Review

Regulation of dynamin family proteins by post-translational modifications

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Dynamin superfamily proteins comprising classical dynamins and related proteins are membrane remodelling agents involved in several biological processes such as endocytosis, maintenance of organelle morphology and viral resistance. These large GTPases couple GTP hydrolysis with membrane alterations such as fission, fusion or tubulation by undergoing repeated cycles of self-assembly/disassembly. The functions of these proteins are regulated by various post-translational modifications that affect their GTPase activity, multimerization or membrane association. Recently, several reports have demonstrated variety of such modifications providing a better understanding of the mechanisms by which dynamin proteins influence cellular responses to physiological and environmental cues. In this review, we discuss major post-translational modifications along with their roles in the mechanism of dynamin functions and implications in various cellular processes.

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1. Introduction

Dynamins are a family of large GTPases which carry out essential cellular functions with their nomenclature reflecting dynamic, molecular motor-like properties. They are involved in various processes like vesicle scission, fission and fusion of organelles, vacuolar trafficking, microbial resistance and nuclear remodelling (Vater *et al.* 1992; Wilsbach and Payne 1993; Janzen *et al.* 2000; Ishihara *et al.* 2004; Praefcke and McMahon 2004; Kuravi *et al.* 2006; Rahaman *et al.* 2008). All the known dynamin family proteins perform their function by fission, fusion or tubulation of target membranes (figure 1). Based on domain architecture, the dynamin superfamily is broadly divided into two groups, classical dynamin and dynamin-related proteins (Drps). The common features shared by all dynamins and Drps are the presence of a large N-terminal GTPase domain, a middle domain (MD) and a GTPase effector domain (GED) (figure 1a). While GTPase domain binds and hydrolyses GTP, MD and GED play important roles in oligomerization and regulation of GTPase activity. Classical dynamin contains two additional domains namely, pleckstrin homology domain (PHD) involved in membrane binding and a proline-rich domain (PRD) containing several SH3-binding sites important for protein–protein interactions (Hinshaw 2000). Although lacking a PH domain, all the known Drps associate with membranes to perform their functions.

Dynamin was discovered as a microtubule associated protein and its role in endocytosis was established when mutation in the protein translated from *shibire* locus was

Keywords. Dynamin; endocytosis; GTPase; mitochondrial dynamics; post-translational modification

Abbreviations used: CaMKIα, calmodulin-dependent protein kinase 1alpha; Cav1, Caveolin1; CDK, cyclin-dependent kinase; Drp, dynamin-related protein; eNOS, endothellial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular regulated kinase; GED, GTPase effector domain; GTP, guanosine triphosphate; HDAC, histone deacetylase; JNK, Janus kinase; MAM, mitochondria-associated ER membrane; MD, middle domain; Mfn, mitofusin; NO, nitric oxide; NOS, nitric oxide synthase; OMM, outer mitochondrial membrane; PHD, Pleckstrin homology domain; PKA, protein kinase A; PKC, protein kinase C; PRD, proline-rich domain; ROS, reactive oxygen species; SH3, Src homology 3; SIRT, sirtuin

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correlated with a conditional temperature sensitive paralytic phenotype in *Drosophila melanogaster* (Shpetner and Vallee 1989; Van der Bliek and Meyerowitz 1991; Chen *et al.* 1991). During clathrin-mediated endocytosis, classical dynamin plays both regulatory role in early endocytosis (bud formation and invagination) and a mechanical role in later stages during vesicle scission (Sever *et al.* 2000; Song *et al.* 2004). However, a recent report demonstrates its role in mitochondrial fission, a function different from canonical vesicle scission (Lee *et al.* 2016). Dynamin polymerizes to form a collar-like structure around the neck of the clathrin coated endocytic vesicles and subsequent GTP hydrolysis dependent conformational changes result in membrane deformation required for vesicle scission (Bashkirov *et al.* 2008; Pucadyil and Schmid 2008; Liu *et al.* 2013; Cocucci *et al.* 2014). Dynamin family proteins perform wide range of biological functions across species. Despite performing diverse functions, the members of this family exhibit similar underlying mechanisms. They undergo cycles of assembly–disassembly on the target membrane, regulated by binding and hydrolysis of GTP, resulting in membrane curvature change (Carr and Hinshaw 1997; Liu *et al.* 2011). The regulation of this cycle is critical for biological function of all dynamin family proteins. The vast array of protein post-translational modifications provides the cell a dynamic and rapid way of regulating various essential processes in response to cellular stimuli. The proteins of this family undergo a variety of post-translational modifications such as phosphorylation, nitrosylation, ubiquitylation, sumoylation, acetylation and regulate their functional properties including membrane recruitment, self-assembly, GTPase activity and interactions with protein partners (Table 1). Loss of these regulations manifests several physiological disorders underscoring the importance of these modifications in various cellular processes (Züchner *et al.* 2004, 2005; Bitoun *et al.* 2005, 2009; Durieux *et al.* 2010). In this review, we highlight the regulatory roles and mechanisms of major post-translational modifications of dynamin family proteins and their implications in cellular functions (summarized in table 1).

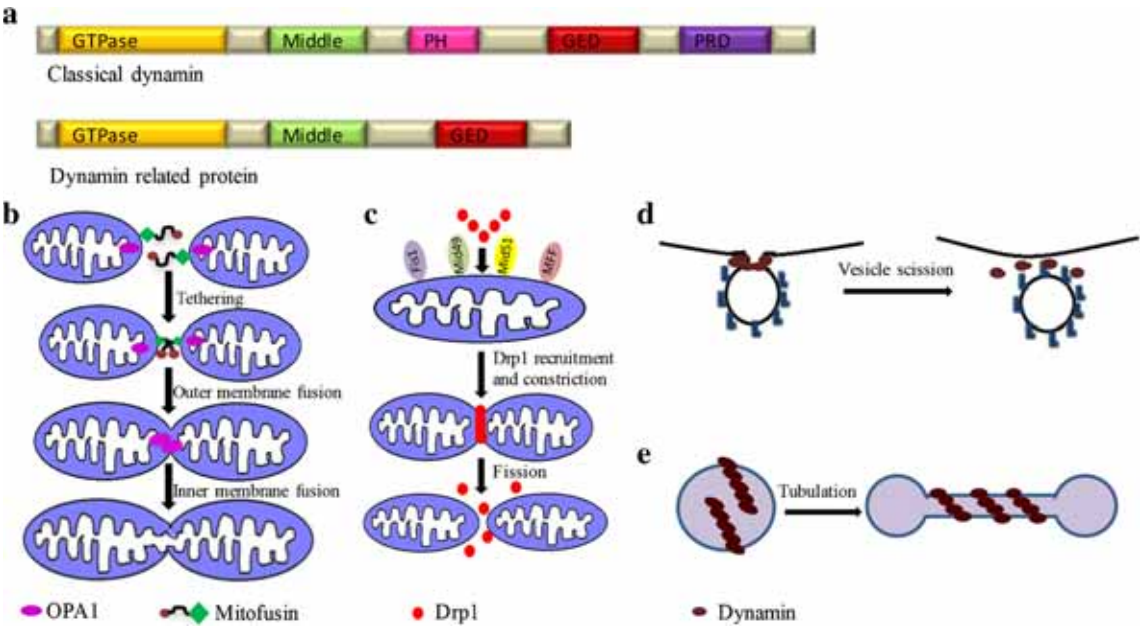

Figure 1. Dynamin family proteins and Membrane remodelling. (**a**) Domain structure of classical dynamin (top) and dynamin-related proteins (bottom). Domains are indicated in different colours. Drps lack PH and PRD; (**b**) mitochondrial fusion by mitofusins and OPA1. Mitofusins mediate tethering and fusion of outer mitochondrial membrane where as OPA1 brings about inner mitochondrial membrane tethering and fusion; (**c**) mitochodrial fission by Drp1. Drp1 is recruited to fission sites by adapter proteins such as Fis1, Mff, Mid49, Mid51 and fragments mitochondria by constriction of mitochondrial membrane; (**d**) clathrin-mediated endocytosis by classical dynamin. Dynamin oligomerizes at the neck of clathrin coated vesicles and drives vesicle scission upon GTP hydrolysis; (**e**) tubulation of spherical vesicles by dynamin. Dynamin binds to vesicles and tubulates upon GTP hydrolysis.

