Mitochondrial Dynamics in Physiology and Pathology of Myelinated Axons

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Abstract

Mitochondria play essential roles in neurons and abnormal functions of mitochondria have been implicated in neurological disorders including myelin diseases. Since mitochondrial functions are regulated and maintained by their dynamic behavior involving localization, transport, and fusion/fission, modulation of mitochondrial dynamics would be involved in physiology and pathology of myelinated axons. In fact, the integration of multimodal imaging in vivo and in vitro revealed that mitochondrial localization and transport are differentially regulated in nodal and internodal regions in response to the changes of metabolic demand in myelinated axons. In addition, the mitochondrial behavior in axons is modulated as adaptive responses to demyelination irrespective of the cause of myelin loss, and the behavioral modulation is partly through interactions with cytoskeletons and closely associated with the pathophysiology of demyelinating diseases. Furthermore, the behavior and functions of axonal mitochondria are modulated in congenital myelin disorders involving impaired interactions

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between axons and myelin-forming cells, and, together with the inflammatory environment, implicated in axonal degeneration and disease phenotypes. Further studies on the regulatory mechanisms of the mitochondrial dynamics in myelinated axons would provide deeper insights into axo–glial interactions mediated through myelin ensheathment, and effective manipulations of the dynamics may lead to novel therapeutic strategies protecting axonal and neuronal functions and survival in primary diseases of myelin.

Keywords
Axonal degeneration · Demyelination · Localization · Remyelination · Transport

10.1 Introduction

Myelin, the multilamellar ensheathment around axons, plays essential roles in facilitating fast saltatory conduction and conserves the space and possibly energy required for nerve conduction (Trapp and Stys 2009; Nave 2010a, b). The ensheathment by myelin-forming cells divides axons into multiple segments with differential morphology, molecular distribution, and accessibility to extracellular molecules, and alters axonal microenvironment and metabolism (Arroyo and Scherer 2000; Poliak and Peles 2003). In addition, myelin is essential for long-term maintenance of axonal integrity, and the loss as well as congenital defects of myelin lead to neurological disorders involving degeneration and loss of axons (Nave and Trapp 2008; Nave 2010a, b). In this context, the regulation of axonal metabolism, functions, and survival are closely associated with axonal mitochondria in physiology and pathology of the nervous system.

Mitochondria play essential roles in almost all cells in the nervous system and critical for cellular metabolisms such as energy production and Ca^{2+} homeostasis. Mitochondrial functions are important in neurons and associated with neurological disorders involving neuronal and axonal degeneration and loss (Wallace 2005; Lin and Beal 2006). Mitochondria are dynamic organelles and characterized in neurons by their characteristic distribution, transport, and fusion/fission (Chan 2006; Saxton and Hollenbeck 2012). The dynamic behavior is regulated by specialized molecules, and interaction with the other organelles including endoplasmic reticulum (ER) is also critical for maintenance of mitochondrial functions (Hayashi et al. 2009; Sheng and Cai 2012; Lamb et al. 2013; Friedman and Nunnari 2014; Tatsuta et al. 2014; Misgeld and Schwarz 2017). Therefore, the regulatory mechanisms of mitochondrial dynamics and organelle interactions have been implicated in pathophysiology of myelin diseases.

This chapter will focus on the characteristic behavior of mitochondria in axons, and provide overview of recent studies on the dynamics of mitochondria in myelinated axons as well as their alterations in myelin diseases. Furthermore, the mechanisms and roles of the behavior and alterations in physiology of myelinated axons and pathophysiology of myelin diseases are also discussed.

10.2 General Behavior of Axonal Mitochondria

Mitochondrial behavior in axons is different from that in the soma of neurons and the other cells, and characterized by two major populations, stationary and motile. The majority of axonal mitochondria are stationary, immobile during entire observation period, and enriched in regions such as growth cones and synaptic terminals, which are considered to have higher metabolic demand (Fig. 10.1a) (Saxton and Hollenbeck 2012; Sheng and Cai 2012). Other mitochondria are motile, which are generally small, and their movement is in anterograde and retrograde directions (Fig. 10.1a). It has been established that these two populations are interchangeable. Motile mitochondria stop and accumulate at the specific regions and static mitochondria start moving when the axonal metabolic states are altered. For example, mitochondria were enriched near the growth cones under axonal growth, while stopping of the axonal growth diminished the mitochondrial accumulation in the distal axons (Morris and Hollenbeck 1993). Therefore, the overall regulation of the two motile and stationary populations is critical to determine mitochondrial distribution within the axons.

