showing the distributions of the instantaneous velocities of microtubules chosen randomly from the total observed microtubule population shown in (D). No significant difference was detected between the instantaneous velocities of moving microtubules between control and actin-depleted axons. F, The combined data from (D) and (E) depicting microtubule velocity change as a function of transport directionality shows a significant increase in the mean microtubule transport velocities that is bidirectional (*P < 0.01; two-tailed t test). A small but insignificant increase is seen in comparing control and latrunculin-treated instantaneous velocities. Bar, 5 µm. Reprinted with permission from the Journal of Neuroscience (Hasaka and others 2004).
lenging neurons depleted of dynein heavy chain and/or actin filaments to perform a variety of growth-related tasks in order to tease out specific roles for this category of microtubule transport. Of course, we are also interested in identifying the specific kinesin-related proteins that participate in the anterograde and retrograde transport of microtubules (Fig. 1B).

Do Actin and Motor-Dependent Forces Affect the Longer Immobile Microtubules?

It seems unlikely that the motor-driven mechanisms that transport microtubules would distinguish the short microtubules from the longer microtubules and only impinge upon the short ones (Baas and others 2005). We suspect

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Fig. 4. Outward microtubule transport requires actin filaments when microtubule density is low. A, A schematic representation of the drug treatment regime used in this experiment. Microtubules are drawn in red. B, Control neuron (actin filaments present) after a 15-min exposure to vinblastine (following treatment with and removal of nocodazole) in which short microtubules (white) were seen to invade distal regions where actin filaments (green) were also localized. Arrows in (B) denote several examples of microtubules that have moved outward to the cell periphery and into processes. Fluorescently labeled actin filaments (C) and microtubules (D) in a neuron that has been partially depleted of actin filaments using a lower drug concentration. Microtubules invade peripheral regions of the neuron where actin filaments remain. Arrows in (C) and (D) denote regions where microtubules and actin filaments colocalized. Actin staining (E) and microtubules (F) in a neuron that has been mostly depleted of actin filaments. Short microtubules were seen in the cytoplasm (F), but they were not transported to the cell periphery and many appeared curved and not outwardly oriented as in control cells (B). Arrows in (F) denote examples of maloriented and/or curved microtubules. Bar, 10 µm. Reprinted with permission from the Journal of Neuroscience (Hasaka and others 2004).
that the same forces that transport the short microtubules are generated on the longer microtubules but cannot move them owing to the drag imposed on them by cross-links with other microtubules and cytoplasmic elements. If this is the case, these forces, although not moving the longer microtubules in a concerted fashion, are likely to affect them nonetheless. We think of this much like an isometric exercise in which no movement occurs of either of the two players, even though there is a great deal of force generated between them (Baas and Buster 2004). A very popular model of the 1980s for the axonal cytoskeleton invoked a static “tensegrity” relationship...
Fig. 6. Anterograde microtubule transport is suppressed in dynein heavy chain (DHC)-depleted neurons. A, Time-lapse images reveal a microtubule moving in the anterograde direction through the photobleached region. Red arrows mark the leading and trailing ends of the microtubule. 

B, The frequencies (events/min) of anterograde microtubule transport were significantly decreased in DHC siRNA–treated axons (P < 0.05, χ²) both at four and seven days after siRNA transfection. However, frequencies of retrograde movements were not significantly affected. 

C, Histogram showing that there is no significant difference between control and DHC-depleted neurons with regard to lengths of the moving microtubules. D and E, Histograms depict mean velocity distributions of microtubule movements. 

F and G, Histograms depict instantaneous velocity distributions of randomly chosen microtubules from the population depicted in D and E, respectively. Bar, 5 μm. Reprinted with permission from the Journal of Cell Biology (He and others 2005).
Fig. 7. A schematic model for axonal elongation and retraction based on opposing forces generated by cytoplasmic dynein and myosin-II. Dynein-driven forces transport short microtubules anterogradely either against the F-actin network or longer microtubules, but it is the dynein-driven forces between the long immobile microtubules and the F-actin network that are most relevant to whether the axon retracts. In the elongating axon (A), dynein-driven forces between the long microtubules and the F-actin network offset the myosin-II-driven contractility of the actin network, thus preventing axonal retraction and allowing the axon to grow. In the retracting axon (B), a reduction of dynein-driven forces between the long microtubules and the F-actin network relieves the attenuation of the myosin-II-driven contractility, resulting in retraction of the axon. Alternatively, the myosin-II-based forces may increase and thereby overpower the dynein-based forces, thus causing the axon to retract (not shown).
between the microtubules and the actin filaments. Our modified view of this model is that the relationship is not static but rather involves forces generated by molecular motors (Baas and Ahmad 2001). Shifts in these forces, in this view, can have profound effects on the disposition of both the microtubule array and the actin cytoskeleton.

