Mitochondria at the Synapse

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Synapses are packed with mitochondria, complex organelles with roles in energy metabolism, cell signaling, and calcium homeostasis. However, the precise mechanisms by which mitochondria influence neurotransmission remain undefined. In this review, the authors discuss pharmacological and genetic analyses of synaptic mitochondrial function, focusing on their role in Ca^{2+} buffering and ATP production. Additionally, they will summarize recent data that implicate synaptic mitochondria in the regulation of neurotransmitter release during intense neuronal activity and link these findings to the pathogenesis of neurodegenerative diseases that feature disrupted synaptic mitochondria, including amyotrophic lateral sclerosis and hereditary spastic paraplegia. NEUROSCIENTIST 12(4): 291–299, 2006. DOI: 10.1177/1073858406287661

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Neurons possess a unique architecture for integrating and transmitting information. Dense dendritic arborizations such as those found in Purkinje neurons allow the cell to receive numerous inputs while long processes allow motor neurons to extend to distal targets. These features optimize contacts with effectors but increase the length along which signals are transmitted, placing greater metabolic demands on these cells. Accordingly, neurons are enriched with energy-generating mitochondria especially at synapses, the specialization where neuronal communication occurs (Shepherd and Harris 1998). However, the exact purpose for the high density of mitochondria at synapses has remained elusive.

Mitochondria are dynamic, mobile organelles undergoing continuous reshaping and transport (Hollenbeck 1996). In response to neuronal stimulation, synaptic mitochondria redistribute and enhance their activity (Miller and Sheetz 2004), suggesting that sustained neurotransmission is an energetically demanding process. Furthermore, mitochondrial distribution and morphology differ between tonic and phasic neuromuscular junctions (NMJs) of crayfish, indicating a possible mitochondrial contribution to divergent synaptic release properties (Nguyen and others 1997). Hence, movement to and sequestration of mitochondria at synapses appears important for regulation of synaptic strength.

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Mitochondrial Calcium Regulation and Synaptic Function

How could mitochondria affect the firing properties of synapses? Apart from their central role in cellular metabolism, mitochondria are integration stations for signaling and calcium regulation (Fig.1). Hence, the potential influence of mitochondria on synaptic efficacy may be diverse. Because calcium is a second messenger important for neuronal plasticity, attention has focused on the capacity of mitochondria to buffer synaptic calcium levels.

Mitochondria buffer cytoplasmic calcium through a sophisticated system of ion transporters and pores. Initially, the hyperpolarized mitochondrial inner membrane potential (~–200 mV) provides a large driving force for calcium entry (Rizzuto and others 2004). Calcium crosses the inner membrane through undefined uniporters (Rizzuto and others 2004). Although calcium uptake by mitochondria is highly favorable, it is balanced by the low affinity of the uniporter for calcium, which limits calcium accumulation when cytosolic levels are below ~1 μM. Alternatively, Na^+/Ca^{2+} and H^+/Ca^{2+} antiporters extrude calcium in a concentration-dependent manner (Rizzuto and others 2004). Using this complex machinery, mitochondria are thought to shape calcium signals for diverse purposes.

Upon stimulation, the presynapse may experience large intracellular calcium rises acting locally to elicit vesicle fusion, or globally to influence various signaling pathways. Along with plasma membrane Ca^{2+}-ATPases, and the endoplasmic reticulum (ER), mitochondria are thought to modulate these processes by regulating calcium levels (Rizzuto 2001). At the calyx of Held (Billups and Forsythe 2002) and the mouse NMJ (David and Barrett 2003), mitochondria appear to be the main calcium buffers, as there is minimal uptake into the ER during rigorous stimulation, supporting a role for mitochondria in regulating synaptic calcium levels.

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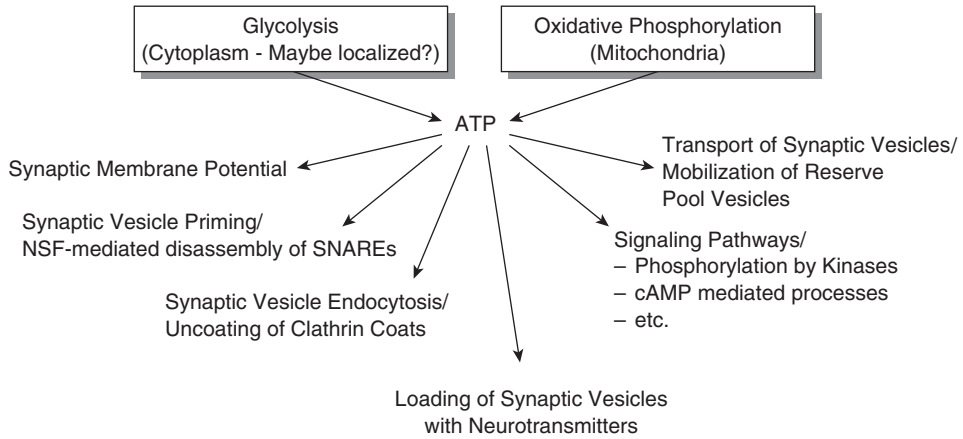