The movement of axonal mitochondria is mediated by specific motor proteins and their adaptor molecules, which regulate direction and cessation of their movement (Fig. 10.1b). The motor proteins include kinesin and dynein with distinct or common

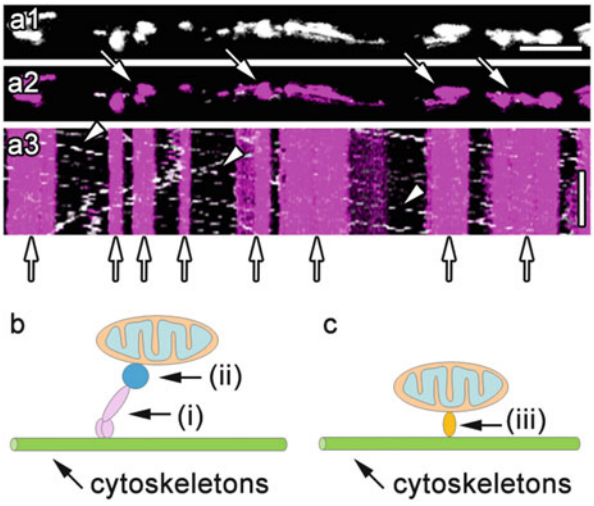

Fig. 10.1 Motile and stationary mitochondria in axons. The first image of the time-lapse imaging showing fluorescently labeled mitochondria in an axon of cultured dorsal root ganglion (**a1**). The stationary mitochondrial profiles are colored magenta (**a2**, arrows) and the kymograph of the time-lapse imaging (**a3**) shows vertically appear stationary mitochondria (**a3**, arrows) and diagonal trajectories of motile mitochondria (**a3**, arrowheads). Bars: 10 μm (horizontal) or 20 sec (vertical). Schemes showing a motile mitochondrion with the motor (**b**, i) and adaptor (**b**, ii) proteins on a track of cytoskeletons, and a stationary mitochondrion with a tethering molecule (**c**, iii) on the scaffold of cytoskeletons

adaptor complex such as Miro and Milton in *Drosophila* and RhoT1/2 and trafficking protein kinesin-binding (TRAK) 1/2 in mammals (Schwarz 2013). While kinesins and dyneins are motors on microtubule tracks, myosins are responsible for the slower bidirectional movement on actin filaments, although myosins may be associated with tethering rather than transport of axonal mitochondria (Morris and Hollenbeck 1995; Pathak et al. 2010). The mitochondrial movement is modulated by multiple mechanisms, including Ca^{2+} and glucose, and cytosolic Ca^{2+} binds to EF-hands of Miro or RhoT1/2, induces mitochondrial detachment from microtubule tracks and stops mitochondrial movement (Saotome et al. 2008; Wang and Schwarz 2009; Pekkurnaz et al. 2014). The local inhibition of mitochondrial movement results in increase of mitochondrial sizes in axons (Chada and Hollenbeck 2004; Macaskill et al. 2009).

Mitochondrial localization is mediated by regulation of mitochondrial docking as well as the local inhibition of mitochondrial movement. Apart from the role of myosin as a potential mitochondrial tether to the actin filament, syntaphilin is another mitochondrial docking molecule, which binds mitochondria to microtubule scaffolds (Fig. 10.1c) (Kang et al. 2008; Pathak et al. 2010). The loss of syntaphilin significantly increased mitochondrial motility while its overexpression decreased number of motile mitochondria (Kang et al. 2008). The impairment in mitochondrial tethering via syntaphilin perturbed axonal arborization, and increased the variability of synaptic transmission (Courchet et al. 2013; Sun et al. 2013). These results indicate that mitochondrial tethering plays significant roles in axonal functions and morphogenesis.

