Mitochondrial Dynamics and Peripheral Neuropathy

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Peripheral neuropathy is perhaps the archetypal disease of axonal degeneration, characteristically involving degeneration of the longest axons in the body. Evidence from both inherited and acquired forms of peripheral neuropathy strongly supports that the primary pathology is in the axons themselves and points to disruption of axonal transport as an important disease mechanism. Recent studies in human genetics have further identified abnormalities in mitochondrial dynamics—the fusion, fission, and movement of mitochondria—as a player in the pathogenesis of inherited peripheral neuropathy. This review provides an update on the mechanisms of mitochondrial trafficking in axons and the emerging relationship between the disruption of mitochondrial dynamics and axonal degeneration. Evidence suggests mitochondria are a "critical cargo" whose transport is necessary for proper axonal and synaptic function. Importantly, understanding the regulation of mitochondrial movement and the consequences of decreased axonal mitochondrial function may define new paths for therapeutic agents in peripheral neuropathy and other neurodegenerative diseases.
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The study of mitochondrial dynamics, defined as the fusion, fission, and movement of mitochondria, is a rapidly developing field with broad implications for metabolism and cell biology research. Many excellent reviews on the broader topic of mitochondrial dynamics in cellular and mitochondrial function have recently been presented (Meeusen and Nunnari 2005; Okamoto and Shaw 2005; Santel 2006; Chan 2006). This update focuses on the emerging role of altered mitochondrial dynamics in peripheral nerve diseases. Peripheral sensory and motor neurons are the most polarized cells in the body—their axons are up to 1 meter in length, with the majority of their mass and energy expenditure at sites far distant from the cell body. Therefore, they are poised to be selectively susceptible to disruption of the proper transport of cytoskeletal components, vesicles, and organelles. Of these organelles, mitochondria are perhaps the most critical cargo traveling in axons, because they support the tremendous requirement for axonal ATP necessary for maintenance of ionic gradients for firing action potentials, maintenance of the axonal cytoskeleton, vesicle mobilization for synaptic transmission, and axonal transport itself (Hollenbeck and Saxton 2005). Mitochondria further play an important role in the regulation of axonal calcium homeostasis, particularly at the neuromuscular junction (Ly and Verstreken 2006), and are either directly or indirectly implicated in the pathogenesis of many neurodegenerative diseases as the arbiters of apoptotic cell death and generators of reactive oxygen species (Lin and Beal 2006).

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Clinical Insights into the Mechanisms of Peripheral Neuropathy

Peripheral neuropathy is a clinical term used to broadly indicate any abnormality of peripheral nerve. Consequently, there are a myriad of forms of peripheral neuropathy, and likewise, many different mechanisms of peripheral nerve disease ranging from autoimmune, vascular, toxic, and metabolic diseases to inherited disorders of Schwann cell myelination or axonal stability. Of these various insults to which peripheral nerves are susceptible, the recent discovery of many genes involved in the inherited neuropathies (known as Charcot-Marie-Tooth disease) have provided a unique opportunity to understand the critical molecular pathways involved in peripheral axon stability and length-dependent peripheral nerve disease (Zuchner and Vance 2006).

Length-Dependent Peripheral Neuropathies—Archetypal Diseases of Axonal Transport?

An important concept in peripheral neuropathy is that many types are characteristically length dependent—that is, the longest axons in the body are affected first and most profoundly. The length-dependent distribution in peripheral neuropathies supports the concept that the

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Table 1. Genetic Disorders of the Axonal Cytoskeleton or Transport Apparatus That Result in Length-Dependent Peripheral Axon Degeneration[a]