Fig. 1. ATP generating and consuming processes at the synapse. Both glycolysis and mitochondria produce ATP at the synapse where it is used to fuel numerous energy-consuming processes. Glycolysis only produces about 10% of total cellular ATP, but compartmentalized synthesis may directly fuel specific synaptic processes such as the maintenance of the synaptic membrane potential or reloading of synaptic vesicles with neurotransmitters. On the other hand, mitochondria are thought to produce more than 90% of cellular ATP, yet mitochondrial-produced ATP specifically limits mobilization of specific synaptic vesicles during intense neuronal activity (see Figs. 2 and 3). SNARE = soluble NSF attachment protein receptor.

Interestingly, at different synapses, calcium regulation is likely mediated by a cooperative partnership between the mitochondria and ER (Rizzuto and others 2004), deemphasizing the role of mitochondria as the sole organelle involved in calcium regulation. Thus, the contribution of mitochondrial calcium regulation to synaptic function requires further study and may vary between synapse types.

Numerous studies addressing the influence of synaptic mitochondrial calcium buffering have relied on pharmacological tools that interfere with uptake or efflux of calcium (Table 1). Repetitive stimulation opens plasma membrane calcium channels evoking a rise in $[Ca^{2+}]_{cyto}$, that is tempered by buffering mechanisms. Impairing mitochondrial calcium uptake using drugs that either disrupt the mitochondrial potential (i.e., antimycin or CCCP [carbonyl cyanide chlorophenyl hydrazone]; see Table 1) or inhibit the calcium uniporter (i.e., ruthenium red) results in rapid rises in $[Ca^{2+}]_{cyto}$ during repetitive stimulation (Tang and Zucker 1997). Functionally, this elevation in calcium is thought to contribute to the synaptic depression also observed at these drug-treated synapses (Nguyen and others 1997; Billups and Forsythe 2002). Furthermore, by blocking both Na^+-dependent and independent mitochondrial calcium efflux using TPP^+, it was shown that slow release of mitochondrial calcium is necessary for synaptic strengthening (Tang and Zucker 1997). These findings indicate that mitochondrial calcium buffering functionally impacts synaptic transmission.

Recently, unbiased genetic screens designed to isolate genes affecting synaptic function in *Drosophila* have isolated mutants with markedly reduced synaptic mitochondria, providing further insights into the role of mitochondria in calcium buffering and neurotransmission. In these mutants, the disruption of mitochondrial distribution results from lesions in Milton (Stowers and others 2002) and dMiro (Guo and others 2005), proteins mediating anterograde mitochondrial transport, as well as mutations in Dynamin-related protein (Drp1), a protein that affects synaptic localization by mediating mitochondrial fission (Li and others 2004; Verstreken and others 2005). In addition, *stress sensitive B* (*sesB*) mutants, which affect the mitochondrial ATP/ADP translocase (Trotta and others 2004), were also used to study synaptic mitochondria. *milton* photoreceptors display impaired synaptic transmission (Stowers and others 2002); however, more quantitative analyses at the third instar NMJ have not been done, likely due to early lethality of these mutants. With regard to the other mutants, each exhibit synaptic depression during intense activity, confirming an important role for mitochondria in synaptic function (Trotta and others 2004; Guo and others 2005; Verstreken and others 2005).

In sharp contrast to the pharmacological experiments cited above, the effects of reduced mitochondria on synaptic calcium levels were minimal at both *drp1* and *dmiro* mutant NMJs. Although resting calcium levels in *drp1* and *dmiro* mutants were doubled, basal neurotransmitter release during mild stimulation was not affected (Guo and others 2005; Verstreken and others 2005). Furthermore, 30 seconds of rigorous stimulation leads to an elevation of $[Ca^{2+}]_{cyto}$ in both mutants that is not different from controls. Only when *drp1* mutants were stimulated longer than 30 seconds did the $[Ca^{2+}]_{cyto}$ rise above control levels (Verstreken and others 2005). Contrarily, mutations in the *Drosophila* sarco-endoplasmic reticulum calcium ATPase responsible for calcium uptake into the ER show sharp rises in $[Ca^{2+}]_{cyto}$ when intensely stimulated (Sanyal and others 2005). However, these high $[Ca^{2+}]_{cyto}$ levels rapidly settle with continued stimulation. These data suggest that the ER acts as an immediate calcium sink while mitochondria may only be involved in