In addition to the transport and localization, mitochondria undergo dynamic morphological changes via fusion and fission. The fusion of two mitochondrial segments generates one large daughter mitochondrion where free exchange of soluble and membrane-bound molecules takes place within the daughter mitochondrion (Busch et al. 2006; Liu et al. 2009). Mitochondrial fission generates two small daughter mitochondria from one large mitochondrion, and one of the two daughter mitochondria could be dysfunctional and selectively degraded for mitochondrial quality control (Twig et al. 2008). The fusion and fission of mitochondria are regulated by distinct sets of GTPases, including mitofusins (Mfn1, Mfn2) and dynamin-related protein 1 (Drp1) on outer membranes and optic atrophy 1 (OPA1) on inner membranes (Hoppins et al. 2007). The fusion and fission of mitochondria are generally critical for neurological disorders, and genetic mutations of Mfn2 and OPA1 cause Charcot–Marie–Tooth type 2 and autosomal dominant optic atrophy, respectively (Alexander et al. 2000; Delettre et al. 2000; Zuchner et al. 2004). Loss of Drp1 in rodents was embryonic lethal, and mutation in human caused severe neurodevelopmental defects (Waterham et al. 2007; Ishihara et al. 2009).

Collectively, regulation of mitochondrial movement, localization, and morphology are critical for functional maintenance and survival of axons and neurons. Understanding the dynamic aspects of mitochondria in myelinated axons would provide insights regarding how myelination affects axonal homeostasis both in physiology and in pathology of the nervous system.

10.3 Mitochondrial Regulation in Myelinated Axons

The ensheathment by myelin in the nervous system of vertebrate causes extreme specialization of axons (Poliak and Peles 2003). The gaps of the insulating compact myelin sheath are the highly specialized domains called nodes of Ranvier. Voltage-dependent Na^+ channels are clustered on the axolemma of the nodes and required for saltatory conduction. The nodes are flanked by paranodes, which have unique cytoplasmic loops of myelin-forming cells. The paranodes contain the junctional complex between axons and myelin-forming cells and limit diffusion of extracellular as well as axolemmal molecules (Mackenzie et al. 1984; Bhat et al. 2001; Boyle et al. 2001; Perkins et al. 2008; Rosenbluth 2009; Mierzwa et al. 2011; Shroff et al. 2011). K^+ channels are enriched in the juxtaparanodal regions, which are about 15 μm long, adjacent to the paranodal regions, and sometimes with invaginations of plasma membranes into the axons (Spencer and Thomas 1974; Griffin and Price 1981).

Conventionally, it was believed that axonal mitochondria were in general accumulated in the nodes with some variance in different tracts (Hollenbeck and Saxton 2005; Chen and Chan 2006). However, the recent development of the methods for three dimensional (3D) ultrastructural analyses enabled high-throughput acquisition of serial electron microscopic images and complete reconstruction of all mitochondria in individual axons (Briggman and Bock 2012; Ohno et al. 2015; Nguyen et al. 2016; Thai et al. 2016). Application of these advanced methods revealed that mitochondria in myelinated axons were enriched in internodal regions rather than the nodes (Fig. 10.2a, b) (Ohno et al. 2011). The abundant mitochondria in the internodal regions are consistent with the notion that the energy substrate for axonal mitochondria is provided through myelin sheath as a form of lactate or glucose and thereby myelin-forming cells maintain axonal integrity (Nave 2010a, b; Brown et al. 2012; Saab and Nave 2017). The transport of lactate may be mediated by distinct transporters such as monocarboxylate transporter 1 (MCT1), whose deficiency in oligodendrocytes impaired axonal survival in the central nervous system (Funfschilling et al. 2012; Lee et al. 2012). In addition, the Na^+/K^+ ATPase, which maintains ion gradient of Na^+ and K^+ in an energy-dependent manner, was enriched on axolemma of the internodes (Young et al. 2008; Trapp and Stys 2009). Therefore, enrichment of axonal mitochondria in the internodal regions suggests that mitochondrial energy production in myelinated axons predominates in regions with the supply of energy substrate and higher metabolic demand under basal conditions (Perge et al. 2009). The molecular mechanisms regulating mitochondrial distribution in internodes are unclear. However, time-lapse observation in vitro demonstrated stationary mitochondria were substantially increased during myelin formation (Kiryu-Seo et al. 2010). It is possible that molecules such as syntaphilin, which support mitochondrial docking and increase of stationary mitochondria, are involved in the internodal enrichment. Such modulation of mitochondria-associated proteins in different segments of myelinated axons may be associated with signal transduction between axons and myelinating glia, which involves posttranslational modification of cytoskeletal proteins in axons (Sousa and Bhat 2007).