	Gene	Disease	Comments
Human			
Axoskeletal components or modulators	*Neurofilament light chain*	Axonal Charcot-Marie-Tooth	
	Gigaxonin	Giant axonal neuropathy	Hair changes, central involvement,and other systemic features
	Heat shock 27kDa protein 1	Axonal Charcot-Marie-Tooth	Can have selective motor involvement
	Atlastin	Hereditary spastic paraparesis (SPG3A) +/– peripheral neuropathy	Can involve both central and peripheral axons
	Motor proteins		
	Kinesin family member 1b	Axonal Charcot-Marie-Tooth	
Mouse			
Axoskeletal components or modulators	*Dystonin/BPAG1*	Dystonia musculorum (dt)	Selective sensory involvement
	Tubulin-specific chaperone E	Progressive motor neuropathy (pmn)	Selective motor involvement
	Intermediate filaments (neurofilaments, peripherin)	Transgenic mouse models	
	Motor proteins		
	Dynein (*Dync1h1*)	Legs at odd angles (loa), cramping (cra1)	
	Dynamitin	Transgenic mouse model	

a. Please refer to Zuchner and Vance (2006), Duncan and Goldstein (2006), and Reid (2003) for additional discussion and references.

major site of pathology is in the axon itself, rather than the cell body. Furthermore, it suggests that shorter axons are either less susceptible or better able to compensate for certain insults than are longer axons, leading to the degeneration of the distal regions of the longest axons first. One of the most widely studied hypotheses for the selective susceptibility of neurons to various insults is that they have a unique requirement for axonal transport to support their proper function (Duncan and Goldstein 2006). A logical extension of this hypothesis is that the longest axons in the body (those in the peripheral nervous system) should be most sensitive to a global defect in axonal transport.

In support of the hypothesis that long axons are most susceptible to a generalized defect in axonal transport, there are now many examples of genetic diseases in both humans and mice in which either primary or secondary defects in axonal transport lead to length-dependent degeneration of peripheral motor or sensory axons (Table 1). These can be placed into two major categories: 1) mutations in genes which affect the axonal cytoskeleton (microtubules or intermediate filaments), leading to a secondary disruption of axonal transport (Perez-Olle and others 2005), and 2) mutations in components of the microtubule-based motor complexes themselves (kinesin, dynein), which directly disrupt axonal transport. Of course, there are some important caveats to the theory that peripheral axons are affected in these genetic disorders simply because they are long. First, not all hereditary neuropathies involve both sensory and motor axons equally, despite similar length, and the nature of this selectivity remains to be understood. Second, upper motor neurons from the brain to the spinal cord are not commonly affected in length-dependent inherited neuropathies, even though some are nearly as long. In fact, a different set of genes involved in axonal transport (*KIF5A*) and cytoskeletal modulation (*Spastin*) are implicated in the selective length-dependent degeneration of central axons in the hereditary spastic paraparesis syndromes (Reid 2003). This suggests that although regulation of cytoskeletal integrity and axonal transport are linked to length-dependent axon degeneration in both central and peripheral neurons, the particular proteins involved may be distinct.

Therefore, there is growing evidence to support that disruption of axonal transport can lead to distal axonal degeneration selectively in the peripheral nervous system and that the vulnerability to transport disruption is length dependent. Given that disruption of axonal transport by altering cytoskeletal "tracks" or microtubule motor proteins can lead to a length-dependent neuropathy, are some

transported cargos more critical than others? Recent evidence that a common form of axonal Charcot-Marie-Tooth disease may be caused by selective disruption of axonal mitochondrial transport suggests that disruption of mitochondrial trafficking alone may be sufficient to cause length-dependent degeneration of peripheral axons (Baloh and others 2007).

Mitochondrial Localization in Peripheral Axons

Although mitochondria are present throughout axons, it has been recognized for some time that they are not randomly distributed, but rather, are more highly concentrated in specific axonal regions that typically have a high demand for energy in the form of ATP (Fig. 1). These include the distal region of the initial segment (YC Li and others 2004), the nodes of Ranvier in myelinated fibers (Berthold and others 1993), and the neuromuscular junction (Lichtman and Sanes 2003) or sensory end organs (Watanabe 2004). Furthermore, they become differentially localized in pathologic states, as they are observed to accumulate in demyelinated axonal segments (Mutsaers and Carroll 1998) and in regions of disrupted axo-glial junctions (Einheber and others 2006).