Table 1. A Selection of Pharmacological and Genetic Tools to Study Synaptic Mitochondria

Drug[a]	Proposed Site of Action	Proposed Effects	Comments
MPP (1-methyl-4-phenylpyridinium)	Complex I of the ETC (electron transport chain)	Reduces mitochondrial ATP production	Protonophores are sometimes used to test for Ca^{2+} uptake by mitochondria; however, they may also lead to ATP depletion. Hence, the observed increased cytoplasmic Ca^{2+} upon stimulation could also be due to a defect in ATP-dependent Ca^{2+} extrusion from the synaptic terminal.
Rotenone	Complex I of the ETC (high affinity)	Reduces mitochondrial ATP and phosphocreatine production	
3-NPA (3-nitropropionic acid)	Complex II of the ETC	Reduces mitochondrial ATP, phosphocreatine production and reduces NADH (nicotinamide adenine dinucleotide) generation	
Malonate	Complex II of the ETC	Reduces mitochondrial ATP production and reduces NADH generation	
Antimycin A	Complex III of the ETC (high affinity)	Reduces mitochondrial ATP production	
Myxathiazol	Complex III of the ETC	Reduces mitochondrial ATP production	
Cyanide	Complex IV of the ETC (low affinity)	Reduces mitochondrial ATP production	
Azide	Complex IV of the ETC	Reduces mitochondrial ATP production	
CCCP (carbonyl cyanide chlorophenyl hydrazone)	Mitochondrial membrane	A weak acid that dissipates the proton gradient across the inner mitochondrial membrane and releases Ca^{2+}	
Oligomycin	ATP synthase (complex V of the ETC and F_0 ATPase) – (high affinity)	Blocks mitochondrial ATP synthesis, but high concentrations also inhibit the Na^+/K^+ ATPase; in addition, Ca^{2+} transport and O_2 slightly increase	
Nigericin	K^+/proton-exchanger	Decreases the pH across mitochondrial membrane (when high K^+ is applied) and leads to an increase in mitochondrial membrane potential; also partly depolarizes the plasma membrane and shortcuts the Na^+/K^+ ATPase	Drugs lead to collapse of mitochondrial potential resulting in dissipation of accumulated Ca^{2+}. The collapsed potential will also lead to reversion of ATP synthase, which depletes cytoplasmic ATP and reduces Na^+ and Ca^{2+} extrusion from the cytoplasm. Drugs also acidify the cytoplasm, which depletes transmitters from synaptic vesicles.
Valinomycin	K^+ uniporter	Collapses the mitochondrial membrane potential. Also leads to mitochondrial swelling and induces ion permeability across all membranes	
Veratridine	Voltage-activated Na^+ channel	Increases respiration driving cycling of Na^+ and K^+ over the plasma membrane but also induces ion permeability across all membranes	
Ionomycin	Ca^{2+}/proton exchanger	In elevated extracellular Ca^{2+}, leads to a collapse of the mitochondrial membrane potential. Also induces ion permeability across all membranes.	
A-23187	Ca^{2+}/proton exchanger	In elevated extracellular Ca^{2+}, leads to a collapse of the mitochondrial membrane potential. Also induces ion permeability across all membranes.	

(continued)

Table 1. (continued)

Drug[a]	Proposed Site of Action	Proposed Effects	Comments
TPP^+ (tetraphenyl-phosphonium)	Mitochondrial membrane	Prevents mitochondrial Ca^{2+} efflux. High concentrations lead to mitochondrial swelling and depolarization of the mitochondrial membrane resulting in reduced ATP production.	
RU-360 (Ruthenium amine)	Ca^{2+} uniporter (nonspecific)	Reduces Ca^{2+} uptake of mitochondria	
Ruthenium red	Ca^{2+} uniporter	Reduces Ca^{2+} uptake of mitochondria, but only specific at low concentrations on isolated mitochondria. Also blocks NMDA receptors and voltage-activated Ca^{2+} channels.	