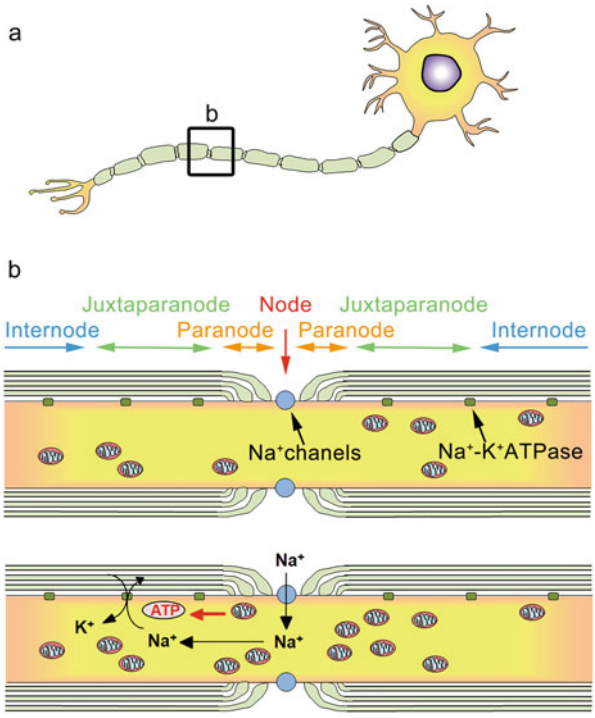

Fig. 10.2 Mitochondrial behavior in myelinated axons. The myelinated axon of a neuron (**a**) is marked with a rectangle and magnified (**b**). There are multiple segments called nodes, paranodes, juxtaparanodes, and internodes (**b**). Mitochondria are enriched in juxtaparanodes and internodal regions (**b**, upper panel), while mitochondria are stopped and increased near the node upon nerve conduction (**b**, lower panel)

In addition to the mitochondrial enrichment in internodal regions, small and short mitochondria were accumulated in a fraction of nodal regions, and the nodal accumulation was mediated by stopping of motile mitochondria in a manner dependent on axonal electrical activity and Ca^{2+} (Fig. 10.2b) (Zhang et al. 2010; Ohno et al. 2011). The metabolism of myelinated axons upon nerve conduction is distinct from that in unmyelinated axons. Myelin ensheathment enables rapid saltatory conduction by concentrating Na^+ channels in the nodal regions and is considered to conserve energy as well as space for nerve conduction (Ritchie 1995). Activation of nodal Na^+ channels upon nerve conduction increased Na^+ concentration in nodal axoplasm (Fleidervish et al. 2010). Repetitive axonal conduction requires an energy-dependent exchange of axoplasmic Na^+ for extracellular K^+ through Na^+/K^+ ATPases, which was enriched in the juxtaparanodal and internodal regions in human brain tissues (Young et al. 2008). Given that nerve conduction was impaired in genetic disorders of mitochondrial functions caused by mutations of mitochondrial DNA (Kaufmann et al. 2006; Horga et al. 2014), activity-dependent mitochondrial stopping and localization in the nodal and paranodal axoplasm would be important in order to meet the energy demand of saltatory nerve conduction.

The distribution and motility of mitochondria in myelinated axons appear to be regulated in response to the metabolic alterations of the axons. The adaptive response of mitochondrial behavior is considered to be more important in myelin diseases particularly in demyelinating disorders. The alterations, mechanisms, and

the roles of mitochondrial dynamics in demyelinating axons will be discussed in the next section.

10.4 Mitochondrial Alterations in Demyelinated Axons

Axonal degeneration, commonly observed in demyelinating diseases (Fig. 10.3a, b), is characterized by axonal swelling and loss and contributes to permanent neurological deficits (Trapp and Nave 2008). The mitochondrial alterations have been implicated in the pathophysiology of demyelinating diseases (Mahad et al. 2015). Demyelination poses a major challenge to axons since axons lose support from myelin-forming cells. Upon demyelination, Na^+ channels were redistributed along the axons, expression of Na^+ channel isoforms was changed, and confinement of Na^+ influx was modified (Craner et al. 2004a; b). The redistribution of Na^+ channels may increase energy consumption necessary for the exchange of Na^+ and K^+ upon nerve conduction (Waxman 2008). In addition, the demyelinated axons are exposed to inflammatory environment, which is common in demyelinating diseases such as multiple sclerosis or animal models such as experimental autoimmune encephalomyelitis (EAE), and often includes toxic mediators including nitric oxide (NO). NO can diffuse into demyelinated axons, and inhibit mitochondrial ATP production. All these factors perturb the energy metabolism of demyelinated axons and would lead