In the initial segment of the axon and the nodes of Ranvier, the mitochondria likely support ATP production necessary to maintain ionic gradients via the Na^{+}-K^{+} ATPase (Erecinska and Silver 1994). In neuromuscular junctions, mitochondria are involved in ATP production to support synaptic vesicle loading, mobilization, and recycling (Murthy and De Camilli 2003) and to maintain calcium homeostasis by direct Ca^{2+} sequestration (David and others 2003) and the generation of ATP to support plasma membrane Ca^{2+}-ATPases (Rizzuto 2001). Given the high numbers of mitochondria in sensory organs of the skin, including corpuscles and free nerve endings, they likely support similar demands for ATP and Ca^{2+} regulation in these critical organs as well.

Regulatory Mechanisms of Mitochondrial Localization and Transport

Movement of mitochondria in tissue culture cells has been recognized since the early part of the 20th century (Lewis and Lewis 1914), and more recently, the use of fluorescent dyes and mitochondrially targeted fluorescent proteins has allowed the analysis of mitochondrial movement in axons in culture and in live mice (Hollenbeck 1996; Misgeld and others 2007). At first glance, mitochondrial movement in axons appears haphazard, with individual mitochondria frequently stopping and changing direction, and at any given time, the majority of mitochondria (~90%) are immobile (Fig. 2). Mitochondria are predominantly transported along microtubules via the ATP-dependent motor proteins kinesin and dynein; however, they can also be transported bidirectionally for short distances along actin filaments via myosin motors (Hollenbeck 1996). Given

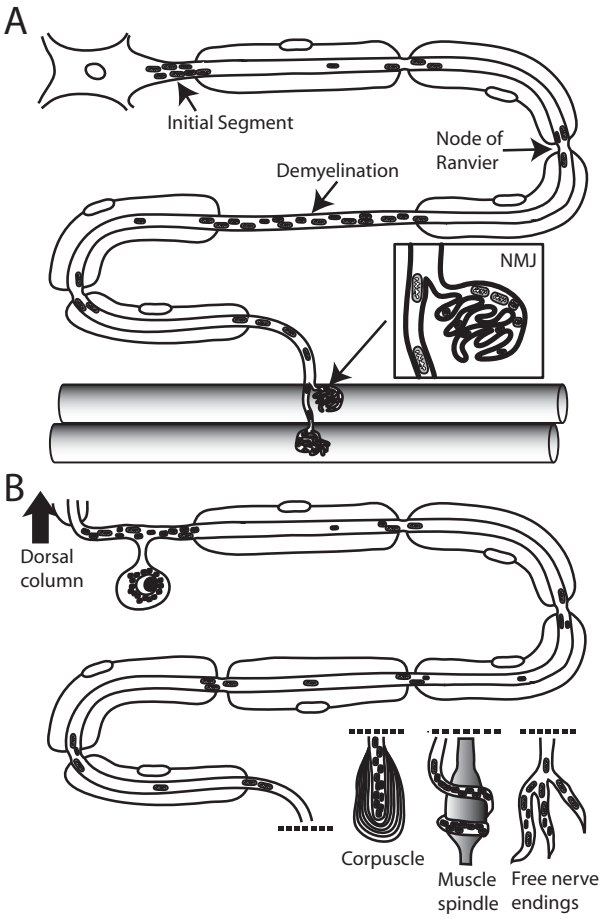

Fig. 1. Regions of mitochondrial localization in peripheral neurons. *A*, Schematic of a motor neuron highlighting axonal regions in which mitochondria are preferentially located. Mitochondria are highly concentrated at sights of high energy demand and ion flux, including the axon initial segment, nodes of Ranvier, and the neuromuscular junction (NMJ—inset). Furthermore, they become concentrated at sites of axonal injury, demyelination, and altered axo-glial interactions. *B*, A similar distribution of mitochondria is seen in myelinated peripheral sensory neurons, again with relatively increased numbers of mitochondria at nodes of Ranvier and distal sensory organs, including corpuscles, muscle spindles, and free nerve endings (see text for references).

the selective localization of mitochondria to sites of high ATP demand and those requiring tight regulation of Ca^{2+} homeostasis, the question arises—what are the mechanisms that regulate the transport and docking of mitochondria at these sites? Recent evidence in this rapidly moving field points to a set of linkers and adaptor proteins that regulate the attachment of mitochondria to motors or anchors in response to various intracellular stimuli including local Ca^{2+} concentration and various signaling cascades (Hollenbeck and Saxton 2005).