Gene	Proposed Function	Proposed Effects
dmiro	GTPase that regulates axonal mitochondrial transport[b]	Mutants lack mitochondria in neuromuscular synapses and show an accumulation in the cell bodies. Ca^{2+} buffering is not significantly affected during high-frequency stimulation. Although the frequency of spontaneous release events is increased, basal-evoked transmission is unaffected. Mutants fail to maintain normal transmitter release during intense stimulation.
drp1	Dynamin-like protein required for mitochondrial fission and synaptic localization[c]	Mutants show dramatic reduced mitochondria at neuromuscular synapses and in photoreceptor synapses. As in *dmiro* mutants, Ca^{2+} buffering is not significantly affected during high-frequency stimulation. Basal neurotransmitter release is also unaffected, but mutants fail to maintain normal levels of transmitter release during intense stimulation. Additional analyses using *drp1* mutants show that synaptic mitochondria generate ATP for the Myosin complex to mobilize reserve pool vesicles during intense activity.
milton	Kinesin heavy chain interacting adaptor required for axonal mitochondrial transport[d]	Mutants lack mitochondria in photoreceptor synapses (and presumably other synapses as well) leading to desynchronization of transmitter release from these tonic synapses as gauged by a defective electroretinogram (ERG; similar to ERGs from *dmiro*, *drp1*, and *sesB* mutants).
sesB	Mitochondrial ADP/ATP translocase involved in ATP synthesis[e]	Mitochondria are not absent from mutant terminals, but mitochondrial ATP production is presumably impaired. Mutant flies show defective ERG responses, and at the neuromuscular junction, neurotransmission during intense activity is reduced likely because vesicle cycling is defective, similar to the defects observed in *drp1* mutants.

[a]Most of the data relevant to pharmacology of synaptic mitochondria can be found in an excellent review: Nicholls and Budd (2000), Physiol Rev 80:315.
[b]*dmiro* mutations are described in Guo and others (2005).
[c]*drp1* mutations are described in Verstreken and others (2005).
[d]*milton* mutations are described in Stowers and others (2002).
[e]*sesB* mutations are described in Trotta and others (2004).

calcium buffering during prolonged stimulation at the *Drosophila* NMJ. Therefore, the loss in mitochondrial calcium buffering capacity is not likely responsible for the physiological defects observed at mutant synapses. Rather, other aspects of mitochondrial function may be important for preserving synaptic transmission.

Energy and Neurotransmission

In addition to calcium buffering, mitochondria support the molecular machinery for aerobic metabolism and produce 90% of cellular ATP used ubiquitously to fuel various processes. More modest levels of ATP are generated by glycolysis in the cytosol (Mathews and van Holde 1995, 448–50). Perhaps the dependence of neurotransmission on energy supply is not surprising given ATP's role in maintaining synaptic membrane potential, and fueling numerous steps of the vesicle cycle. The synapse relies on ATP for priming and to drive NSF-mediated disassembly of SNARE (soluble NSF attachment protein receptor) complexes. In addition, various steps in vesicle retrieval require ATP, including scission, uncoating, and refilling of vesicles with neurotransmitters (Murthy and De Camilli 2003). Furthermore, vesicular transport to the synaptic terminal along actin highways depends on myosin-ATPases (Ryan 1999; Fig. 1).

Given the intimate involvement of ATP in numerous steps of the vesicle cycle, one might presume that reduced ATP levels would lead to many defects in synaptic mechanisms. However, until recently, this had not been rigorously tested in vivo, nor is it known to what extent these synaptic processes depend on glycolytic versus mitochondrial ATP. Evidence from various genetic and pharmacological studies indicates a requirement for both aerobic and anaerobically derived ATP in synaptic function. For instance, drugs (i.e., protonophores) that depolarize the mitochondrial membrane not only abolish the driving force for calcium uptake but also disrupt the proton-motive-force that drives ATP synthesis (David and Barrett 2003). Interestingly, the synaptic depression observed at protonophore-treated synapses can be alleviated by administering glucose to stimulate glycolytic ATP production (Calupca and others 2001), suggesting energy depletion compromises synaptic function. In line with this, *Drosophila nubian* mutants, which affect phosphoglycerate kinase (PGK), required for a terminal step of glycolysis, have a threefold reduction in neuronal ATP levels and display an activity-dependent depression in neurotransmission (Wang and others 2004). Together, these findings confirm a role for ATP-energy in synaptic function.