A key example of this is observed with the phenomenon of axonal retraction. Axonal retraction is a physiologically important event, crucial for pruning overgrown axons during the development of the nervous system (Gallo and others 2002; He and others 2002; Burnstein and Lichtman 1999). It is known that the retraction of the axon requires actin filaments (Ahmad and others 2000; Baas and Ahmad 2001; Gallo and others 2002). We have shown that functional inhibition of myosin-II prevents axonal retraction, whereas functional inhibition of cytoplasmic dynein tends to promote retraction (Ahmad and others 2000). Prior inhibition of myosin-II prevents retraction when dynein is inhibited. These studies suggest that the forces generated by cytoplasmic dynein offset those generated by myosin-II. Our view of this scenario is that the cortical actin cytoskeleton is highly contractile, using myosin-II as a motor for the contractility. Long microtubules interact with the cytoplasmic dynein that is associated with the actin cytoskeleton, imposing forces that oppose the contractility of the

Fig. 8. Schematic model for how dynein-driven forces between microtubules and actin bundles in the growth cone can contribute to growth cone turning. Short microtubules are rapidly transported by cytoplasmic dynein, either along the F-actin network, bundles of actin filaments, or longer microtubules. Comparable forces between actin bundles and the long microtubules are most relevant to growth cone turning. Long microtubules invade the peripheral region of the growth cone and individual filopodia specifically in the direction of growth cone turning. This invasion involves the dynamics of the microtubules (Suter and others 2004; Zhou and Cohan 2004; Watanabe and others 2005). The model speculates that dynein-driven forces play a role in assisting the microtubules to align with and functionally engage the actin bundles during the turning of the growth cone. Not shown in the figure is the possibility that dynein may also play a role in the “capture” of the plus ends of the microtubules at the cortex as has been suggested for certain kinds of motile cells (Dujardin and others 2003).
actin meshwork, thus offsetting the forces generated by myosin-II (Fig. 7). According to this model, which we are now further testing, axons can retract physiologically by either enhancing the myosin-driven forces or dampening the dynein-driven forces (Baas and Ahmad 2001).

More speculative at present is a potential role for these forces in the association of microtubules and actin bundles during growth cone turning. We have reported that stalled growth cones contain looped bundles of microtubules that fracture into short, highly mobile pieces when the growth cone resumes its motility (Dent and others 1999). The mobility of these short microtubules demonstrates that motor-driven forces are active in the region of the growth cone, and we suspect that these same forces also play roles in the alignment and distribution of the longer microtubules as they invade discrete regions of the cone necessary for it to turn in a particular direction (Fig. 8). To date, most studies have focused on the assembly dynamics of the microtubules in the turning growth cone, with little evidence for any bona fide motion of the polymers, at least in the anterograde direction (Schaefer and others 2002). Microtubules and actin filaments have been observed to coassemble (Rochlin and others 1999; Dent and Kalil 2001), suggesting that there may be no role for motors in their alignment. It is our contention, however, that cytoplasmic dynein (and perhaps other motors) is important for integrating the microtubule with the actin bundle in a force-dependent fashion comparable to the manner by which microtubules interact with the cortex in other cell types. Without such forces, we speculate that the two filament systems would be unable to functionally engage and that the microtubules would slip backward from the actin bundle in much the same way that the entire microtubule array retreats during axonal retraction. Studies are under way to test this predication.

Some Closing Thoughts

It was not terribly long ago that live-cell imaging techniques were not sufficiently developed to permit the visualization of microtubule transport in living cells. Back then, it was not uncommon for cell biologists to posit that neurons probably do not transport their microtubules. Today, however, we know that microtubule transport is not only robust in neurons but also a theme broadly utilized across cell types. With regard to microtubule-actin interactions, it is apparent that these two filament systems can interact in a number of different ways related to their assembly properties and molecules that link or interact with both. Not all microtubule-actin interactions in the neuron involve motor-driven forces, and not all motor-driven forces on the microtubules involve actin. Surprisingly, not all motor interactions with microtubules and actin involve force generation (Chuang and others 2005). Even so, the available data indicate that force generation between actin and microtubules is responsible for at least one category of microtubule transport in the axon, and the same forces may be critical for regulating essential features of the cytoskeleton underlying events such as axonal retraction and growth cone turning. We look forward to performing a variety of functional studies that will test the merits of these speculations.

References