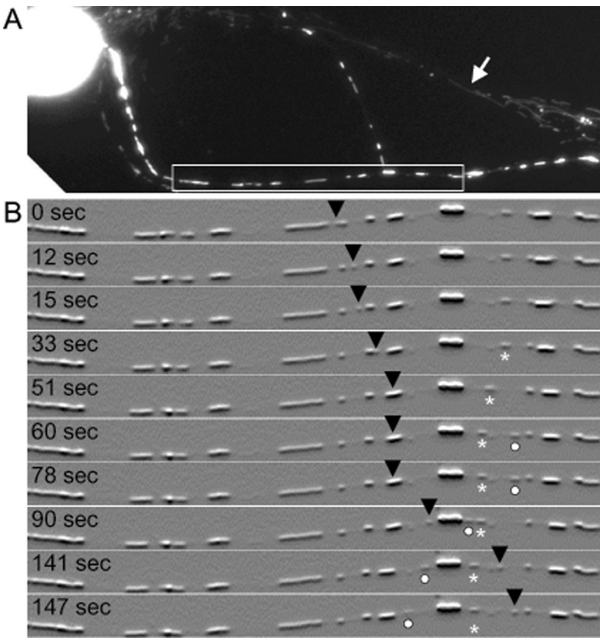

Fig. 2. Movement of axonal mitochondria in a dorsal root ganglion (DRG) neuron. *A*, Fluorescence image of a cultured DRG neuron expressing a mitochondrial-targeted fluorescent protein to visualize mitochondria (for methods, see Baloh and others 2007). The soma is on the left of the image and is completely white because it is filled with fluorescent mitochondria. The axon shown in the time series below is highlighted with a white outline. Note the elongated mitochondria in the neighboring fibroblast (white arrow) as compared with those in the axon. *B*, Time series of an axon from the top panel showing a typical pattern of mitochondrial movement over several minutes of imaging. The axon is represented with the emboss filter (Adobe Photoshop) to make it easier to visualize mitochondria. Most mitochondria are immobile, whereas others undergo brief movements, stop, then continue. Mobile mitochondria are typically short tubules, whereas stationary mitochondria are usually longer. For clarity, several moving mitochondria are highlighted (arrowhead, asterisk, white circle).

Linkers and Adaptor Proteins

Milton was the first protein identified that can function as a mitochondria-specific linker protein for attachment to microtubule motors (Stowers and others 2002). It was found in a screen for *Drosophila* with defective photoreceptor synapse function and hence named for the famed British poet who became blind late in his life. *Milton* mutants have photoreceptor terminals devoid of mitochondria but with normal quantities of synaptic vesicles. Milton binds to the heavy chain of kinesin-1 and is not directly attached to the mitochondrial membrane, but rather interacts via an outer mitochondrial membrane GTPase named Miro (mitochondrial Rho-GTPase), which was also found in a screen for *Drosophila* mutants with defective synaptic function in photoreceptors (Glater and others 2006; Guo and others 2005). *dMiro* mutants have locomotor abnormalities and die prematurely, and mitochondria accumulate in the soma and are absent from distal motor axons. Importantly, mitochondria are also abnormally distributed in other tissues including muscle; however, the locomotor defect and survival could be rescued by selectively restoring neuronal expression of dMiro (Guo and others 2005). This indicates that at least in *Drosophila*, there is indeed selective vulnerability of neurons over other cell types to a global defect in mitochondrial trafficking.

Regulatory Stimuli

Given the role of mitochondria in regulating intracellular Ca^{2+}, it is not surprising that mitochondrial movement has been shown to be regulated by Ca^{2+} itself. In cultured myoblasts, elevations in intracellular Ca^{2+} decrease mitochondrial movement (Yi and others 2004), and Ca^{2+} entry from excitotoxic doses of glutamate decreases both dendritic and axonal mitochondrial transport in cultured cortical neurons (Chang and others 2006). Although the mechanism remains uncertain, the presence of two Ca^{2+}-binding EF hand motifs in the Miro proteins makes them excellent candidates to modulate mitochondrial movement in response to intracellular Ca^{2+} (Fransson and others 2006). Other pathways that likely influence mitochondrial transport include GSK3β phosphorylation of Kinesin-1, phosphatidyl-inositol signaling, and NGF-TrkA signaling (for references, see Hollenbeck and Saxton 2005). The specificity of these signals for regulating mitochondrial trafficking versus other organelles, and how the adaptors and linkers integrate these signals to direct axonal traffic will be an exciting field to follow in the years to come.