Interestingly, recent studies suggest that energy production is compartmentalized at synapses to mediate specific processes. For instance, glycolytic enzymes, PGK, and glyceraldehyde phosphate dehydrogenase (GAPDH) are enriched on synaptic vesicles and provide energy locally to drive vesicular uptake of glutamate (Ikemoto and others 2003). This process is thought to rely on glycolytic ATP because iodoacetate, a GAPDH inhibitor, suppresses accumulation of glutamate into vesicles, while oligomycin, a mitochondrial ATP-synthetase inhibitor, had minimal effects on refilling. In addition, GADPH associates with $GABA_A$ receptors at the plasma membrane and supplies focal ATP for receptor phosphorylation to sustain fast inhibitory neurotransmission (Laschet and others 2004). Hence, in neurons, glycolytic energy may be locally produced and preferred for "rapid" events, consistent with the role of anaerobically derived ATP in skeletal muscle (Heilmeyer and others 1990).

Mitochondrial Energy Fuels Reserve Pool Mobilization

The contribution of mitochondrial ATP to synaptic function is not well defined. However, analyses of mitochondrial-deficient *drp1* mutant *Drosophila* NMJs helped elucidate this issue. First, to determine whether reduced neurotransmission during intense stimulation in *drp1* is ATP-dependent, mutant NMJs were supplemented with exogenous ATP. This partially rescued the synaptic defect strongly implicating mitochondrial metabolism as a key factor in maintaining neurotransmission (Verstreken and others 2005).

Gradual synaptic depression could potentially arise from ATP depletion by compromising metabolically demanding processes such as exocytosis, endocytosis, or vesicle mobilization. At the fly NMJ, these processes can be functionally separated. *Drosophila* NMJs harbor two distinct vesicle pools, an exo-endo cycling pool (ECP) that cycles with mild stimulation and a reserve pool (RP) that is recruited only with intense stimulation (>10 Hz) (Kuromi and Kidokoro 2005; Verstreken and others 2005; Fig. 2). To test the role of mitochondria in the function of the ECP and RP, various labeling protocols using FM1-43, a fluorescent dye that is internalized in newly forming endocytic vesicles, were used on control and *drp1* mutant synapses. FM1-43 uptake and unloading of ECP vesicles proceeded normally in *drp1* mutant synapses even if these synapses were intensely stimulated, indicating that the inability of *drp1* to maintain normal neurotransmission does not arise from defects in endocytosis or exocytosis of ECP vesicles. Rather, the defect to maintain transmission during intense stimulation is due to a failure to mobilize RP vesicles in the absence of synaptic mitochondria as evidenced by a failure to exocytose or internalize FM1-43 into this vesicle subset in mutant NMJs. Interestingly, the defect in RP function is alleviated by adding exogenous ATP, suggesting that mitochondrial ATP is limiting for mobilization of RP vesicles (Verstreken and others 2005).

What are the precise molecular targets of mitochondrial ATP? Although the mechanisms mediating vesicle mobilization are not entirely known, several studies implicate protein kinase A (PKA) and myosin-light-chain (MLC) as distinct players in this process (Ryan 1999; Kuromi and Kidokoro 2005). In the emerging model for vesicle mobilization, PKA serves to untether vesicles (Kuromi and Kidokoro 2005), and once untethered, myosin-powered transport along actin cables delivers vesicles to release sites (Ryan 1999; Fig. 3). Myosin ATPase activity is triggered by the sequential activation of myosin-light-chain-kinase (MLCK) and phosphorylation