2. Phosphorylation

Protein phosphorylation is a ubiquitous strategy adopted by cells to modulate wide range of biological processes. Addition of a phosphate group to a protein imparts a negative

Table 1. Post-translational modifications and their effects on dynamin superfamily proteins

Modification	Protein	Residues	Effect on function	Mechanism
Phosphorylation	Dynamin 1	Tyr 597	Promotes clathrin-mediated endocytosis	Increases self-assembly and GTP hydrolysis
	Dynamin 2	Tyr 597	Promotes caveolae-mediated scission	Increases translocation to plasma membrane
		Tyr 231	-	Enhances Dyn2-Cav1 interaction
		Ser 774/778	Inhibit endocytosis	Impair interaction with Amphiphysin and Syndapin, inhibit its membrane recruitment
	Yeast Vps1	Ser 599	Promotes vesicle scission from trans golgi	Enhances interaction of Vps1 with Amphiphysin partner Rvs167
	Drp1	Ser 585(Rat) or Ser616(Human)	Promotes mitochondrial fission during mitosis.	Facilitates interaction with other adaptor proteins
		Ser 637	Inhibits mitochondrial fission	Inhibits interaction of GED and GTP binding domain and reduces GTPase activity
		Ser 600	Promotes mitochondrial fission	Facilitates Drp1 association with adapter Fis1
		Ser 635(Rat) or Ser617(human)	Promotes mitochondrial fission during apoptosis	Dissociates sequestered Drp1 from microtubule and recruits to mitochondria
	Mfn2	Ser 27	Promotes mitochondrial fission during apoptosis	Promotes ubiquitylation of Mfn2 by E3 ligase Huwe1 and targets for proteasomal degradation
		Thr 111/Ser442	Promotes mitochondrial fragmentation during apoptosis	Phosphorylated form acts as a receptor for E3 ligase Parkin
	Mfn1	Thr562	Promotes mitochondrial permeabilization and apoptosis	Promotes recruitment of BAK to mitochondrial membrane
S-nitrosylation	Dynamin1	Cys 86/Cys 607	Stimulates endocytosis	Induces self-assembly and GTPase activity
	Drp1	Cys 644	Promotes mitochondrial fission and causes synaptic damage	Promotes Drp1 dimerization, self-assembly andstimulates GTPase activity
Ubiquitylation	Drp1	-	Induces mitochondrial fragmentation	Promotes Drp1 association with mitochondria
	Fzo1	Lys 398	Inhibits mitochondrial fusion	Targets for proteasomal degradation
		Lys 464	Promotes mitochondrial fusion	Stabilizes Drp1 on the mitochondrial membrane
Sumoylation	Drp1	Lys 594,Lys 597, Lys 606, Lys 608	Promotes Cyt C release from inter mitochondrial membrane space during apoptosis	Stabilises Drp1 association with mitochondria and facilitates Bax oligomerization on mitochondria
Acetylation	OPA1	Lys 926, Lys 931	Promotes mitochondrial fragmentation	Inhibits GTPase activity of OPA1
	Mfn1	Lys 491	Inhibits mitochondrial fusion	Targets Mfn1 for MARCH-V-mediated proteasomal degradation

charge and changes its chemical property, often leading to changes in its structure and function. Dynamin phosphorylation was first identified two and half decades back as one of the major regulators of endocytosis where protein kinase C (PKC)-mediated phosphorylation enhanced its GTPase activity and acted as a trigger for rapid synaptic vesicle recycling (Robinson *et al.* 1993). Subsequently, a number of reports detailing phosphorylations and their roles in regulating dynamin functions became available.

Being an indispensable part of endocytic process, regulatory function of dynamin has been demonstrated in ligand activated receptor endocytosis and caveolae-mediated endocytosis. In both the processes, phosphorylation at Y597 (tyrosine 597) of dynamin (dynamin 1 and 2) enhances endocytosis by triggering self-assembly and subsequent GTP hydrolysis. However, constitutive endocytosis remains unaffected suggesting phosphorylation can be used as a very specific regulator for a particular process (Ahn *et al.* 2002). In case of caveolae-mediated endocytosis, Y597 phosphorylation enhances the association of Dynamin2 with Caveolin1 (Cav1). As for example, binding of albumin to GP60 triggers immediate phosphorylation of Src and Src-dependent tyrosine phosphorylations of dynamin 2 at Y231 in the GTPase domain and at Y597 in the PH domain. While both the phosphorylations are required for interaction of dynamin2 with Cav1, only phosphorylated Y597 is required for translocating dynamin to plasma membrane and is essential for scission of caveolae (Shajahan *et al.* 2004). Dynamin phosphorylations also play important roles in regulation of endocytosis during vesicle recycling in neurons. With highly specialized membrane structures and higher rates of membrane recycling and turn over, nerve cells have greater demands of membrane trafficking. During synaptic vesicle endocytosis, dynamin interacts via PRD with many SH3 domain containing adapter proteins such as amphiphysin, syndapin and endophilins, and this interaction is essential for its recruitment to the site of vesicle scission (David *et al.* 1996; Takei *et al.* 1999; Qualmann and Kelly 2000; Gad *et al.* 2000; Hill *et al.* 2001; Kim and Chang 2006). During formation of synaptic vesicles in post-mitotic neurons, phosphorylation of dynamin1 at residues S774 and S778 by CDK5 renders a conformational change and inhibits membrane recruitment by impairing its interaction with amphiphysin and syndapin. Calceneurin-mediated dephosphorylation promotes dynamin recruitment to the site of vesicle scission by facilitating interaction with amphiphysin (Tan *et al.* 2003; Anggono *et al.* 2006; Armbruster *et al.* 2013). The intricate balance between phosphorylation and dephosphorylation of dynamin 1is essential to modulate the rate of endocytosis in neuronal cells in response to synaptic stimuli.

Classical dynamins are exclusively present in metazoans. Clathrin-mediated endocytic trafficking in yeast is mediated by a dynamin-related protein Vps1 (Vacuolar protein sorting 1), also involved in protein trafficking between trans-golgi network and endosomes (Vater *et al.* 1992; Ekena *et al.* 1993; Nannapaneni *et al.* 2010). Similar to dynamin 1, Vps1 undergoes regulation by cycles of phosphorylation and dephosphorylation (Smaczynska-de Rooij *et al.* 2015). It assembles into higher order ring like structures followed by phosphorylation at S599 by cyclin associated kinase Pho85. This phosphorylation facilitates its interaction with amphiphysin Rvs167. However, during the final step of scission, dephosphorylation of S599 is required to facilitate dissociation of Vps1-Rvs167 which in turn brings about the downstream processes for the release of vesicle, signifying the importance of phosphorylationin regulating protein interaction and progression of endocytosis.

