Critical dependence of neurons on mitochondrial dynamics
Hsiuchen Chen and David C Chan

The selective disruption of certain cell types — notably neurons — in diseases involving mitochondrial dysfunction is thought to reflect the high-energy requirements of these cells, but few details are known. Recent studies have provided clues to the cellular basis of this mitochondrial requirement. Mitochondria are regionally organized within some nerve cells, with higher accumulations in the soma, the hillock, the nodes of Ranvier and the nerve terminal. In the synaptic region, mitochondria regulate calcium and ATP levels, thereby maintaining synaptic transmission and structure. Defects in mitochondrial dynamics can cause deficits in mitochondrial respiration, morphology and motility. Moreover, mutations in the mitochondrial fusion genes Mitofusin-2 and OPA1 lead to the peripheral neuropathy Charcot-Marie-Tooth type 2A and dominant optic atrophy. Perhaps it is the strict spatial and functional requirements for mitochondrial function in neurons that cause defects in mitochondrial fusion to manifest primarily as neurodegenerative diseases.

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Introduction
Mitochondria are critical for many cellular functions — including oxidative phosphorylation, intermediary metabolism, calcium buffering and apoptosis regulation — that are important to all cells. Yet mitochondrial diseases generally affect only a subset of tissues, most notably muscle and neurons. Why are these cells more prone to mitochondrial insult? In this review, we describe recent studies that have furthered our understanding of why neurons are particularly dependent on mitochondria. In particular, we explore how defects in mitochondrial dynamics can lead to neuronal disease.

Mitochondria and neurons
The prevalence of neuronal diseases associated with mutations in mitochondrial genes indicates an important functional relationship between mitochondria and neurons (Table 1). Classic mitochondrial encephalomyopathies caused by mtDNA mutations are often characterized by neurological symptoms [1,2]. Prominent are those associated with movement, such as ataxia, dysarthria and peripheral neuropathy. Mutations in mitochondrial proteins that are encoded in the nucleus have also been linked to neurodegenerative diseases [3]. One form of hereditary spastic paraplegia (HSP) is caused by mutations in paraplegin, an ATPase with proteolytic function that resides in the mitochondrial inner membrane. Mouse models of HSP indicate that mitochondria in neurons suffer ultrastructural abnormalities prior to axonal degeneration [4]. A second form of HSP is due to mutations in the mitochondrial heat shock protein HSP60 [3]. Certain familial forms of amyotrophic lateral sclerosis and Parkinson’s disease are also caused by abnormal mitochondrial proteins.

The earliest ultrastructural studies on synapses noted a remarkable concentration of mitochondria at the nerve terminal [5]. Recent experiments have indicated that this regionalized accumulation of mitochondria is indeed functionally important. Rat hippocampal cells demonstrate a positive correlation between the presence of mitochondria in growing dendrites and the extent of dendritic spine development [6]. In Drosophila, several mutants with depletion of mitochondria from axonal synapses show neuronal defects and early lethality. Axonal transport of mitochondria in Drosophila requires the Milton protein [7]. In photoreceptors lacking Milton, mitochondria are concentrated in the neuronal body and missing in the axons, whereas other organelles such as synaptic vesicles accumulate at the synapses as usual. Synaptic structure is normal except for the dearth of mitochondria, and yet the mutant flies are blind. Like Milton, Drosophila Miro (dMiro) is required for axonal transport of mitochondria [8**]. In mutants of dMiro, mitochondria also accumulate in neuronal bodies, resulting in larval locomotor defects and early pupal lethality. In contrast to mutant Milton synapses, dMiro mutants exhibit abnormal synaptic bouton structure at neuromuscular junctions. Finally, mutations in drp1, a mitochondrial fission gene, result in elongated mitochondria, which are mostly absent from the synapses [9**]. The resulting uncoordinated flies have morphologically normal neuromuscular boutons, but prolonged excitation reveals a defect in mobilizing reserve pool vesicles. These defects appear to result from insufficient ATP production, and to a lesser extent from misregulation of calcium levels.