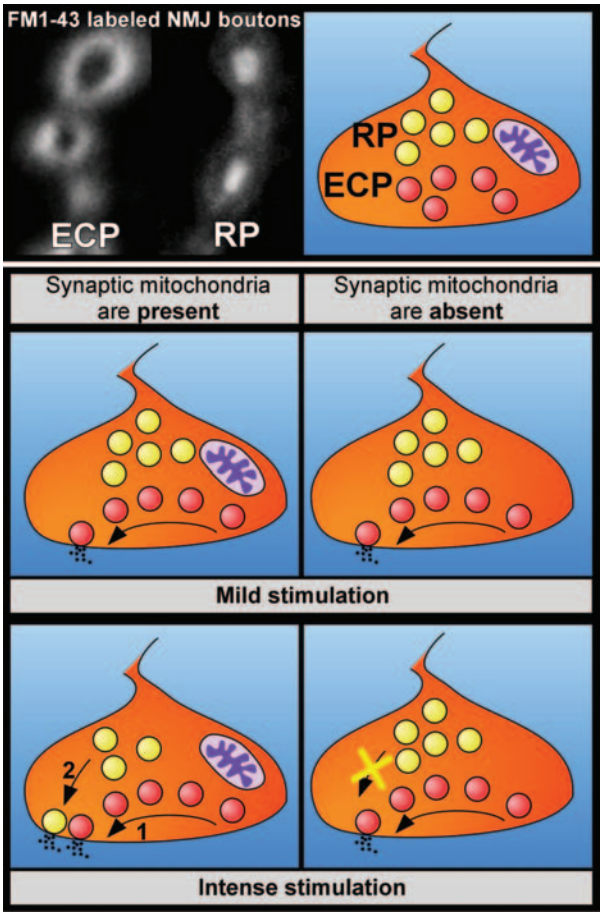

Fig. 2. Reserve pool and exo-endo cycling pools maintain neurotransmitter release. *Top*, *Drosophila* neuromuscular junctions (NMJs) harbor at least two functionally distinct synaptic vesicle pools, a reserve pool (RP), and an exo-endo cycling pool (ECP), and defects in mobilization or filling of either pool leads to defects in neurotransmitter release. Using specific stimulation protocols in the presence of a dye that is internalized into newly forming synaptic vesicles (FM1-43), it is possible to specifically label each pool at the synapse (*top left*, and schematized on the *right*; yellow vesicles represent the RP, and red vesicles are the ECP). *Bottom*, ECP and RP vesicles are deployed for exocytosis depending on the stimulation paradigm. At low-frequency stimulation, only ECP vesicles participate in exocytosis, whereas during more intense stimulation (>10 Hz), ECP as well as RP vesicles are exocytosed (*bottom left*). ECP exocytosis is not significantly affected by the absence of mitochondria at the synapse, and neurotransmitter release during low-frequency stimulation proceeds fine (*bottom right*). However, because RP vesicle mobilization during strong stimulation depends mostly on mitochondrial ATP, synapses fail to maintain normal levels of transmitter release during intense activity (*bottom right*).

of MLC. In support of this, when MLCK inhibitors are used to impair myosin-mediated transport at *drp1* synapses, ATP supplementation no longer rescues the RP defect at *drp1* synapses (Verstreken and others 2005). Hence, mitochondrial ATP is particularly limiting for actin-myosin-mediated transport of RP vesicles.

It is surprising that when synapses are nearly depleted of mitochondria, most aspects of the vesicle cycle proceed normally; however, enough ATP may be produced at *drp1* synapses to fulfill these functions. At *drp1* synapses, about 5% to 10% of the mitochondria remain and glycolysis is presumably intact, suggesting that *drp1* synapses produce ~10% of normal ATP levels (Verstreken and others 2005). Therefore, the residual ATP produced at *drp1* mutant synapses may be adequate for rapidly cycling ECP vesicles, possibly by optimally localizing glycolysis enzymes. In contrast, vesicle mobilization from the RP is an energy-consuming process, requiring a high supply of ATP apparently offered by local mitochondria.

These in vivo studies at the fly NMJ reveal a functional specificity for mitochondria that is nonintuitive given their multiple roles in energy metabolism, calcium handling, and cell signaling. However, it remains unclear whether these findings are constrained to *Drosophila* NMJs or if they also occur in other synapse types. In addition, the functions of mitochondria at synapses with diminutive reserve pools, such as cone photoreceptors, remain elusive as well (Rea and others 2004). In addition, some studies indicate that these organelles may have little physiological influence on the release properties of hippocampal neurons (Waters and Smith 2003), possibly because these synapses harbor only a small RP. Nonetheless, cumulative evidence from pharmacologic and genetic perturbation of mitochondria indicates a clear modulatory role for these organelles at a variety of synapses, possibly by regulating the function of RP vesicles (Fig. 3).

Mitochondria and Neurodegenerative Disease

Mitochondrial dysfunction has emerged as a hallmark of various neurodegenerative diseases, opening the possibility that mitochondrial deficits may lead to earlier functional defects prior to the onset of degeneration. To exert their synaptic roles, mitochondria must arrive from the soma to distal synapses via microtubule-mediated transport (Hollenbeck 1996) and constantly reconfigure to meet synaptic needs (Okamoto and Shaw 2005). Interestingly, impairments in these aspects of mitochondrial dynamics have arisen as common themes in the pathology of numerous neurodegenerative diseases. Unlike lesions that perturb the core mitochondrial machinery, and hence somatic mitochondrial function, interruption of mitochondrial distribution mechanisms may preferentially affect the function of distal synapses.

