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SYMPOSIUM REVIEW

Interactions between mitochondria and the transcription factor myocyte enhancer factor 2 (MEF2) regulate neuronal structural and functional plasticity and metaplasticity

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Abstract The classical view of mitochondria as housekeeping organelles acting in the background to simply maintain cellular energy demands has been challenged by mounting evidence of their direct and active participation in synaptic plasticity in neurons. Time-lapse imaging has revealed that mitochondria are motile in dendrites, with their localization and fusion and fission events regulated by synaptic activity. The positioning of mitochondria directly influences function of nearby synapses through multiple pathways including control over local concentrations of ATP, Ca^{2+} and reactive oxygen species. Recent studies have also shown that mitochondrial protein cascades, classically associated with apoptosis, are involved in neural plasticity in healthy cells. These findings link mitochondria to the plasticity- and metaplasticity-associated activity-dependent transcription factor myocyte enhancer factor 2 (MEF2), further repositioning mitochondria as potential command centres for regulation of synaptic plasticity. Intriguingly, MEF2 and mitochondrial functions appear to be intricately intertwined, as MEF2 is a target of mitochondrial apoptotic caspases and, in turn, MEF2 regulates mitochondrial genome transcription essential for production of superoxidase and hydrogen peroxidase. Here, we review evidence supporting mitochondria as central organelles controlling the spatiotemporal expression of neuronal plasticity, and attempt to disentangle the MEF2–mitochondria relationship mediating these functions.

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Abbreviations Akt1, RAC-alpha serine/threonine-protein kinase; AMPAR, AMPA receptor; Apaf-1, apoptotic protease activating factor 1; Arc, activity-regulated cytoskeleton-associated protein; BAD, Bcl-2 associated death promoter; BAX, Bcl-2 associated X protein; Bcl-2, B cell lymphoma 2; Bcl-xl, B cell lymphoma extra-large; BDNF, brain-derived neurotrophic factor; CaMK, Ca^{2+}/calmodulin-dependent kinase; CDK5, cyclin-dependent kinase 5; CREB, cAMP response element-binding protein; Drp1, dynamin-1-like protein; ERK5, extracellular signal regulated kinase; FMRP, fragile X mental retardation protein; FOXO3a, forkhead box-O3a; GSK3β, glycogen synthase kinase-3β; HDAC, histone deacetylase; Jnk, *c-jun* N-terminal kinase; Lgi1, leucine-rich glioma-inactivated; LTD, long-term depression; LTP, long-term potentiation; MAPK, p38 mitogen-activated protein kinase; MEF2, myocyte enhancer factor 2; MHCI, major histocompatibility complex I; MIRO1, mitochondrial Rho GTPase 1; mtDNA, mitochondrial genome;

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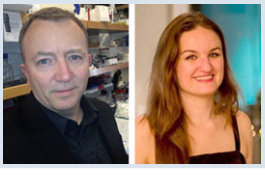

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mtHsp70, mitochondrial heat shock protein 70; ND6, NADH dehydrogenase 6; NMDAR, NMDA receptor; NRF2, nuclear factor (erythroid-derived 2); Pcdh10, protocadherin 10; PKD, protein kinase D; PP1, protein phosphatase 1; PSD-95, post-synaptic density-95; ROS, reactive oxygen species; SRF, serum response factor; SynGAP, synaptic Ras GTPase-activating protein; WAVE1, Wiskott–Aldrich syndrome protein-family verprolin homologous protein 1; XIAP, X-linked inhibitor of apoptosis protein.

Introduction

Mitochondria are traditionally known as organelles responsible for maintaining cellular energy demands. In addition to converting energy substrates into ATP, mitochondria also participate in reactive oxygen species (ROS) metabolism, Ca^{2+} signalling and apoptosis (Mattson *et al.* 2008). Such functions have been the primary focus of studies of mitochondria in neurons, which have particularly high energy and calcium homeostasis demands. In neurons, the vast majority of ATP produced by mitochondria is directed towards maintaining transmembrane Na^{+}/K^{+} ion gradients required for synaptic and intracellular ion-based activity, synaptic transmission involving presynaptic activity-driven neurotransmitter vesicular release and recycling, and postsynaptic receptor trafficking (Attwell & Laughlin, 2001; Rangaraju *et al.* 2014). Mitochondria are also critical for re-establishing Ca^{2+} homeostasis through sequestration following neural activity-associated intracellular Ca^{2+} transients (Pivovarova *et al.* 2002; Levy *et al.* 2003).