Dynamics of mitochondria
In a typical mammalian cell, the mitochondria are highly dynamic and undergo continual fusion and fission [10].
These processes control not only the overall morphology of the mitochondrial population, but also its proper function. Three proteins have been shown to be central to the fusion of mammalian mitochondria (Figure 1a). The mitofusins, Mfn1 and Mfn2, are essential GTPases localized to the mitochondrial outer membrane \[11–13\]. Deletion of either Mfn1 or Mfn2 results in mitochondrial fragmentation, although low levels of mitochondrial fusion remain. Deletion of both mitofusins abolishes all mitochondrial fusion [14,15\]. Mitofusins are required on adjacent mitochondria during fusion and form complexes in trans that tether mitochondria together. The third protein required for fusion is OPA1, a dynamin-related GTPase [15\*,16]. OPA1 is localized to the intermembrane space, with tight association with the inner membrane [17–19]. Given that classical dynamin self-assembles to modify plasma membrane structure, it is possible that OPA1 plays an important role in control of inner membrane structure.

A separate machinery mediates mitochondrial fission. Fission requires Fis1, a mitochondrial outer membrane protein, and Drp1, another dynamin-related GTPase [10\*] (Figure 1b). Inhibition of either protein results in elongation and increased interconnectivity of mitochondrial tubules [20,21]. Whereas Fis1 is localized uniformly on mitochondria, Drp1 oligomerizes to puncta on the mitochondrial surface, and some of these puncta proceed to mitochondrial fission. Again, given classical dynamin’s proposed mechanochemical role in fission of endocytic vesicles, it is thought that Drp1 puncta wrap around mitochondrial tubules and use GTP hydrolysis to drive the constriction necessary for fission [21].

Both fusion and fission are important for mitochondrial function. When mitochondrial fusion is completely abrogated, by removal of mitofusins or OPA1, cells grow poorly and have greatly reduced respiratory function \[15\*\]. Clearly, these results indicate that mitochondrial fusion is important for the health of the mitochondrial population and of the entire cell. Mitochondrial dynamics is thought to support mitochondrial function by allowing the mixing and exchange of small molecules, proteins and mtDNA [22]. Mutations in mitochondrial fusion proteins would lead to mitochondrial dysfunction by preventing this ability of mitochondria to cooperate. In addition, mitochondrial morphology defects have secondary effects on the transport of mitochondria. Both fission mutants with elongated mitochondria and fusion mutants with small, rounded mitochondria seem to demonstrate movement defects [9\*,11]. Finally, fission has been shown to be an important component of the mitochondrial apoptosis pathway [23]. In many models of programmed cell death, mitochondria fragment during the early stages of apoptosis, and inhibition of Drp1 or Fis1 can ameliorate cell death. In contrast, mitochondrial fusion molecules can demonstrate protective effects in programmed cell death [23–25]. Given the above, it is not surprising that defects in mitochondrial dynamics lead to neurological disease.

### Mitochondrial dynamics and Charcot-Marie-Tooth disease

The predominant gene affected in Charcot-Marie-Tooth (CMT) disease type 2A is Mfn2 [26,27,28\*]. CMT refers to a group of hereditary peripheral neuropathies that affect both motor and sensory nerves [29,30]. All patients demonstrate motor defects in at least the lower extremities, but other clinical aspects vary greatly. Over time, the primary neuronal defects can lead to muscle atrophy. Genetically, CMT is heterogeneous with at least 24 different genes associated with the disease, and both dominant and recessive modes of inheritance. A combination of clinical signs, electrophysiology, histopathology and genetics is used to subdivide CMT. Some forms are termed demyelinating, characterized by primary defects in Schwann cells, while other forms are termed axonal and involve the neurons themselves. With rapid advances in identifying disease genes and their cellular functions, a mechanistic understanding of these neurodegenerative diseases can be anticipated.