Mitochondria are dynamic tubulo-vesicular organelles and maintenance of mitochondrial morphology is essential for normal cellular function (Chan 2006). Mitochondrial morphology is maintained by highly regulated fission and fusion events driven mainly by dynamin-related proteins (Chen and Chan 2005; Youle and van der Bliek 2012). Among all dynamin family proteins, the regulation of Drp1, the central regulator of mitochondrial fission is understood in great detail. Several post-translational modifications regulate Drp1 activity and its translocation onto mitochondria (Pitts *et al.* 1999; Chang and Blackstone 2010). Phosphorylation of Drp1 confers opposing effects on mitochondrial morphology depending on cellular conditions, position of residues modified and the kinases involved. Loss of regulation of mitochondrial dynamics is implicated in various degenerative neurological disorders such as Charcot-Marie-Tooth neuropathy, hereditary optic atrophy, Alzheimer's disease, Parkinson's disease and Huntington's disease (Alexander *et al.* 2000; Chan 2006; Davies *et al.* 2007; Knott and Bossy-Wetzel 2008; Liesa *et al.* 2009). S585 (serine 585) of Drp1 is phosphorylated by multiple kinases. In addition to CDK1 during mitosis, this residue can be phosphorylated by CDK5, PKA and calmodulin dependent protein kinase-1 alpha (CaMKIα) in neuronal cells. ERK1/2 and PKC delta also control phosphorylation of this residue under high-glucose condition and oxidative stress condition respectively (Han *et al.* 2008; Yu *et al.* 2010; Qi *et al.* 2011). Phosphorylation of mammalian Drp1 by CDK1/cyclinB at S585 (S585 in rat/S616 in human) stimulates mitochondrial fission during mitosis without affecting its GTPase activity. Loss of this phosphorylation results in reduced mitochondrial fission and is suggested to be by interfering the interaction of Drp1 with adapter proteins and subsequent recruitment of mitochondrial fission machinery onto the fission foci (Taguchi *et al.* 2007). Post-mitotic neurons have higher levels of CDK5 activity than CDK1 in physiological condition and CDK5 predominantly regulates post-mitotic neuronal signals (Su and Tsai 2011). Phosphorylation of Drp1 by a neuron specific CDK5 has implications in multiple processes such as synaptic plasticity, survival and

development of neurons. In case of both dividing neuronal cells and non-dividing mature neurons, CDK5 translocates from nucleus to cytoplasm before entering S phase and requires association with p35 or p39 for activation (Tsai *et al.* 1994; Humbert *et al.* 2000). This activation of CDK5 has been implicated in regulation of various neuronal processes including neuron development, axonal transport and synaptic activity. CDK5-mediated phosphorylation of Drp1 at S585 in neuronal cells induces mitochondrial fragmentation post injury (Jahani-Asl *et al.* 2015). However, an earlier report demonstrates that CDK5-mediated phosphorylation on the same residue inhibits mitochondrial fragmentation by interfering its oligomerization suggesting that downstream effects of phosphorylation of Drp1 at S585 are possibly determined by additional modifications in response to cellular cues (Cho *et al.* 2014). How phosphorylation of the same residue exhibits opposite effect remains to be explored. To perform fission activity at the mitochondrial foci, Drp1 requires specific intra molecular and inter molecular interactions, and are regulated by post-translational modifications. One such means of regulation comes from PKA-mediated phosphorylation of S637 in GED (Chang and Blackstone 2007). This residue is conserved across several species and provides a common means for regulating Drp1 function. PKA-mediated phosphorylation at S637 perturbs interaction of GED with the GTP-binding domain, interfering in both GTPase activity and higher order oligomerization. Inhibition of mitochondrial fission by loss of interaction between the GED and GTP binding domain suggests a direct correlation between Drp1 GTPase activity and mitochondrial fission. Activation of apoptotic signals leads to dephosphorylation of S637 by calcineurin and translocation of Drp1 to mitochondria. Drp1 phosphorylation by PKA and dephosphorylation by calceneurin act as a switch to respond intracellular calcium changes and subcellular distribution of mitochondria (Cereghetti *et al.* 2008). Calcium/calmodulin dependent kinase 1 alpha (CaMKIα) regulates neuronal mitochondrial morphology and dynamics by stimulating Drp1 association with Fis1 and promoting fission activity by phosphorylating Drp1 at S600 in an intracellular calcium dependent manner (Han *et al.* 2008). These phosphorylations have a role in recruitment of Drp1 to mitochondrial target site without affecting its activity.

Drp1 being a crucial player in mitochondrial morphology maintenance is regulated at multiple levels. Along with post-translational modifications, alternative splicing products of Drp1 control its distribution. Drp1 heteromers exist in dynamic equilibrium between cytosolic, cytoskeletal and mitochondrial pools. Reversible phosphorylation at a conserved serine residue S635 (Rat-635 or Human-617) and microtubule dynamics are responsible for differential localization of Drp1. Phosphorylation of alternatively spliced Drp1 products by CDK1/cyclinB during mitosis and by CDK5 in interphase releases Drp1 from microtubule and induces mitochondrial fission in response to apoptotic stimuli (Strack *et al.* 2013).

Phosphorylation of mitofusins, the outer mitochondrial membrane fusion Drps, regulate mitochondrial quality control during mitophagy by facilitating the recruitment of various ubiquitin ligases. For example, activation of pro apoptotic Janus kinase (JNK) in response to cellular stress leads to phosphorylation of Mfn2 at residue S27. This phosphorylation triggers ubiquitylation and proteasomal degradation of Mfn2 by E3 ligase Huwe1, promoting mitochondrial fragmentation and apoptotic cell death (Leboucher *et al.* 2012). Also, phosphorylation of Mfn2 at T111 and S442 by PINK1 leads to ubiquitylation of Mfn2 by Parkin (E3 ubiquitin ligase) during mitophagy and has implications in quality control of cardiac mitochondria (Chen and Dorn 2013). Phosphorylation of Mfn1 at T562 by ERK has been shown to regulate mitochondrial morphology and permeabilization during apoptosis by promoting recruitment and oligomerization of BAK on mitochondrial membrane (Pyakurel *et al.* 2015).

3. S-nitrosylation

S-nitrosylation has emerged as a potential mechanism for regulation of protein function in cellular signal transduction and provides a means for regulation of redox based physiologcal processes (Broillet 1999). Nitrosylation involves transfer and covalent addition of a nitroso (NO) group to the reactive cysteine thiol. This is mediated by direct interaction of nitric oxide synthase (NOS) with the target protein. Addition of NO exerts its biological effects through S-nitrosylation of proteins involved in many processes such as endocytosis, vascular homeostasis, blood pressure control, DNA repair, host defence, ion channel regulation and neuro-transmission by affecting stability of protein function and their localization (Gould *et al.* 2013). Nitrosylation of dynamin1 at a single conserved cysteine residue C607 in PH domain by endothelial NO synthase (eNOS) enhances self-assembly, GTPase activity and membrane recruitment during receptor-mediated endocytosis (Wang *et al.* 2006). Dynamin plays a critical role during endothelial cell based angiogenesis and vascular homeostasis. In addition to C607 in PH domain, nitrosylation of C86 in the GTPase domain regulates endocytosis and endothelial cell survival. Nitrosylation at C607 induces self-assembly and GTPase activity of dynamin possibly by promoting its interaction with eNOS and stabilizing dynamin at the plasma membrane. This is essential for endocytic receptor internalization during endothelial cell based angiogenesis.

Regulation of dynamin by nitrosylation thus is a novel strategy (other than canonical cGMP-PKG pathway) adopted

by endothelial cells in response to cell survival signals and is essential for maintaining the balance between cell survival and apoptosis (Kang-Decker *et al.* 2007). In contrast to endothelial cells, dynamin over expression inhibits cell survival signals in epithelial cells suggesting regulation of cell survival by S-nitrosylated dynamin is cell type specific (Fish *et al.* 2000).