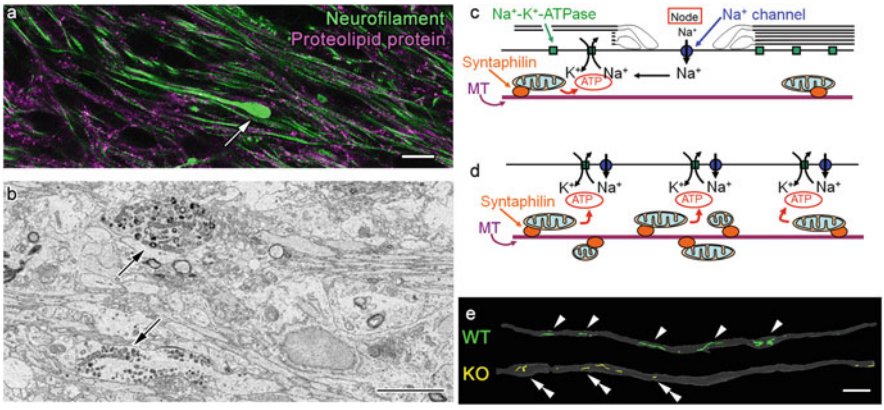

Fig. 10.3 Axons and mitochondria in demyelinated lesions. Immunostaining for an axonal marker, neurofilament, and a myelin marker, proteolipid protein (**a**), and an electron micrograph (**b**) in mouse corpus callosum demyelinated with cuprizone feeding shows axonal swellings (**a**, **b**, arrows) with numerous axoplasmic organelles (**b**, arrows). A scheme showing stationary mitochondria, enriched in internodal regions of myelinated axons (**c**) and increased in demyelinated axons (**d**), are tethered to microtubules with syntaphilin. The three-dimensional reconstruction of serial electron microscopic images, which are obtained from axons demyelinated under cuprizone feeding shows mitochondrial number and volume in a syntaphilin knockout axon (KO, double arrowheads) is less than that in a wild-type axon (WT, arrowheads). Bars 5 μm

to axonal degeneration mediated by the accumulation of Na^+ and Ca^{2+} (Trapp and Stys 2009).

The mitochondrial distribution, behavior, and lifecycle are modulated upon demyelination in order to maintain axonal integrity and functions. In human brain tissues of patients with demyelinating diseases, the mitochondrial size and numbers were significantly increased (Mahad et al. 2009; Witte et al. 2009; Zambonin et al. 2011). The increase was prominent compared with adjacent normal-appearing white matter, and also the consistent increase of mitochondrial mass was observed in demyelinated axons of animal models (Mutsaers and Carroll 1998; Sathornsumetee et al. 2000; Zambonin et al. 2011). The live imaging studies of fluorescently labeled mitochondria in demyelinated axons in vitro revealed that the majority of increased mitochondrial profiles in demyelinated axons were stationary and these static population increased in their sizes (Kiryu-Seo et al. 2010; Ohno et al. 2014). The expression of mitochondrial tethering molecule, syntaphilin, was increased in demyelinated axons of human multiple sclerosis patients and a demyelinating mouse model produced by cuprizone feeding (Fig. 10.3c, d), and the genetic ablation of syntaphilin impaired volume increase of mitochondria in demyelinated axons and led to exacerbation of axonal degeneration (Mahad et al. 2009; Ohno et al. 2014). Since the lack of syntaphilin did not augment neurological symptoms in the inflammatory demyelination model of EAE, the mitochondrial tethering could be more beneficial in demyelination with less inflammation (Joshi et al. 2015). Indeed, the impairment of mitochondrial respiratory chain components in acute demyelinating lesions was implicated in the tissue damages (Mahad et al. 2008). These studies support the concept that the enrichment of functional mitochondria is the common adaptive response of axons against demyelination, mediated by molecular interaction between mitochondria and cytoskeletons, and beneficial for the survival of demyelinated axons.