CMT2A is an autosomal dominant, axonal form of CMT. At least 18 mutations in 22 families have been identified in Mfn2 [26,27,28\*]. Although mutations can be found throughout the gene, most mutations cluster in the

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**Table 1**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Function</th>
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<tr>
<td>Charcot-Marie-Tooth type 2A</td>
<td>Mfn2</td>
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<tr>
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<td>Familial Parkinson’s</td>
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GTPase region, underscoring the importance of this region to Mfn2 function. Another subtype of CMT that includes optic atrophy, termed hereditary motor and sensory neuropathy type VI (HMSN VI), is also caused by mutations in Mfn2 [31]. Six different mutations have been identified, only one of which has been isolated from CMT2A patients. This combination of neuromuscular and sensory defects is reminiscent of syndromes caused by mtDNA mutations.

Finally, CMT4A — a demyelinating, recessive form of CMT — is caused by mutations in ganglioside-induced differentiation-associated protein-1 (GDAP1) [32]. Interestingly, some families with mutations in GDAP1 present with an axonal form of CMT that closely resembles CMT2 [33]. In accordance with these mixed clinical presentations, GDAP1 is expressed in both neurons and Schwann cells. Like Mfn2, GDAP1 is a mitochondrial outer membrane protein that may be involved in mitochondrial dynamics [34]. Cell culture studies demonstrate mitochondrial fragmentation upon overexpression of GDAP1 and tubulation upon RNAi-mediated knockdown of GDAP1, suggesting that GDAP1 functions to promote mitochondrial fission. CMT mutations attenuate the ability of GDAP1 to fragment mitochondria.

Mitochondrial dynamics and dominant optic atrophy

Dominant optic atrophy (DOA), the most commonly inherited optic neuropathy, generally presents in childhood as bilateral loss of visual acuity associated with the clinical finding of optic nerve pallor, an indication of degeneration [35]. This disease is caused by loss of retinal ganglion cells (RGCs), which line the front of the retina and give rise to the fibers of the optic nerve. Three loci (OPA1, OPA4, OPA5) have been linked with DOA [36–39]. OPA1 is by far the most common cause and the only one whose gene product has been identified. Many mutations in OPA1 have now been associated with DOA [40]. Most of these mutations result in OPA1 truncation, suggesting that the dominant inheritance pattern is due to haploinsufficiency. This mechanism is supported by a case in which a microdeletion removed one copy of OPA1 [41]. Many missense mutations have also been reported, and it is possible that some of these disease alleles have dominant-negative effects, given the characteristic property of dynamin-related proteins to self-assemble. However, even if a dominant-negative mechanism underlies certain OPA1 disease alleles, it is likely that significant OPA1 activity remains in DOA patients. Given the severe consequences of OPA1 loss in cultured cells [15/24], complete loss of OPA1 activity is likely to be incompatible with life.

How does reduced OPA1 function lead to DOA? This remains to be definitively determined, but several observations are consistent with a mitochondrial basis. First, monocytes from a DOA patient were reported to have aggregated mitochondria, suggesting a defect in mitochondrial distribution within cells [38]. The histopathology of mitochondria in patient RGCs has not been reported, but RNAi knockdown of OPA1 in cultured RGCs leads to a similar aggregation of mitochondria in both the soma and neurites [42]. Second, DOA patients have been reported to have lower rates of ATP synthesis in skeletal muscle [43] and fewer copies of mtDNA [44]. These defects in patient tissues are consistent with cell culture experiments, indicating that loss of OPA1 leads to severe cellular dysfunction, including poor cell growth.
reduced mitochondrial membrane potential, reduced cellular respiration and even cell death [15**,24].

As with CMT, several observations hint at additional parallels between optic atrophy and classic mitochondrial diseases. In one pedigree, DOA is additionally associated with hearing loss, ptosis (drooping of the upper eyelids) and ophthalmoplegia (paralysis of the eye muscles) [45]. The extraocular movement deficits are suggestive of skeletal muscle defects, a hallmark of mitochondrial myopathies. Two other forms of optic atrophy are also due to mitochondrial dysfunction. Leber’s hereditary optic neuropathy (LHON) is a classic mtDNA disease caused by mutations in Complex I of the respiratory chain. 3-methylglutaconic aciduria type III, a recessive form of optic atrophy, is caused by mutations in OPA3, which appears to be a mitochondrial protein [46]. Intriguingly, this disease also causes later-onset symptoms including movement defects, ataxia and cognitive decline, suggesting broader neuronal involvement.