The Relationship Between Mitochondrial Fission, Fusion, and Movement

Up to this point, the discussion of mitochondrial dynamics has focused on mitochondrial movement. However, in accordance with their name (Greek: *mitos*—thread, *khondros*—grain), mitochondria are constantly fusing with and breaking apart from one another to generate various morphologies (Lewis and Lewis 1914). The cadre of proteins that mediate mitochondrial fusion and fission has been characterized in detail in recent years (Okamoto and Shaw 2005). The primary mediators for mitochondrial fission include the proteins Drp1 and Fis1. Fis1 resides on the outer mitochondrial membrane, whereas Drp1 is a dynamin-family GTPase recruited to the mitochondrial membrane from the cytoplasm and functions as an oligomer to cleave one mitochondrion into two (Otsuga and others 1998). Mitochondrial fusion is mediated by the mitofusin proteins (MFN1 and MFN2), which are GTPases located on the outer mitochondrial membrane, and another dynamin-family GTPase associated with the inner mitochondrial membrane, OPA1 (Chen and others 2003; Cipolat and others 2004). Like mitochondrial movement, the balance of mitochondrial fusion and fission varies in different cell types and is regulated in response to different stimuli. For example, prolonged increases in intracellular Ca^{2+} lead to increased fission and fragmentation of the mitochondrial network (Rintoul and others 2003), as does the initiation of apoptosis (Youle and Karbowski 2005).

One important concept in mitochondrial dynamics is that mitochondrial movement and fusion/fission are not completely independent from one another. The most obvious example of this is in the case of mitochondrial fusion. If two mitochondria cannot travel across the cell to dock with one another, they cannot fuse. Therefore, a decrease in mitochondrial movement could result in a secondary decrease in mitochondrial fusion. In contrast, mitochondrial fission is independent of mitochondrial movement—a mitochondrion can sit immobile, recruit Drp1 from the cytoplasm, and undergo fission without requiring movement to do so. Therefore, it is predicted that a decrease in mitochondrial movement would alter the balance of mitochondrial dynamics toward fission.

Another important concept is that at least in axons and dendrites, shorter mitochondria move more often and are more easily transported. In mouse peripheral axons expressing a mitochondrial-targeted fluorescent protein, moving mitochondria are half the size of stationary mitochondria (~1.5 μm vs. 3 μm; Misgeld and others 2007). Furthermore, artificially tilting the balance toward fusion by decreasing Drp1 function leads to decreased numbers of mitochondria in distal neuronal processes of cultured hippocampal neurons (Z Li and others 2004) and decreased mitochondria in neuromuscular junctions in *Drosophila* (Verstreken and others 2005). This may be because longer threadlike mitochondria cannot be transported as efficiently in the narrow passageways along axons and dendrites.

Altered Mitochondrial Dynamics in the Pathogenesis of Peripheral Neuropathy

How then, does the disruption of mitochondrial dynamics relate to the focus of this update, peripheral neuropathy? The connection was made by the observation that dominantly inherited mutations in MFN2 are the most commonly identified cause of the axonal variant of Charcot-Marie-Tooth disease (Zuchner and others 2004). MFN2 is ubiquitously expressed (Rojo and others 2002), and a familiar question in the study of neurodegenerative disease arises—how do mutations in a ubiquitously expressed protein lead to selective degeneration of a specific region of the nervous system, in this case, the distal regions of long peripheral axons? Although still a work in progress, initial experiments suggest that a disruption in mitochondrial transport may explain this selectivity. Cultured sensory neurons expressing disease-mutant MFN2 proteins show delayed transport of mitochondria into distal axons and a decreased frequency of mitochondrial movements, and this transport defect occurred in the setting of normal levels of ATP and normal measures of mitochondrial function (Baloh and others 2007). Therefore, MFN2 disease mutants likely lead to a global disruption of mitochondrial transport, but severe enough to affect only the cells most susceptible to a transport defect—long peripheral axons. Exactly how MFN2 disease mutants disrupt mitochondrial transport remains to be determined, but an interesting possibility is that MFN2 itself functions in the linker-adapter complex between mitochondria and microtubule motors.