In addition, mitochondrial fusion and fission play essential roles for functional regulation and maintenance of mitochondria (Youle and van der Bliek 2012; Friedman and Nunnari 2014). The mitochondrial sizes were increased in demyelinated axons of human tissues and animal models at the light microscopic level (Zambonin et al. 2011). This observation was supported by detailed 3D electron microscopic observation showing that the volume of individual mitochondria was increased in demyelination model produced by cuprizone feeding (Ohno et al. 2014). These results suggested that the increase of mitochondrial volume in demyelinated axons is at least partly mediated by mitochondrial fusion. Although the balanced mitochondrial fusion/fission is critical for neuronal functions and survival, aberrant activation of mitochondrial fission was observed in neurological disorders, and inhibition of mitochondrial fission led to neuroprotection in models of neurological diseases (Barsoum et al. 2006; Grohm et al. 2012; Cho et al. 2013). In fact, inhibition of mitochondrial fission with overexpression of dominant-negative Drp1 protected axons from degeneration induced by aberrant activation of the axonal cation channel, transient receptor potential vanilloid receptor 1 (TRPV1) (Chiang et al. 2015). Since aberrant axoplasmic Ca^{2+} increase may

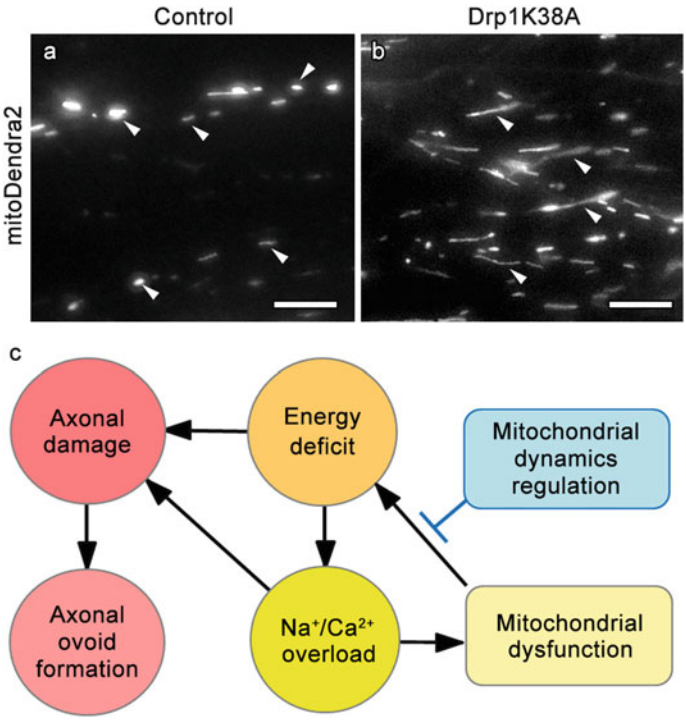

Fig. 10.4 Mitochondrial fission and axonal degeneration. Mitochondria-targeted fluorescent Dendra2 (mitoDendra2) in cultured rat dorsal root ganglion axons shows overexpression of dominant-negative dynamin-related protein1 (Drp1K38A) elongate mitochondrial profiles (**b**, arrowheads) compared with control (**a**, arrowheads). Bars: 5 μm. Regulation of mitochondrial dynamics would ameliorate mitochondrial dysfunction and support survival of axons (**c**)

contribute to the degeneration of demyelinated axons, inhibition of mitochondrial fission could be beneficial for the survival of demyelinated axons (Fig. 10.4).

The inflammatory toxic mediators cause damages in mitochondrial molecules, and mitochondrial segments with the damaged molecules would require turnover and functional maintenance, involving fusion/fission and transport (Smith et al. 1999; Chang and Reynolds 2006; Twig et al. 2008; Saxton and Hollenbeck 2012). Mitochondrial transport was substantially impaired by inflammatory reactions and oxidative stress in demyelinated tissues, as observed in an inflammatory demyelination model (Sorbara et al. 2014). On the other hand, in live imaging studies of demyelinated axons in vitro under the presence of less inflammation and oxidative stress, demyelination increased speed and number of axonal mitochondria (Kiryu-Seo et al. 2010; Ohno et al. 2014). The increased mitochondrial transport in demyelinated axons was at least partly mediated by the expression of activating transcription factor 3 (ATF3) (Kiryu-Seo et al. 2010). The increased mitochondrial transport supported renewal of mitochondrial proteins in the distal portion of neurites, and perturbed axonal transport of mitochondria in demyelinated axons is

associated with accumulation of oxidative stress and impaired axonal integrity (Ferree et al. 2013; Sorbara et al. 2014). Alterations of mitochondrial transport upon demyelination may be regulated as an adaptive response against mitochondrial damage under the noxious environment of demyelinated axons and involves unidentified retrograde signaling toward nuclei from demyelinated axons. Furthermore, environmental perturbation of the response would exacerbate the axonal pathology.