In amyotrophic lateral sclerosis (ALS) and hereditary spastic paraplegia (HSP), genetically heterogeneous diseases primarily affecting motor neurons, multiple disease loci likely affect axonal transport and delivery of mitochondria to synapses (reviewed in Reid 2003; Shaw 2005). Several genetic lesions that cause ALS and HSP affect cytoskeletal elements crucial for maintaining cellular highways used to transport proteins and organelles to synapses (Table 2). Nearly 40% of HSP cases are

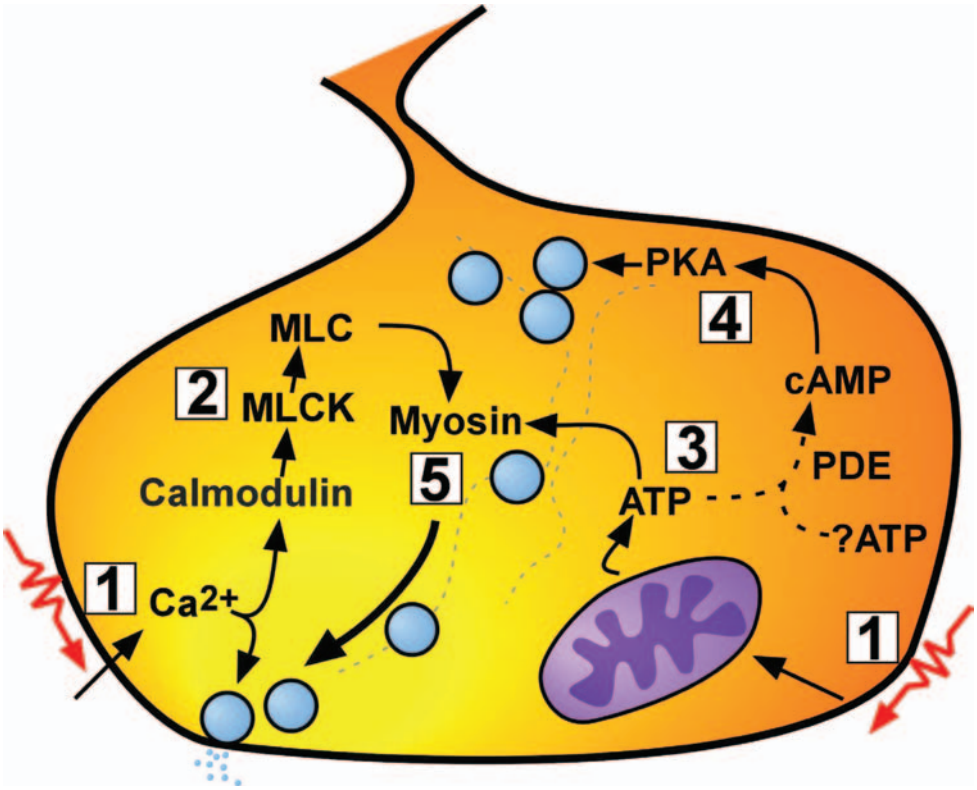

Fig. 3. Synaptic mechanisms that lead to reserve pool (RP) vesicle mobilization. RP vesicle mobilization during high-frequency stimulation is likely regulated by converging pathways. Some of these pathways, suggested by our work and the work of others, are depicted here. 1) Repetitive membrane depolarization leads to a rise in $[Ca^{2+}]_i$ triggering neurotransmitter release and activating calmodulin (Xia and Storm 2005). 2) Calmodulin switches on the enzymatic activity of myosin-light-chain-kinase (MLCK), which in turn phosphorylates and activates myosin-light-chain (MLC), a component of the actin-binding myosin complex. 3) Neuronal activity also increases mitochondrial activity, leading to increased ATP production (Kann and others 2003). 4) Phosphodiesterase (PDE) produces cAMP from ATP. Protein kinase A (PKA) activation by cAMP leads to untethering of RP vesicles, allowing their mobilization (Kuromi and Kidokoro 2000). 5) Untethered vesicles can then be mobilized and transported to release sites over actin cables by the activated myosin-ATPase using mitochondrial ATP.