Many pathogenic microbes and viruses use endocytic processes for cellular entry and dynamin being a key endocytic factor regulates this process. Adenoviral entry to host cells utilizing the endocytic machinery is one prominent example of such processes (Gastaldelli *et al.* 2008; Meier and Greber 2003). The mechanism of early adenoviral infection steps including entry into target cells remained unknown until 2012. Adenoviral entry by clathrin-mediated endocytosis is regulated by NO-mediated S-nitrosylation of dynamin. This infection causes generation of NO followed by nitrosylation of eNOS in epithelial cells. eNOS being the endogenous NO source, directly interact with dynamin2 and nitrosylates C86, C607 residues which enhances self-assembly and GTPase activity of dynamin and favours viral entry into the host cell by accelerating rate of vesicle internalization (Wang *et al.* 1998, 2012; Cao *et al.* 2001).

Although, dynamin2 has six cysteine residues, the specificity of C86 and C607 residues for S-nitrosylation could be attributed to the conformation and accessibility of these sites in dynamin structure. Amino acids His85-Cys86-Lys87 in the GTPase domain of dynamin constitutes a nitrosylation motif and in this motif, the hydrophobic cysteine residue (C86) is exposed to the surface of the molecule (Reubold *et al.* 2005; Kang-Decker *et al.* 2007). Additionally, as proposed by Decker et al, position of C86 in close proximity (within 6Å) to aromatic amino acids (phenylalanine at 83 and 91) could also contribute to potential target for nitrosylation. The electronegative sulphur of cysteine often establishes favourable electrostatic interaction with electropositive hydrogen on aromatic ring. This electrostatic interaction provides protein structure stability in hydrophobic environment (Reid *et al.* 1985; Hao *et al.* 2006). Cysteine aromatic interactions, together with hydrogen bonding to a nearby basic residue provide a general structural motif that makes cysteine residues favourable target for S-nitrosylation (Hao *et al.* 2006).

Neuronal viability is dependent on mitochondrial function to meet high energy requirements of neuronal cells. Normal mitochondrial function is perturbed when the balance between mitochondrial fusion and fission is disturbed, leading to several neurological and metabolic disorders. The regulation of protein function by S-nitrosylation extends to other members of dynamin family. Drp1, major mitochondrial fission protein, is also regulated by S-nitrosylation (Nakamura *et al.* 2010; Wada and Nakatsuka 2016). During several pathophysiological conditions such as Alzhaeimer's disease and Huntington's disease, Drp1 is nitrosylated through a redox-mediated pathway in response to beta amyloid oligomers and promotes mitochondrial fragmentation causing neuronal damage (Cho *et al.* 2009; Haun *et al.* 2013). S-nitrosylation of Drp1 causes dimerization and increase in GTPase activity which accelerates the process of mitochondrial fragmentation contributing to neuronal synaptic damage and cell death. The GED of Drp1 is crucial for its inter and intra molecular interactions and for stimulated GTPase activity. Nitrosylation at C644 promotes Drp1 dimerization, nucleates formation of higher order structures and augments GTPase activity, establishing a mechanistic link between the effect of oligomeric amyloid protein, mitochondrial fragmentation and neuronal damage (Cho *et al.* 2009; Haun *et al.* 2013).

4. Ubiquitylation

The ubiquitylation machinery of the cell is conventionally thought to be associated with protein degradation by proteasomal pathway. However, ubiquitylation plays other roles such as regulation of protein activity, protein-protein interaction, protein localization, protein stability and lysosomal degradation (Komander and Rape 2012). Dynamin-related proteins are regulated by ubiquitylation as well. Ubiquitin ligases such as Rsp5, Skp1/Cullin/F-box (SCF) complex are involved in shaping mitochondrial morphology in yeast (Fisk and Yaffe 1999; Fritz *et al.* 2003; Altmann and Westermann 2005). Maintenance of mitochondrial morphology by MARCH-V-mediated ubiquitylation of mitochondrial fission/fusion machineries presents an example of such regulation in mammals (Nakamura *et al.* 2006; Yonashiro *et al.* 2006). Initially, MARCH-V was thought to promote mitochondrial fusion. However, ubiquitylation of Drp1 by MARCH-V was later found to induce mitochondrial fragmentation by its recruitment onto the fission site (Yonashiro *et al.* 2006). The RING CH-V domain is the active structural component of MARCH-V since either expression of RING CH-V domain mutant or absence of MARCH-V leads to aberrant assembly of Drp1 on mitochondrial fission sites (Karbowski *et al.* 2007). Mitochondrial fission is also regulated by MARCH-V-mediated ubiquitylation of an adapter protein MiD49, which sequesters Drp1. Drp1 is released upon proteasomal degradation of ubiquitylated MiD49 which in turn promotes mitochondrial fission (Xu *et al.* 2015; Palmer *et al.* 2011). Recently, another ubiquitin E3 ligase Parkin is demonstrated to ubiquitylate and degrade Drp1 which confers cellular protection from mitochondrial dysfunction during apoptosis induced by oxygen glucose deprivation and reperfusion (OGDR), and has implications in Parkinson's disease (Tang *et al.* 2016).

Mitochondrial fusion as well is regulated by ubiquitylation. MARCH-V interacts with Mfn1, Mfn2 and regulates mitochondrial morphology (Park *et al.* 2010; Nakamura

et al. 2006). Althoughboth Mfn1 and Mfn2 are essential, they play distinct roles in mitochondrial fusion (Eura *et al.* 2003; Chen *et al.* 2003). While Mfn1 mediates mitochondrial docking and fusion, Mfn2 tethers mitochondria (Ishihara *et al.* 2004; Koshiba *et al.* 2004). Ubiquitylation and proteasomal degradation of Mfn1 by MARCH-V leads to mitochondrial fragmentation in a cell cycle dependent manner (Park and Cho 2012). Ubiquitylation by MARCH-V is also associated with degradation of Mfn2 under hypoxic condition. A recent report has demonstrated that a cytosolic ubiquitin binding deacetylase HDAC6 interacts with and prevents MARCH-V-mediated degradation of Mfn2 and maintains mitochondrial connectivity. This confers a protective role under hypoxic stresses which might be associated with neurodegenerative diseases (Kim *et al.* 2015). In addition to proteasomal degradation, ubiquitylation of Mfn2 by MARCH-V also plays a role in maintaining mitochondria associated ER membrane (MAM) (de Brito and Scorrano 2008). MARCH-V specifically ubiquityates mitochondrial Mfn2 but not the Mfn2 present in ER. Polyubiquityation of Mfn2 at K192 by MARCH-V upregulates GTPase dependent oligomerization, essential for MAM association and tethering of ER with mitochondria (Sugiura *et al.* 2013). Failure in MAM function is linked with neuronal diseases like Alzheimer's disease where significant increase in both MAM function and ER-mitochondrial connectivity are observed (Estela *et al.* 2012; Hedskog *et al.* 2013). Development of pathogenesis of such neurodegenerative conditions could be attributed to the lack of elimination of dysfunctional mitochondria as a result of defect in the regulation of mitochondrial proteins by MARCH-V-mediated ubiquitylation.

Mitochondrial outer membrabe fusion protein Fzo1 (yeast homolog of mitofusin), represents another example where ubiquitylation plays a role different from proteasomal degradation (Anton *et al.* 2013). Fzo1 is ubiquitylated at residues K398 and K464 which are deubiquitylated by deubiquitylases Ubp2 and Ubp12 respectively in a stage specific manner. While Fzo1 ubiqutylation at K398 has a canonical role in proteasomal degradation leading to inhibition of mitochondrial fusion, Mdm30-mediated ubiquitylation at K464 confers stability promoting mitochondrial fusion.