Remyelination, the restoration of the myelin sheath, is protective for axonal survival and neurological deficits (Franklin and Ffrench-Constant 2008). The remyelination reverses the mitochondrial alterations upon demyelination, and the sizes of mitochondrial profiles were decreased compared with demyelinated axons in brain tissues of multiple sclerosis patients (Zambonin et al. 2011). The stationary mitochondria were decreased while more mitochondria were mobile in remyelinated axons compared with demyelinated axons in vitro (Kiryu-Seo et al. 2010; Zambonin et al. 2011). However, when compared with myelinated axons, mitochondrial mass in remyelinated axons is still slightly larger. Further studies are required for elucidating the metabolic states and demands of remyelinated axons, which will provide further insights about the molecular mechanisms associated with the incomplete reversal of mitochondrial behavior upon remyelination.

10.5 Alterations of Axonal Mitochondria in Models of Congenital Myelin Disorders

Congenital defects of myelin structures and functions lead to various phenotypes from almost normal development and aging to severe neurodevelopmental defects and premature death. These phenotypes are at least partly attributable to the abnormal myelin ensheathment, which results in impaired nerve conduction and alterations of axo–glial interactions affecting organelle dynamics and metabolism (Nave 2010a, b). Abnormal dynamics of mitochondria are often implicated in the pathophysiology of the congenital myelin disorders.

The severe phenotypes are observed among the disorders caused by mutations in proteolipid protein (PLP). The types of mutations in the Plp gene on X-chromosome cause wide ranges of symptoms including severe disorders of Pelizaeus-Merzbacher disease (PMD) to the much milder form of spastic paraplegia type 2 (SPG2) (Willard and Riordan 1985; Saugier-Veber et al. 1994). Animal models for these PLP-associated disorders are also established and contributed to our understanding of the pathological mechanisms (Yool et al. 2000; Inoue 2005). In myelin-deficient (MD) rats, a model of PMD, where myelin formation is disrupted by a point mutation in the Plp gene (Csiza and de Lahunta 1979; Boison and Stoffel 1989), oligodendrocytes ensheath axons but fail to produce compact myelin sheath. Although these axons in MD rats had node-like regions with concentrated Na^+ channels, molecular distribution of paranodal proteins, such as contactin and contactin-associated proteins (Caspr), and saltatory conduction were significantly affected (Waxman et al. 1990; Arroyo et al. 2002). In the electron microscopic analyses, mitochondrial densities and areas occupied by mitochondria were

significantly increased in axons of MD rats (Dentinger et al. 1985). In addition, live imaging analyses in organotypic slice cultures revealed that the stationary mitochondria were increased in these axons of MD rats, and the mitochondrial motility was not affected by the axonal electrical activity around the node-like regions (Ohno et al. 2011). These findings are consistent with the concept that increased mitochondrial mass in demyelinated axons is largely stationary, and myelin formation and saltatory conduction is critically involved in the modulation of mitochondrial transport and localization at the nodal regions (Mahad et al. 2009; Kiryu-Seo et al. 2010).

PMD is also caused by the duplication of the Plp gene, and animals with extra copies of Plp are informative to understand the role of PLP as well as the pathophysiology of PMD (Griffiths et al. 1998a, b). The animal models with different dosages of Plp gene demonstrated that higher PLP dosage causes severer and often lethal phenotypes involving impaired myelin formation and oligodendrocyte degeneration, and lower dosage leads to progressive demyelination with axonal degeneration (Kagawa et al. 1994; Readhead et al. 1994). In demyelinated axons of mice overexpressing PLP, mitochondrial density was significantly increased in axons of optic nerves, and mitochondrial respiratory functions were also upregulated in the demyelinated axons of PLP mutants (Hogan et al. 2009). Mutation in the other myelin genes also causes a severe deficit of myelin formation in the nervous system, and myelin basic protein (MBP) is partially deleted and myelin formation is impaired in *shiverer* mice (Roach et al. 1985). Analyses with electron microscopy and enzyme histochemistry revealed increased mitochondrial density and mitochondrial cytochrome c activity in spinal cord axons of *shiverer* mice lacking myelin chronically (Andrews et al. 2006). These findings are consistent with the findings in demyelinated axons of human multiple sclerosis patients (Mahad et al. 2009), and increased mitochondrial volume and functions would be the common response against the loss of myelin irrespective of the cause.