caused by lesions in Spastin, a putative ATPase that binds microtubules via its N-terminus and is thought to be involved in microtubule dynamics (Reid 2003; see Table 2). Also, abnormal perinuclear clusters of mitochondria have been observed in neurons expressing mutant Spastin protein, supporting a role in mitochondrial localization and transport (McDermott and others 2003). Interestingly, *Drosophila spastin* mutant flies show severe defects in movement and neurotransmission (Sherwood and others 2004), suggesting that mitochondrial defects could possibly underlie impaired neurotransmission at these synapses. Furthermore, Troyer's syndrome, a variant of HSP, is caused by mutations in *spastin*, which shares sequence similarity with the microtubule binding N-terminus of *spastin*, indicating it may also influence cytoskeletal dynamics (Reid 2003; see Table 2). In addition, defective neurofilaments also trigger these progressive neuropathies. Neurofilaments are an integral component of the cytoskeleton controlling axon caliber, cell shape, and organelle transport. In nearly 1% of sporadic ALS cases, the neurofilament heavy-chain is disrupted (Shaw 2005; see Table 2). Similarly, in ALS mouse models, neurons exhibit abnormal neurofilament organization and reduced rates of axonal transport (Shaw 2005). Furthermore, mutations in the neurofilament light-chain have been identified in peripheral neuropathy, Charcot Marie Tooth 2 (CMT2), and lead to defects in the distribution of mitochondrial and other proteins in cell culture models of the disease (Shy 2004; see Table 2). Hence, cytoskeletal defects identified in numerous neurological disorders may impact synaptic mitochondrial localization.

Impairment of mitochondrial transport in many of these disorders may also be caused by defects in the molecular motors driving transport of organelles and proteins. For instance, mutations in Dynactin, which is necessary for retrograde microtubular transport, cause a lower motor neuron disorder in humans (Shaw 2005; see Table 2). In addition, kinesins involved in anterograde transport of mitochondria and other cellular components are implicated in CMT2 and HSP (Reid 2003; Shy 2004; see Table 2). At least in the case of HSP, the predominant effect on the lower limbs may reflect increased vulnerability of neurons with long axons to impaired mitochondrial

Table 2. Neurodegenerative Disease Loci That Potentially Affect Synaptic Mitochondria

	Disease Symbol	Chromosomal Location	Gene	Function
Hereditary spastic paraplegia (HSP)[a]	Spastic paraplegia gene 4 (SPG4)	2p21-p24	Spastin	Cytoskeletal
	SPG20	13q12.3	Spastin	Cytoskeletal
	SPG10	12q13	Kinesin family protein–5A (KIF5A)	Molecular motor subunit
Charcot-Marie-Tooth (CMT)[b]	CMT2E	8p21	Neurofilament light chain	Cytoskeletal
	CMT2A-1	1p35-p36	KIF1B	Molecular motor subunit
	CMT2A-2	1p35-p36	Mitofusin 2	Mitochondrial fission
Amyotrophic lateral sclerosis (ALS)[c]	Sporadic ALS	22q12.1-q13.1	Neurofilament heavy chain	Cytoskeletal
Other[d]	Lower motor neuron disease	2p13	Dynactin p150 subunit	Molecular motor subunit
	Autosomal dominant optic atrophy	3q28	Optic atrophy 1 (OPA1)	Mitochondrial fission

[a]Recent advances in HSP can be found in a review, Reid (2003).
[b]Also, refer to Shy (2004) for further information regarding CMT.
[c]A thorough discussion of ALS pathogenesis can be found in the review, Shaw (2005).
[d]For further information on the involvement of dynactin in motor neuron disease, please consult references within Shaw (2005). The involvement of OPA1 in autosomal-dominant optic atrophy is discussed in Alexander and others (2000).

transport and/or energy metabolism at the synapse. This evidence highlights a link between impaired mitochondrial transport, stemming from cytoskeletal network perturbations and defective molecular motors, in some neurodegenerative diseases that may potentially lead to synaptic defects.

In addition to cytoskeletal transport, proper mitochondrial distribution depends on a balance between fission and fusion of the mitochondrial membranes. For instance, the most common form of hereditary blindness, autosomal dominant optic atrophy, is caused by mutations in Optic atrophy 1, a dynamin GTPase that opposes Drp1 function by mediating mitochondrial fusion (Alexander and others 2000; see Table 2). Aside from progressive retinal degeneration, patients also produce less mitochondrial ATP (Lodi and others 2004). Similarly, the most common genetic lesion in CMT2 affects mitofusin 2, another component of the mitochondrial fusion complex, and leads to misdistribution of mitochondria (Shy 2004; see Table 2). Hence, failure to dynamically resculpt mitochondria also compromises organelle localization and neuronal function.

Although the pathogenesis of these neurodegenerative disorders ultimately pivots on the initiation of degenerative and apoptotic programs, early manifestations of synaptic dysfunction due to mitochondrial loss may have an impact in disease progression. In light of recent findings that indicate a specific function for mitochondria at synapses (Verstreken and others 2005), it will be interesting to see whether the mutations implicated in these disorders lead to common synaptic defects prior to neuronal degeneration, particularly defects in RP function. Such studies may illuminate novel therapeutic targets for these debilitating neurological diseases.

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