5. Sumoylation

SUMO is an acronym for Small Ubiquitin-like Modifier and the sumoylation pathway uses a defined sequence of enzyme catalyzed reactions that bear a close resemblance to the ubiquitin pathway. However, sumoylated proteins play role distinct from that of ubiquitylation. Like the ubiquitin pathway, conjugation of SUMO also involves three principal enzymes; specific E1 for activating (SAE1/SAE2), E2 conjugating enzymes (Ubc9 enzymes) that catalyze formation of isopeptide bonds between the C terminal of SUMO moiety and N terminal of the amino group of the target lysine residue, and SUMO E3 ligases (RanBP2 and proteins of the PIAS family) (Pichler *et al.* 2002; Wang Yonggang 2009). The mammalian SUMO exists in four isoforms (SUMO 1-4) which have defined cellular locations (Su and Li 2002; Bohren *et al.* 2004). SUMO 1,2 and 3 are known to sumoylate Drp1 at residues K594, K597, K606, K608 in the B domain and affect its activity (B domain is absent in dynamin and evolutionarily is the most variable region of the Drp1 structure) (Figueroa-Romero *et al.* 2009). At the onset of mitosis, sumoylation of Drp1 by SUMO-1 stabilizes the association with the mitochondrial membrane preventing rapid cycling between cytosol and mitochondria (Harder *et al.* 2004). Drp1 sumoylation is reversible, and the SUMO moiety is removed by the isopeptidase SENP5 (Zunino *et al.* 2007). During apoptosis, sumoylation plays a new role by stabilizing Drp1 on mitochondrial membrane which leads to outer mitochondrial membrane (OMM) remodelling. This facilitates oligomerization of pro-apoptotic Bax onto the mitochondrial membrane resulting in OMM permeabilization and release of cytochrome C from inter membrane space (Montessuit *et al.* 2010; Wasiak *et al.* 2007). Drp1 gets sumoylated by SUMO-2/3 as well and this sumoylation plays an opposite role as described above. Unlike sumoylation by SUMO-1, SUMO-2/3 renders Drp1 partitioned in the cytosol away from the mitochondrial membrane during oxygen glucose deprived stress condition and inhibits apoptosis (Guo *et al.* 2013). The balance between Drp1 population at the mitochondria and in the cytosol is maintained by sumoylation by SUMO-2/3 and desumoylation by SENP3.

MxA, an anti-viral dynamin-related protein also undergoes sumoylation at K48. However, the modification does not affect MxA oligomerization or viral entry (Brantis-de-Carvalho *et al.* 2015). Oligomerization is mainly driven by interaction of MxA stalk structure formed by MD and GED (Gao *et al.* 2010). Since residue K48 is not part of the stalk structure, modification on this residue may not influence oligomerization. However, if sumoylation at this residue affect any other property of MxA needs to be addressed.

6. Acetylation

Lysine acetylation is a reversible post-translational modification of proteins that targets large macromolecular complexes involved in diverse cellular processes such as chromatin remodelling, cell cycle, splicing, nuclear transport, and actin nucleation (Choudhary *et al.* 2009, 2014; Zhao *et al.* 2010). Acetylation also plays a role in conjunction with other post-translational modifications where it allows a post-translationally modified protein to get

identified by its target. Although infrequent, regulatory role of acetylation has been extended to dynamin family of proteins.

OPA1 is involved in the mitochondrial dynamics maintenance primarily as a fusion Drp. Apart from the fusion activity, OPA1 plays important role in cristae maintenance and oxidative phosphorylation in mitochondria which is crucial for the cell survival. Pathological stress during cardiac hypertrophy leads to hyper-acetylation of OPA1 that reduces its GTPase activity and inhibits mitochondrial fusion. In hypertrophic cells, acetylation of OPA1 at K926 and K931 leads to an abnormal distribution of mitochondria mainly due to mitochondrial fragmentation arising from inhibition of mitochondrial fusion. Sirtuins (SIRT) are a class of histone deacetylases (HDACs) which interact with many proteins involved in the mitochondrial dynamics including OPA1 (Jing *et al*. 2011; Samant *et al.* 2014). Deacetylation by SIRT3 enhances the activity of OPA1 possibly by influencing intra molecular interaction. Moreover, deacetylation of OPA1 by SIRT3 regulates restoration of mitochondrial DNA copy number and oxidative damage by ROS generation (Samant *et al.* 2014).

During cellular stress, mitochondria undergo hyperfusion as a protective mechanism for cell survival. The mitochondrial fusion proteins Mfn1, Mfn2 and OPA1 are upregulated during such conditions. During cellular stress, Mfn1 is activated causing hyperfusion of mitochondria to adapt to the cell environment. However, when fusion exceeds to a lethal level and Mfn1 accumulates, MARCH-V ubiquityates Mfn1 for degradation. Recognition of accumulated Mfn1 by MARCH-V requires tagging of Mfn1 by acetylation. Only the acetylated Mfn1 at K491 is identified by MARCH-V for degradation. This report is the first demonstration of acetylation regulating stability of a mitochondrial dynamics protein which serves as an essential mechanism for maintaining mitochondrial homeostasis (Park *et al.* 2014).

7. Conclusion

With ever-increasing information on post-translational modifications, the mechanism and regulation of dynamin function are becoming more clear. A number of post-translational modifications have been identified across the dynamin family of proteins that critically regulate their functions. Although the members of dynamin superfamily undergo differential post-translational modifications to perform different biological processes, they follow similar underlying mechanisms such as modulation of GTPase activity, self-assembly, interaction with other proteins and recruitment to the target membranes. Post-translational modifications have both stimulatory and inhibitory effects on dynamin function depending on the cellular environment. Investigation of regulation of dynamin proteins presents interesting findings where a particular modification has opposing effects on protein function depending on the location of residue on the target protein and upstream signals. Some aspects of dynamin functions are also controlled by interplay between combination of different modifications, for example phosphorylation/acetylation followed by ubiquitylation regulates protein quality control. It is interesting to note that the same modification such as phosphorylation of mitochondrial fission protein Drp1 at S585 exerts opposing effects on mitochondrial dynamics, mechanism of which needs to be addressed. Post-translational modifications of dynamin family proteins have implications in many pathophysiological conditions. Understanding their regulation therefore would help addressing the mechanistic details of dynamin functions which in turn would help designing therapeutic interventions.

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References

Ahn S, Kim J, Lucaveche CL, Reedy MC, Luttrell LM, Lefkowitz RJ and Daaka Y 2002 Src-dependent tyrosine phosphorylation regulates dynamin self-assembly and ligand-induced endocytosis of the epidermal growth factor receptor. *J. Biol. Chem.* **277** 26642–26651.

Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A, Rodriguez M, Kellner U, *et al*. 2000 OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat. Genet.* **26** 211–215.

Altmann K and Westermann B 2005 Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae. *Mol. Biol. Cell.* **16** 5410–5417.