Hints that mitochondria play more than passive roles in neural function came from findings that their localization in dendritic arbors is activity-dependent (Li *et al.* 2004; Chang *et al.* 2006; MacAskill *et al.* 2009). The movement of mitochondria allows differential spatiotemporal energy supplies and Ca^{2+} homeostasis, which affects local synapse function and plasticity. Furthermore, synaptic transmission may trigger neighbouring mitochondria to locally activate the so-called 'mitochondrial apoptotic cascade'. While well studied for their role in programmed cell death, these apoptotic proteins have recently been found to be activated under normal physiological conditions. For example, glutamatergic synaptic transmission through NMDA receptors (NMDARs), at levels that induce plasticity but not excitotoxicity, evokes Ca^{2+} influx, which triggers mitochondrial activation of caspase-3 and its degradation of synaptic proteins and transcription factors. One of the caspase-3 targets is the transcription factor myocyte enhancer factor 2 (MEF2), recently implicated in neuronal morphological and functional plasticity and metaplasticity (Chen *et al.* 2012). MEF2 is particularly interesting as it has been found to regulate the transcription of nuclear genes involved in strengthening and weakening of synapses, as well as the mitochondrial gene *ND6* critical to cellular respiration and production of superoxidase and hydrogen peroxidase. Altogether, this emerging model places mitochondria and their inter-relationship with MEF2 as central regulators of neuronal plasticity.

Mitochondrial dynamics and neural activity

In the dendritic arbors of neurons, mitochondria are primarily localized to dendritic shafts in close proximity to the neck of filopodia and dendritic spines (Fig.1) (Cameron *et al.* 1991; Popov *et al.* 2005). However, mitochondrial localization and movement in dendrites varies with neuronal maturation and activity. Mitochondria exhibit a complex array of movement behaviours. They can be localized at synapses for brief or long periods, pause at synaptic sites as they travel unidirectionally along processes, or shuttle bidirectionally between multiple neighbouring synapses (Chang *et al.* 2006). In younger neurons, mitochondria undergo more frequent fission and fusion events, are more motile, are smaller and occupy fewer processes. As neurons mature and establish stable dendritic morphologies and synapses, mitochondria move less and elongate to simultaneously interact with multiple neighbouring synapses over longer periods (Chang & Reynold, 2006).

These mitochondrial dynamics are modulated by synaptic activity. Mitochondria are recruited to active synapses and increased neuronal activity is associated with reduced mitochondrial motility, while lower activity is correlated with increased movement (Li *et al.* 2004; Chang *et al.* 2006; MacAskill *et al.* 2009). Neuronal activity increases the percentage of mitochondria in dendritic filopodia (Fig. 1) and spines, and the ratio of fission to fusion events (Morris & Hollenbeck, 1995; Li *et al.* 2004; MacAskill *et al.* 2009). Fission events may function to reduce the size of mitochondria to allow their traffic into small diameter dendritic protrusions. Dendritic mitochondria can move to and pause at synapses before undergoing fission, leaving a fragment at the synapse while the remaining parent fragment moves elsewhere (Chang *et al.* 2006). These findings support a model in which the localization of a limited number of mitochondria shifts to best suit the energy and Ca^{2+} homeostasis demands across synapses in the dendritic arbor.

A number of activity-dependent mechanisms have been identified to mediate mitochondrial localization and motility in dendrites. Stimulation of NMDARs with exogenous or synaptically released glutamate leads to the stabilization of mitochondria at postsynaptic sites (MacAskill *et al.* 2009). Local Ca^{2+} influx through

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NMDARs or activation of neurotrophic input regulates mitochondrial motility via several GTPases, including the mitochondrial rho GTPase 1 (Miro1) (Guo *et al.* 2005), dynamin-1-like protein (Drp1), OPA1 and mitofusins (Alexander *et al.* 2000; Frank *et al.* 2001; Li *et al.* 2004). Specifically, Miro1 links mitochondria to KIF5 motor proteins, allowing mitochondria to move along microtubules, while intracellular synaptic Ca^{2+} transients inhibit the Miro1–KIF5 complex resulting in motility arrest (MacAskill *et al.* 2009). Mitochondrial movement into dendritic spines and filopodia is facilitated by actin binding to the mitochondrial outer membrane protein WAVE1 (Wiskott–Aldrich syndrome protein-family verprolin homologous protein 1; Sung *et al.* 2008).