What about mitochondrial fusion, the primary function of MFN2? Perhaps long peripheral axons are somehow selectively susceptible to loss of mitochondrial fusion, which may serve as a protective mechanism for mitochondrial stress (Chan 2006). Although the contribution of defective fusion from MFN2 disease mutants remains to be determined, an important clue to answering this question again arises from clinical observation. OPA1, located on the inner mitochondrial membrane, has no known homologue and is absolutely required for mitochondrial fusion (Chen and others 2005). Loss of both alleles of OPA1 leads to early embryonic lethality in mice, and presumably, also in humans (Alavi and others 2007; Davies and others 2007). Loss of one allele leads to dominantly inherited optic atrophy via haploinsufficiency, and fibroblasts from some patients with OPA1 mutations show a fragmented mitochondrial network consistent with decreased mitochondrial fusion (Olichon and others 2007). The fact that no OPA1 patients with peripheral neuropathy have been reported argues strongly that a fusion defect alone will not lead to distal peripheral axon degeneration. Interestingly, although patients with OPA1 mutations do not develop peripheral neuropathy, a subset of Charcot-Marie-Tooth (CMT) patients with MFN2 mutations does develop optic atrophy (Zuchner and Vance 2006). Given that about half of the neuropathy-associated MFN2 mutations examined also have a defect in mitochondrial fusion (Detmer and Chan 2007), it can be proposed that, whereas all disease-associated MFN2 mutations lead to decreased mitochondrial transport and thereby peripheral neuropathy, the subset of mutations that also lead to optic atrophy do so because they additionally disrupt mitochondrial fusion (Fig. 3).

It is important to note that GDAP1 (ganglioside differentiation induced associated protein-1) is another protein mutated in axonal CMT that is attached to the outer mitochondrial membrane and appears to be involved in mitochondrial fission (Niemann and others 2005). Interestingly, most GDAP1 mutations that cause CMT are recessively inherited and presumably lead to loss of GDAP1 function and decreased mitochondrial fission. Further work will need to determine if loss of mitochondrial fission in these cases could lead to a situation analogous to that seen in Drp1 mutants in *Drosophila*, resulting in decreased transport of mitochondria to distal axons possibly from inefficient transport of hyperfused mitochondria (Verstreken and others 2005).

Abnormal Mitochondrial Localization as a Pathway to Axonal Dysfunction and Degeneration

Regardless of the mechanism by which it occurs, disruption of proper mitochondrial localization would certainly disrupt axonal and synaptic dysfunction and likely ultimately result in axonal degeneration (Fig. 4). Experiments investigating the mechanism of anoxic injury to axons have defined a molecular pathway of axonal degeneration resulting from mitochondrial dysfunction (Stys and others 1992). The drop in ATP levels from dysfunctional mitochondria