Anggono V, Smillie KJ, Graham ME, Valova VA, Cousin MA and Robinson PJ 2006 Syndapin I is the phosphorylation-regulated dynamin I partner in synaptic vesicle endocytosis. *Nat. Neurosci.* **9** 752–760

Anton F, Dittmar G, Langer T and Escobar-Henriques M 2013 Two deubiquitylases act on mitofusin and regulate mitochondrial fusion along independent pathways. *Mol. Cell.* **49** 487–498

Armbruster M, Messa M, Ferguson SM, De Camilli P and Ryan TA 2013 Dynamin phosphorylation controls optimization of endocytosis for brief action potential bursts. *eLife*. doi:10.7554/eLife.00845

Bashkirov PV, Akimov SA, Evseev AI, Schmid SL, Zimmerberg J and Frolov VA 2008 GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. *Cell.* **135** 1276–1286

Bitoun M, Maugenre S, Jeannet P-Y, Lacène E, Ferrer X, Laforêt P, Martin J-J, Laporte J, *et al*. 2005 Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat. Genet.* **37** 1207–1209

Bitoun M, Bevilacqua JA, Eymard B, Prudhon B, Fardeau M, Guicheney P and Romero NB 2009 A new centronuclear

myopathy phenotype due to a novel dynamin 2 mutation. *Neurology.* **72** 93–95

Bohren KM, Nadkarni V, Song JH, Gabbay KH and Owerbach D 2004 A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. *J. Biol. Chem.* **279** 27233–38

Brantis-de-Carvalho CE, Maarifi G, Boldrin PEG, Zanelli CF, Nisole S, Chelbi-Alix MK and Valentini SR 2015 MxA interacts with and is modified by the SUMOylation machinery. *Exp. Cell Res.* **330** 151–163

Broillet MC 1999 S-nitrosylation of proteins. *Cell. Mol. Life Sci.* **55** 1036–1042

Cao S, Yao J, McCabe TJ, Yao Q, Katusic ZS, Sessa WC and Shah V 2001 Direct interaction between endothelial nitric-oxide synthase and dynamin-2. Implications for nitric-oxide synthase function. *J. Biol. Chem.* **276** 14249–14256

Carr JF and Hinshaw JE 1997 Dynamin assembles into spirals under physiological salt conditions upon the addition of GDP and ??-phosphate analogues. *J. Biol. Chem.* **272** 28030–28035

Cereghetti GM, Stangherlin A, de Brito OM, Chang CR, Blackstone C, Bernardi P and Scorrano L 2008 Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc. Natl. Acad. Sci. USA* **105** 15803–15808

Chan DC 2006 Mitochondrial fusion and fission in mammals. *Annu. Rev. Cell Dev. Biol.* **22** 79–99

Chang C-R and Blackstone C 2007 Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J. Biol. Chem.* **282** 21583–21587

Chang CR and Blackstone C 2010 Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. in *Annals of the New York Academy of Sciences* **1201** 34–39

Chen H and Chan DC 2005 Emerging functions of mammalian mitochondrial fusion and fission. *Hum. Mol. Genet*. **14** R283–289

Chen Y and Dorn GW 2013 PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science (New York, N.Y.)* **340** 471–475

Chen MS, Obar RA, Schroeder CC, Austin TW, Poodry CA, Wadsworth SC and Vallee RB 1991 Multiple forms of dynamin are encoded by shibire, a drosophila gene involved in endocytosis. *Nature* **351** 583–586

Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE and Chan DC 2003 Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and Are essential for embryonic development. *J. Cell Biol.* **160** 189–200

Cho D-HH, Nakamura T, Fang J, Cieplak P, Godzik A, Zezong G and Lipton SA 2009 S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science* **324** 102–105

Cho B, Cho HM, Kim HJ, Jeong J, Park SK, Hwang EM, Park J-Y, Kim WR, *et al*. 2014 CDK5-dependent inhibitory phosphorylation of Drp1 during neuronal maturation. *Exp. Mol. Med.* **46**, e105

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV and Mann M 2009 Lysine acetylation targets protein complexes and Co-regulates major cellular functions. *Science* **325** 834–840

Choudhary C, Weinert BT, Nishida Y, Verdin E and Mann M 2014 The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat. Rev. Mol. Cell Biol.* **15** 536–550

Cocucci E, Gaudin R and Kirchhausen T 2014 Dynamin recruitment and membrane scission at the neck of a clathrin-coated pit. *Mol. Biol. Cell.* **25** 3595–3609

David C, McPherson PS, Mundigl O and de Camilli P 1996 A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. *Proc. Natl. Acad. Sci. USA* **27293** 331–335

Davies VJ, Hollins AJ, Piechota MJ, Yip W, Davies JR, White KE, Nicols PP, Boulton ME, *et al*. 2007 Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum. Mol. Genet.* **16** 1307–13018

de Brito OM and Scorrano L 2008 Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456** 605–610

Durieux A-C, Prudhon B, Guicheney P and Bitoun M 2010 Dynamin 2 and human diseases. *J. Mol. Med.* **88** 339–350

Ekena K, Vater CA, Raymond CK and Stevens TH 1993 The VPS1 protein is a dynamin-like GTPase required for sorting proteins to the yeast vacuole. *Ciba Found. Symp.* **176** 198–211

Estela A-G, Del Carmen Lara Castillo M, Tambini MD, Guardia-Laguarta C, de Groof Ad J , Madra M, Ikenouchi J, Umeda M, *et al*. 2012 Upregulated function of mitochondria-associated {ER} membranes in Alzheimer disease. *EMBO J.* **31** 4106–4123

Eura Y, Ishihara N, Yokota S and Mihara K 2003 Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J. Biochem.* **134** 333–344

Figueroa-Romero C, Iñiguez-Lluhí JA, Stadler J, Chang C-R, Arnoult D, Keller PJ, Hong Y, Blackstone C, and Feldman EL 2009 SUMOylation of the mitochondrial fission protein Drp1 occurs at multiple nonconsensus sites within the B domain and is linked to its activity cycle. *FASEB J.* **23** 3917–3927

Fish KN, Schmid SL and Damke H 2000 Evidence that dynamin-2 functions as a signal-transducing GTPase. *J. Cell Biol.* **150** 145–154

Fisk HA and Yaffe MP 1999 A role for ubiquitination in mitochondrial inheritance in Saccharomyces cerevisiae. *J. Cell Biol.* **145** 1199–1208

Fritz S, Weinbach N and Westermann B 2003 Mdm30 is an F-box protein required for maintenance of fusion-competent mitochondria in yeast. *Mol. Biol. Cell* **14** 2303–2313

Gad H, Ringstad N, Löw P, Kjaerulff O, Gustafsson J, Wenk M, Di Paolo G, Nemoto Y, *et al*. 2000 Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin. *Neuron.* **27** 301–312.

Gao S, von der Malsburg A, Paeschke S, Behlke J, Haller O, Kochs G and Daumke O, Alexander von der Malsburg, Susann Paeschke, Joachim Behlke, Otto Haller, Georg Kochs, and Oliver Daumke. 2010 Structural basis of

oligomerization in the stalk region of dynamin-like MxA. *Nature* **465** 502–506

Gastaldelli M, Imelli N, Boucke K, Amstutz K, Meier O and Greber U F Greber 2008 Infectious adenovirus type 2 transport through early but not late endosomes. *Traffic* **9** 2265–2278

Gould N, Doulias PT, Tenopoulou M, Raju K and Ischiropoulos H 2013 Regulation of protein function and signaling by reversible cysteine s-nitrosylation. *J. Biol. Chem.* **288** 26473–26479

Guo C, Hildick KL, Luo J, Dearden L, Wilkinson KA, Henley JM, Anderson DB, Wilkinson KA and Henley JM 2013 SENP3-mediated deSUMOylation of dynamin-related protein 1 promotes cell death following ischaemia. *EMBO J.* **32** 1514–1528. doi:10.1038/emboj.2013.65

Han X-JJ, Lu Y-FF, Li S-AA, Kaitsuka T, Sato Y, Tomizawa K, Nairn AC, Takei K, Matsui H and Matsushita M 2008 {CaM} kinase I alpha-induced phosphorylation of Drp1 regulates mitochondrial morphology. *J. Cell Biol.* **182** 573–585

Hao G *et al*. 2006 SNOSID, a proteomic method for identification of cysteine S-nitrosylation sites in complex protein mixtures. *Proc. Natl. Acad. Sci. USA* **103** 1012–1017