Possible mechanisms of neuronal dysfunction

With both CMT2A and DOA, little information is available on the histopathology of mitochondria in disease tissues. In addition, it is unclear what kind of mitochondrial...
dysfunction is caused by the Mfn2 and OPA1 disease alleles. However, on the basis of information about the cellular functions of these proteins, several possibilities can be proposed (Figure 2). First, the mutations in Mfn2 and OPA1 may impair respiratory capacity, by analogy with cells lacking Mfn or OPA1 function [15]. However, these severe effects are only observed with near-complete arrest of mitochondrial fusion, and it is unclear whether the disease mutations would cause such severe defects. Effects of fusion mutants on calcium regulation and other mitochondrial functions have not yet been examined. Second, defects in mitochondrial dynamics can secondarily result in abnormal mitochondrial motility due to improper mitochondrial transport along the cytoskeleton [11] or mitochondrial aggregation [38,42]. Finally, defects in mitochondrial dynamics have been linked to programmed cell death [23–25].

Regardless of the precise mode of mitochondrial dysfunction, it is unclear why the defects in CMT2A and DOA are largely limited to specific neuronal cells, given the widespread expression patterns of both Mfn2 and OPA1. However, histological studies show that these neurons have special features that may provide clues to why these cells are vulnerable to mitochondrial dysfunction [47,48,49]. First, of the cells in the retina, the retinal ganglion cells have by far the most extended processes. The axon of each retinal ganglion cell threads through the inner surface of the retina towards the optic nerve head, where axons from over a million RGCs converge to form the myelinated optic nerve. As with the extremely long motor and sensory neurons affected in CMT, the length of these axons may impose stricter requirements for mitochondrial function. For example, mild defects in transport of mitochondria may have little consequence in most cells but cumulative effects over time may lead to axonal impairment. Second, mitochondria within motor neurons and the optic nerve show a strikingly non-uniform subcellular pattern. Mitochondria are abundant in the cell body and the proximal, unmyelinated axon, the site where action potentials originate. Once the axons become myelinated, mitochondria are sparse, except in unmyelinated areas such as the nodes of Ranvier and of course, the synapses themselves. The significance of this inverse relationship between myelination and mitochondrial accumulation is as yet uncertain, but may reflect a higher requirement for ATP production in non-myelinated areas to maintain neuronal transmission. For example, the Na+,K+-ATPase, an ATP-dependent pump critical to restoring membrane potential, is also concentrated at nodes of Ranvier [50]. Interestingly, demyelination of the feline optic nerve is associated with an increase in mitochondrial number, which returns to normal as remyelination occurs [51]. Because of this regionalized demand for mitochondria, defects in proper subcellular distribution of mitochondria may be especially problematic for certain neurons.

Conclusions
Clearly, mitochondrial function is critical for neuronal function, as demonstrated by the many neuronal symptoms associated with mtDNA mutations. Localization of mitochondria at particular segments of neuronal cells is also critical for neuronal function. This localization requires proper transport and morphology of mitochondria. Because mitochondrial dynamics influences both mitochondrial function and motility, the involvement of two mitochondrial fusion proteins, Mfn2 and OPA1, in neurodegenerative diseases is not surprising. Although CMT and DOA primarily affect different neurons, less common forms of each may share overlapping targets. As the mitochondrial and cellular effects of mitochondrial dynamics are clarified, not only will our basic understanding of neuronal biology improve, but also more avenues for therapeutic interventions will become apparent.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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The relationship between mitochondria and optic neuropathy is explored in this review, with emphasis on basic biology, LHON and DOA, animal models, and possible therapies.