Axons of specific tracts in the PLP mutants have impaired axonal transport, and accumulation of axoplasmic organelles including mitochondria was observed in retinal ganglion axons (Edgar et al. 2010; Ip et al. 2012). The accumulation of organelles was prominent in paranodal regions, and the perturbation of axonal transport was associated with neuroinflammation, since the disruption of transport was rescued in the absence of cytotoxic T cells (Ip et al. 2012). The massive neuroinflammation may be associated with the mitochondrial abnormality in PLP mutant model as well (Nave et al. 1986; Macklin et al. 1987; Moriguchi et al. 1987; Huttemann et al. 2009; Tatar et al. 2010). On the other hand, in the nerve fibers of Caspr mutants, axonal mitochondria with abnormal morphology were accumulated near the nodes (Einheber et al. 2006; Sun et al. 2009). Mutations of Caspr cause impaired paranodal septate-like junctions, abnormal expression or distribution of paranodal and juxtaparanodal molecules, and severe decrease of conduction velocity (Bhat et al. 2001). The abnormal transport, distribution, and functions of mitochondria in myelin mutants would be affected by not only inflammation but also abnormal metabolism derived from impaired myelin structures.

Genetic ablation of myelin-related molecules, such as PLP, 2′,3′-cyclic nucleotide phosphodiesterase (CNP) and myelin-associated glycoprotein (MAG), caused progressive axonal degeneration following myelin formation in the central and peripheral nervous system (Griffiths et al. 1998a, b; Yin et al. 1998; Lappe-Siefke et al. 2003; Nguyen et al. 2009). In these mutant models of myelin-related proteins, axonal swelling with accumulated axoplasmic organelles was commonly observed (Griffiths et al. 1998a, b; Lappe-Siefke et al. 2003; Yin et al. 2006). Biochemical analyses revealed fast axonal transport was impaired (Edgar et al. 2004), and abnormal organelle accumulation in the distal regions of the nodes was often observed in degenerating axons (Griffiths et al. 1998a, b; Yin et al. 2006). In a myelin mutant model with axonal swelling in the distal side of the nodes, time-lapse imaging of live axons in slice cultures indicated impaired transport of mitochondria in distal regions of the nodes, and this transport defects accompanied destruction of microtubules and abnormal microtubule stability in axons (Yin et al. 2006, 2016). Posttranslational modification of axonal cytoskeletons could be perturbed in myelin mutants such as *shiverer* and MAG-deficient mice (Rosenbluth 1980; Inoue et al. 1981; Windebank et al. 1985; Shine et al. 1992; Colello et al. 1994; Kirkpatrick and Brady 1994; Yin et al. 1998; Brady et al. 1999; Nguyen et al. 2009), and therefore the trophic support of myelin ensheathment may include signal transduction, which modulates axonal cytoskeletons and is required for transport of axonal organelles including mitochondria.

Axonal pathology that is independent of the formation and maintenance of myelin ensheathment also involves morphological alterations of axonal mitochondria and associated organelles. The detailed ultrastructural analyses using the 3D reconstruction of serial electron microscopic images revealed that mitochondria in the myelinated axons of optic nerves had an extension of outer membranes, which had close contacts with tubular smooth ER membranes (Yin et al. 2016). In the myelin mutant model with progressive axonal degeneration following myelination, the intimate contacts between mitochondria and ER were decreased when the mitochondria became shorter and the extension was diminished. These structural changes in organellar interactions accompanied abnormal cristae structures and impaired functions of axonal mitochondria (Yin et al. 2016). These results indicate that axonal degeneration in dysmyelinated axons of myelin mutants also involves organelle interactions necessary for mitochondrial homeostasis.

10.6 Summary and Conclusions

The integration of multimodal imaging along with animal and in vitro models enabled more detailed analyses of mitochondrial behavior in myelinated, demyelinated, and dysmyelinated axons. These analyses started to reveal that stationary and motile pools of axonal mitochondria are regulated in nodal and internodal regions in response to metabolic alterations of myelinated axons. In addition, adaptive responses involving cytoskeletal interactions and genetic transcription regulate the behavioral alterations of mitochondria in demyelinated axons

and affect the pathophysiology of demyelinating diseases. Furthermore, congenital myelin disorders modulate behavior and functions of axonal mitochondria, which influence axonal survival and disease phenotypes and are associated with impaired interactions between axons and myelin-forming cells as well as the inflammatory environment of the nervous system. The regulatory mechanisms and effective manipulations of mitochondrial dynamics and functions would provide deeper insights into axo–glial interactions mediated through myelin sheath, and may lead to novel therapeutic strategies protecting axons and neurons in primary diseases of myelin.

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