Mitochondria in the regulation of neuroplasticity

Several recent studies have demonstrated the importance of mitochondria in influencing neuronal structural and functional plasticity, including the regulation of neuronal process outgrowth, dendritic remodelling and neurotransmission. To start, dendritic morphology and neurotransmission are sensitive to the concentration of mitochondria (Stowers *et al.* 2002; Levy *et al.* 2003; Li *et al.* 2004; Guo *et al.* 2005; Verstreken *et al.* 2005; Mattson *et al.* 2008). Reduction of dendritic mitochondrial content leads to loss of synapses and spines, while increasing mitochondrial number or their activity enhances the number and plasticity of synapses (Li *et al.* 2004). Efforts to identify underlying molecular mechanisms have

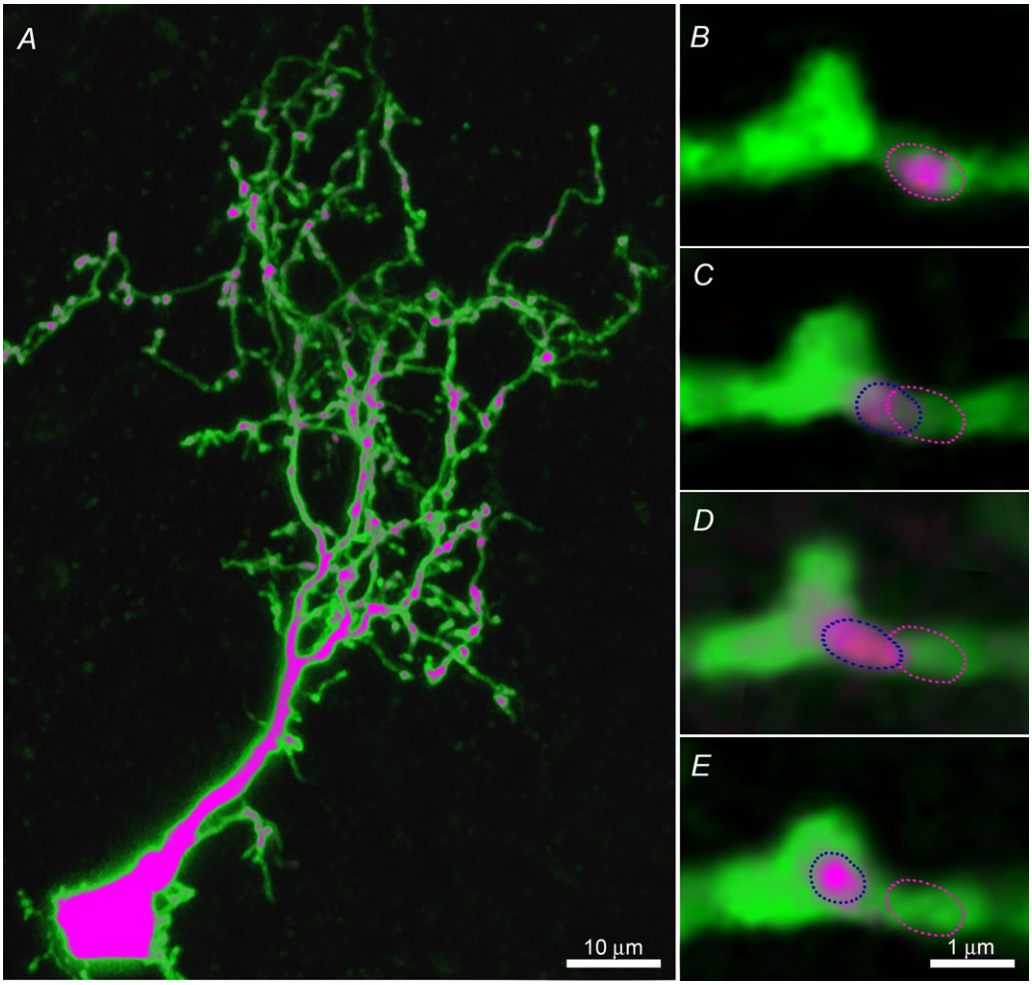

Figure 1. Imaging dendritic mitochondria in the awake developing brain
A, z-stack projection of 48 two-photon images through an entire *Xenopus laevis* tectal neuron expressing GFP as space filler (green) and the mitochondria-specific marker pmito-mOrange2 (overlap shown in magenta) imaged *in vivo* in the unanaesthetized tadpole. pMito labelling is apparent as discreet oblong objects throughout the dendritic arbor, and shows high expression in the soma and proximal dendrite due to summation of discrete objects on multiple image planes through this large volume. *B–E*, *in vivo* time-lapse imaging of dendritic mitochondria (magenta dashed lines show initial position, blue dashed lines indicate movement over time) demonstrates translocation towards filopodia in response to sensory-induced neural activity. Time between panels: 2.5 min.

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linked multiple mitochondrial pathways to the regulation of synaptic function and plasticity. These include mitochondrial respiration, regulation of subcellular Ca^{2+} concentration, redox homoeostasis, apoptotic protein cascades and mitochondrial gene expression.