Harder Z, Zunino R and McBride H 2004 Sumo1 conjugates mitochondrial substrates and participates in mitochondrial fission. *Curr. Biol.* **14** 340–345

Haun F, Nakamura T, Shiu AD, Cho D-H, Tsunemi T, Holland EA, La Spada AR and Lipton SA 2013 S-nitrosylation of dynamin-related protein 1 mediates mutant huntingtin-induced mitochondrial fragmentation and neuronal injury in Huntington's disease. *Antioxid. Redox Signal* **19** 1173–1184

Hedskog L, Pinho CM, Filadi R, Rönnbäck A, Hertwig L, Wiehager B, Larssen P, Gellhaar S, *et al*. 2013 Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proc. Natl. Acad. Sci. USA* **110** 7916–7921

Hill E, Van Der Kaay J, Downes CP and Smythe E 2001 The role of dynamin and its binding partners in coated pit invagination and scission. *J. Cell Biol.* **152** 309–323

Hinshaw JE 2000 Dynamin and its role in membrane fission. *Annu. Rev. Cell Dev. Biol.* **16** 483–519

Humbert S, Dhavan R and Tsai L 2000 P39 activates Cdk5 in neurons, and is associated with the actin cytoskeleton. *J. Cell Sci.* **113** 975–983

Ishihara N, Eura Y and Mihara K 2004 Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J. Cell Sci.* **117** 6535–6546

Jahani-Asl A, Huang E, Irrcher I, Rashidian J, Ishihara N, Lagace DC, Slack RS and Park DS 2015 CDK5 phosphorylates DRP1 and drives mitochondrial defects in NMDA-induced neuronal death. *Hum. Mol. Genet.* **24** 4573–4583

Janzen C, Kochs G and Haller O 2000 A monomeric GTPase-negative MxA mutant with antiviral activity. *J. Virol.* **74** 8202–8206

Jing E, Emanuelli B, Hirschey MD, Boucher J, Lee KY, Lombard D, Verdin EM and Kahn CR 2011 Sirtuin-3 (Sirt3) Regulates Skeletal Muscle Metabolism and Insulin Signaling via Altered Mitochondrial Oxidation and Reactive Oxygen Species Production. *Proc. Natl. Acad. Sci. USA* **108** 14608–14613

Kang-Decker, Ningling, Sheng Cao, Suvro Chatterjee, Janet Yao, Laurence J Egan, David Semela, Debabrata Mukhopadhyay, andVijay Shah 2007 Nitric oxide promotes endothelial cell survival signaling through S-nitrosylation and activation of dynamin-2. *J. Cell Sci.* **120** 492–501

Karbowski M, Neutzner A and Youle RJ 2007 The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. *J. Cell Biol.* **178** 71–84

Kim Y and Chang S 2006 Ever-expanding network of dynamin-interacting proteins. *Mol. Neurobiol.* **34** 129–136

Kim HJ, Nagano Y, Choi SJ, Park SY, Kim H, Yao TP and Lee JY 2015 HDAC6 maintains mitochondrial connectivity under hypoxic stress by suppressing MARCH5/MITOL dependent MFN2 degradation. *Biochem. Biophys. Res. Commun.* **464** 1235–1240

Knott AB and Bossy-Wetzel E 2008 Impairing the mitochondrial fission and fusion balance: a new mechanism of neurodegeneration. in *Annals of the New York Academy of Sciences* pp. 283–292

Komander D and Rape M 2012 The ubiquitin code. *Annu. Rev. Biochem.* **81** 203–229

Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM and Chan DC 2004 Structural basis of mitochondrial tethering by mitofusin complexes. *Science (New York, N.Y.)* **305** 858–862

Kuravi K, Nagotu S, Krikken AM, Sjollema K, Deckers M, Erdmann R, Veenhuis M and van der Klei IJ 2006 Dynamin-related proteins Vps1p and Dnm1p control peroxisome abundance in Saccharomyces cerevisiae. *J. Cell Sci.* **119** 3994–4001

Leboucher GP, Tsai YC, Yang M, Shaw KC, Zhou M, Veenstra TM, Glickman MH and Weissman AM 2012 Stress-induced phosphorylation and proteasomal degradation of mitofusin 2 facilitates mitochondrial fragmentation and apoptosis. *Mol. Cell* **47** 547–557

Lee JE, Westrate LM, Wu H, Page C and Voeltz G 2016 Multiple dynamin family members collaborate to drive mitochondrial division. *Nature* **540** 139–143

Liesa M, Palacín M and Zorzano A 2009 Mitochondrial dynamics in mammalian health and disease. *Physiol. Rev.* **89** 799–845

Liu Y-W, Neumann S, Ramachandran R, Ferguson SM, Pucadyil TJ and Schmid SL 2011 Differential curvature sensing and generating activities of dynamin isoforms provide opportunities for tissue-specific regulation. *Proc. Natl. Acad. Sci. USA* **108** E234–E242

Liu YW, Mattila JP and Schmid SL 2013 Dynamin-catalyzed membrane fission requires coordinated GTP hydrolysis. *PLoS ONE*. **8**

Meier O and Greber UF 2003 Adenovirus endocytosis. *J. Gene Med.* **5** 451–462

Montessuit S, Somasekharan SP, Terrones O,Lucken-Ardjomande S, Herzig S, Schwarzenbacher R, Manstein DJ, Bossy-Wetzel E, *et al*. 2010 Membrane remodeling induced by the dynamin-related protein Drp1 stimulates Bax oligomerization. *Cell* **142** 889–901

Nakamura N, Kimura Y, Tokuda M, Honda S and Hirose S 2006 MARCH-V} is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep.* **7** 1019–1022

Nakamura T, Cieplak P, DH Cho, Godzik A and Lipton SA 2010 S-Nitrosylation of Drp1 links excessive mitochondrial fission to neuronal injury in neurodegeneration. *Mitochondrion* **10** 573–578

Nannapaneni S, Wang D, Jain S, Schroeder B, Highfill C, Reustle L, Pittsley D, Maysent A, *et al*. 2010 The yeast dynamin-like protein Vps1:vps1 mutations perturb the internalization and the motility of endocytic vesicles and endosomes via disorganization of the actin cytoskeleton. *Eur. J. Cell Biol.* **89** 499–508

Palmer CS, Osellame LD, Laine D, Koutsopoulos OS, Frazier AE and Ryan MT 2011 {MiD49} and {MiD51,} new components of the mitochondrial fission machinery. *EMBO Rep.* **12** 565–573

Park Y-Y and Cho H 2012 Mitofusin 1 is degraded at G2/M phase through ubiquitylation by MARCH5. *Cell Div.* **7** 25

Park Y-Y, Lee S, Karbowski M, Neutzner A, Youle RJ and Cho H 2010 Loss of MARCH5 mitochondrial E3 ubiquitin ligase induces cellular senescence through dynamin-related protein 1 and mitofusin 1. *J. Cell Sci.* **123** 619–626

Park, Y-Y, OTK Nguyen, H Kang, and H Cho 2014 MARCH5-mediated quality control on acetylated Mfn1 facilitates mitochondrial homeostasis and cell survival. *Cell Death Dis.* **5** e1172

Pichler A, Gast A, Seeler JS, Dejean A and Melchior F 2002 The nucleoporin RanBP2 has SUMO1 E3 ligase activity. *Cell* **108** 109–120

Pitts KR, Yoon Y, Krueger EW and McNiven MA 1999 The dynamin-like protein DLP1 is essential for normal distribution and morphology of the endoplasmic reticulum and mitochondria in mammalian cells. *Mol. Biol. Cell.* **10** 4403–4417

Praefcke GJK and McMahon HT 2004 The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat. Rev. Mol. Cell. Biol.* **5** 133–147