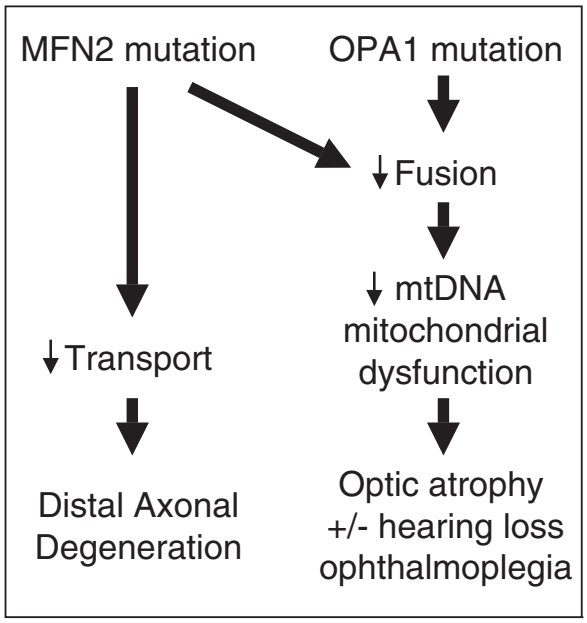

Fig. 3. Flow diagram with a possible explanation for the relationship between abnormal mitochondrial dynamics and disease phenotype. *A*, OPA1 mutations result in decreased mitochondrial fusion, leading to decreased mitochondrial DNA (mtDNA) stability and optic atrophy. These patients also occasionally have external ophthalmoplegia (weakness of the eye muscles), which is characteristic of disorders with decreased mtDNA stability. Mitofusin protein-2 (MFN2) mutations lead to decreased mitochondrial transport (Baloh and others 2007), and therefore, distal axonal degeneration and peripheral neuropathy. Some MFN2 mutations also disrupt mitochondrial fusion (Detmer and Chan 2007), and therefore can have optic atrophy similar to OPA1 patients. However, because OPA1 mutations do not disrupt mitochondrial transport, they do not lead to distal axonal degeneration and peripheral neuropathy.

can lead to decreased Na^+-K^+ ATPase function, resulting in a rise in intracellular Na^+ and reversal of the Na^+-Ca^{2+} exchanger (Waxman 2006). The resulting rise in intra-axonal Ca^{2+} levels, if not buffered, has several deleterious effects that can ultimately lead to axonal degeneration (Raff and others 2002). Short of restoring proper axonal mitochondrial transport, understanding these downstream pathways of axonal degeneration may be the key to devising therapeutic strategies for peripheral neuropathies in which disrupted axonal mitochondrial transport is found to be a primary player.

Summary and Future Directions

In summary, the invaluable insights gained from human genetics research have led to what at first appeared an unlikely marriage—peripheral nerve disease and the control of mitochondrial dynamics. However, as more investigations are performed, it appears that disruption of mitochondrial fission and fusion likely ties into the familiar Achilles heel of the longest cells in the body—their need to effectively transport cellular materials and

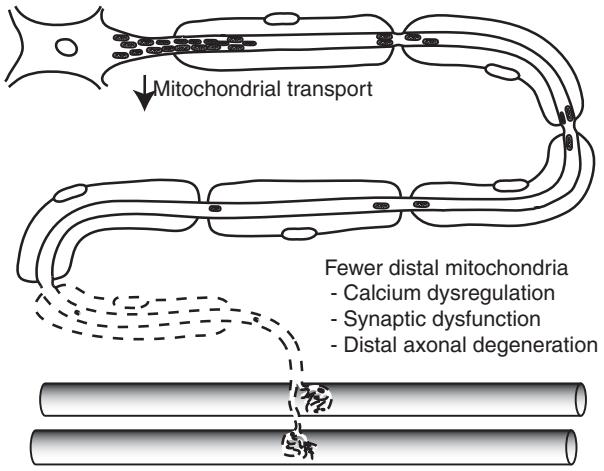

Fig. 4. Schematic diagram demonstrating the potential consequences of abnormal mitochondrial transport in peripheral axons. Mitochondria cluster in proximal axons because of a defect in transport, and a gradient is generated with fewer mitochondria present in distal regions of axons. This can lead to synaptic dysfunction at the neuromuscular junction (Ly and Verstreken 2006), and ultimately, to synapse and axonal degeneration, likely from Ca^{2+} dysregulation via both loss of mitochondrial ATP for maintaining ionic gradients and loss of mitochondrial Ca^{2+} buffering itself (Waxman 2006).

organelles for great distances. Furthermore, it suggests the possibility that abnormal transport of just one critical cargo, the mitochondrion, may be enough to disrupt axonal and synaptic function and lead to axonal degeneration because of a local energy defect present only in the longest axons. Future investigations aimed at determining the contribution of altered mitochondrial transport in more common forms of peripheral neuropathy, and the pathways by which altered transport leads to axonal degeneration, will hopefully provide novel insights into treatments for peripheral nerve diseases.

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