Pucadyil TJ and Schmid SL 2008 Real-time visualization of dynamin-catalyzed membrane fission and vesicle release. *Cell* **135** 1263–1275

Pyakurel A, Savoia C, Hess D and Scorrano L 2015 Extracellular regulated kinase phosphorylates mitofusin 1 to control mitochondrial morphology and apoptosis. *Mol. Cell* **58** 244–254

Qi X, Disatnik M-H, Shen N, Sobel RA, and Mochly-Rosen D 2011 Aberrant mitochondrial fission in neurons induced by protein kinase C{delta} under oxidative stress conditions in vivo. *Mol. Biol. Cell* **22** 256–265

Qualmann B and Kelly RB 2000 Syndapin isoforms participate in receptor-mediated endocytosis and actin organization. *J. Cell Biol.* **148** 1047–1061

Rahaman A, Elde NC and Turkewitz AP 2008 A dynamin-related protein required for nuclear remodeling in tetrahymena. *Curr. Biol.* **18** 1227–1233

Reid KSC, Lindley PF and Thornton JM 1985 Sulphur-aromatic interactions in proteins. *FEBS Lett.* **190** 209–213

Reubold TF *et al*. 2005 Crystal structure of the GTPase domain of rat dynamin 1. *Proc. Natl. Acad. Sci. USA* **102** 13093–13098

Robinson PJ, Sontag JM, Liu JP, Fykse, C Slaughter EM, McMahon H and Südhof TC 1993 Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals. *Nature* **365** 163–166

Samant SA, Zhang HJ, Hong Z, Pillai VB, Sundaresan NR, Wolfgeher D, Archer SL, Chan DC and Gupta MP 2014 SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress. *Mol. Cell. Biol.* **34** 807–819

Sever S, Damke H and Schmid SL 2000 Dynamin: GTP controls the formation of constricted coated pits, the rate limiting step in clathrin-mediated endocytosis. *J. Cell Biol.* **150** 1137–1147

Shajahan AN, Timblin BK, Sandoval R, Tiruppathi C, Malik AB and Minshall RD 2004 Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. *J. Biol. Chem.* **279** 20392–20400

Shpetner HS and Vallee RB 1989 Identification of dynamin, a novel mechanochemical enzyme that mediates interactions between microtubules. *Cell* **59** 421–432

Smaczynska-de Rooij II, Marklew CJ, Allwood EG, Palmer SE, Booth WI, Mishra R, Goldberg MW and Ayscough KR 2015 Phosphorylation regulates the endocytic function of the yeast dynamin-related protein Vps1. *Mol. Cell Biol.* **36** 742–755

Song BD, Leonard M and Schmid SL 2004 Dynamin GTPase domain mutants that differentially affect GTP binding, GTP hydrolysis, and clathrin-mediated endocytosis. *J. Biol. Chem.* **279** 40431–40436

Strack S, Wilson TJ and Cribbs JT 2013 Cyclin-dependent kinases regulate splice-specific targeting of dynamin-related protein 1 to microtubules. *J. Cell Biol.* **201** 1037–1051

Su HL and Li SSL 2002 Molecular features of human ubiquitin-like SUMO genes and their encoded proteins. *Gene.* **296** 65–73

Su SC and Tsai L-H 2011 Cyclin-dependent kinases in brain development and disease. *Annu. Rev. Cell Dev. Biol.* **27** 465–491

Sugiura A, Nagashima S, Tokuyama T, Amo T, Matsuki M, Ishido S, Kudo Y, McBride HM, et *al. 2013* MITOL regulates endoplasmic reticulum-mitochondria contacts via Mitofusin2. *Mol. Cell* **51** 20–34

Taguchi N, Ishihara N, Jofuku A, Oka T, and Mihara K 2007 Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J. Biol. Chem.* **282** 11521–11529

Takei,K, Slepnev VI, Haucke V and De Camilli P 1999 Functional partnership between amphiphysin and dynamin in clathrin-mediated endocytosis. *Nat. Cell Biol.* **1** 33–39

Tan TC, Valova VA, Malladi CS, Graham ME, Berven LA, Jupp OJ, Hansra G, McClure SJ, *et al*. 2003 Cdk5 is essential for synaptic vesicle endocytosis. *Nat. Cell Biol.* **5** 701–710

Tang J, Hu Z, Tan J, Yang S and Zeng L 2016 Parkin protects against oxygen-glucose deprivation/reperfusion insult by promoting Drp1 degradation. *Oxid. Med. Cell Longev.* **2016** 8474303

Tsai LH, Delalle I, Caviness VS, Chae T, and Harlow E 1994 P35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. *Nature* **371** 419–423

Van der Bliek AM and Meyerowitz EM 1991 Dynamin-like protein encoded by the Drosophila shibire gene associated with vesicular traffic. *Nature* **351** 411–414

Vater CA, Raymond CK, Ekena K, Howald-Stevenson I and Stevens TH 1992 The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in Saccharomyces cerevisiae, is a GTPase with two functionally separable domains. *J. Cell Biol.* **119** 773–786

Wada J and Nakatsuka A 2016 Mitochondrial dynamics and mitochondrial dysfunction in diabetes. *Acta Med. Okayama.* **70** 151–158

Wang Yonggang DM 2009 SUMOylation and deSUMOylation at a glance. *J. Cell Sci.* **122** 4249–4252

Wang K, Huang S, Kapoor-Munshi A and Nemerow G 1998 Adenovirus internalization and infection require dynamin. *J. Virol.* **72** 3455–3458

Wang G, Moniri NH, Ozawa K, Stamler JS and Daaka Y 2006 Nitric oxide regulates endocytosis by S-nitrosylation of dynamin. *Proc. Natl. Acad. Sci. USA* **103** 1295–1300

Wang Z, Kim JII, Frilot N and Daaka Y 2012 Dynamin2 S-nitrosylation regulates adenovirus type 5 infection of epithelial cells. *J. Gen. Virol.* **93** 2109–2117

Wasiak S, Zunino R and McBride HM 2007 Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. *J. Cell Biol.* **177** 439–450

Wilsbach K and Payne GS 1993 Vps1p, a member of the dynamin GTPase family, is necessary for Golgi membrane protein retention in Saccharomyces cerevisiae. *EMBO J.* **12** 3049–3059

Xu S, Cherok E, Das E, Li S, Roelofs BA, SX, Polster BM, Boyman L *et al*. 2015 Mitochondrial E3 ubiquitin ligase MARCH5 controls mitochondrial fission and cell sensitivity to stress-induced apoptosis through regulation of MiD49 protein. *Mol. Biol. Cell* **27** 349–359

Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y, Ohmura-Hoshino M, Sada K, *et al*. 2006 A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. *EMBO J.* **25** 3618–3626

Youle RJ and van der Bliek AM 2012 Mitochondrial fission, fusion, and stress. *Science* **337** 1062–1065

Yu T, Jhun BSS and Yoon Y 2010 High glucose stimulation increases reactive oxygen species production through the calcium and MAP kinase-mediated activation of mitochondrial fission. *Antioxid Redox Signal*. **14** 425–437

Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L *et al*. 2010 Regulation of cellular metabolism by protein lysine acetylation. *Science (New York, N.Y.)* **327** 1000–1004

Züchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E *et al*. 2004 Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* **36** 449–451

Verhoeven K, Claeys K, De Jonghe P, Merory J, Oliveira SA *et al*. 2005 Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat. Genet.* **37** 289–294

Zunino R, Schauss A, Rippstein P, Miguel A-N and McBride HD 2007 The {SUMO} protease {SENP5} is required to maintain mitochondrial morphology and function. *J. Cell Sci.* **120** 1178–1188

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