Postsynaptic Ca^{2+} is a critical initiator of long-term potentiation and depression (LTP/LTD) of synaptic transmission, and its spatiotemporal concentration is tightly regulated by uptake and release from mitochondria and other intracellular stores. Ca^{2+} has a complex influence on mitochondrial function. Neuronal activity enhances mitochondrial Ca^{2+} uptake, which stimulates ATP production and increases mitochondrial NAD(P)H levels (Jonas, 2014). Ca^{2+} also influences mitochondrial function by promoting fragmentation of mitochondria triggered by calcineurin-mediated dephosphorylation of Drp1 (Cribbs & Strack, 2007; Cereghetti *et al.* 2008; Wang *et al.* 2011). Local mitochondrial ROS homeostasis also influences synaptic plasticity. Local release of superoxidase and hydrogen peroxide inactivates the LTD-associated serine/threonine protein phosphatases PP1 and calcineurin (or PP2B), which indirectly activates the LTP-associated Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and protein kinase D (PKD) (Fig. 2) (Hongpaisan *et al.* 2004). Thus, local mitochondrial events directly affect key molecular pathways of LTP and LTD.

The role of mitochondria in apoptosis of both neuronal and non-neuronal cells has been an area of extensive investigation, leading to the canonical association of mitochondrial proteins with programmed cell death. During apoptosis, mitochondrial outer membrane permeabilization is precisely controlled by the B-cell lymphoma 2 (Bcl-2) family of proteins. Bcl-2 proteins interact with the mitochondrial membrane to either increase its permeability (inducing apoptosis: BAX and BAD) or to stabilize the membrane and prevent apoptosis (Bcl-2 and Bcl-xL) (Labi *et al.* 2008). Mitochondrial outer membrane permeabilization results in the release of cytochrome *c*. In the cytosol, cytochrome *c* associates with Apaf-1 (apoptotic protease activating factor 1) to form the apoptosome, a multimeric protein complex that recruits and activates the initiator caspase-9. Active caspase-9 next cleaves and activates the executioner caspases-3 and -7. Ultimately, the executioner caspases cleave multiple protein substrates resulting in cell death (Pop & Salvesen, 2009). Critical caspase-3 substrates include the transcription factors MEF2, serum response factor (SRF), cAMP response element-binding protein (CREB), forkhead box-O3a (FOXO3a) and nuclear factor (erythroid-derived 2) (NRF2), the cleavage of which causes loss of their transcriptional activity and pro-survival functions (Ohtsubo *et al.* 1999; Bertolotto *et al.* 2000; François *et al.* 2000; Okamoto *et al.* 2002; Charvet *et al.* 2003).

Recently, mitochondrial apoptotic proteins have been implicated in non-apoptotic roles in healthy cells in a number of neural systems. Caspases-3 and -9, Bcl-xL, and BAX have been implicated in neuronal proliferation and differentiation (Weber & Menko, 2005; Chang *et al.* 2007; Li *et al.* 2010; Ohsawa *et al.* 2010), neurite outgrowth in hippocampal culture (Westphal *et al.* 2010), regulation of developmental pruning of dendrites in Drosophila (Kuo *et al.* 2006; Williams *et al.* 2006), chemotropic guidance of axonal growth cones of retinal ganglion cells in *Xenopus* (Campbell & Holt, 2003), habituation to repetitive bird songs in zebra finches (Huesmann & Clayton, 2006), and LTD in the rodent hippocampus (Li *et al.* 2010).

Mounting evidence finds multiple links between mitochondrial apoptotic pathways and synaptic plasticity. During NMDAR-mediated LTD, Ca^{2+} influx through NMDARs leads to the activation of the phosphatases PP1 and calcineurin (Malenka & Bear, 2004). PP1 and calcineurin dephosphorylate BAD, inducing its translocation to the mitochondrial outer membrane, where activation of BAX by BAD causes the release of cytochrome *c* to the cytosol (Fig. 2) (Wang *et al.* 1999; Jiao & Li, 2011). These events appear to be required for NMDAR-dependent LTD, since BAD, BAX, and caspase-3 knock-out mice all present LTD deficits, and LTD is blocked by pharmacological inhibition of caspases-3 and -9 (Li *et al.* 2010; Li & Sheng, 2012). Furthermore, BAD and BAX activation is necessary and sufficient to activate caspase-3 and induce LTD in hippocampal neurons (Jiao & Li, 2011). In contrast, LTP is unaffected by caspase-3 pharmacological inhibition (Li *et al.* 2010; Jiao & Li, 2011; Olsen & Sheng, 2012).

How the same proteins responsible for neuronal programmed cell death also mediate synaptic plasticity is particularly intriguing. The spatiotemporal extent of mitochondrial activation probably determines whether local homeostasis and plasticity, or apoptosis occurs. During apoptosis, high global levels of active caspase-3 cleave and degrade proteins essential for cell survival. In contrast, during LTD, localized and low caspase-3 activation affects synaptic AMPA receptor (AMPAR) anchoring and trafficking by cleaving the AMPAR subunits GluR1 (GluA1) (Lu *et al.* 2002) and GluR4 (GluA4) (Chan *et al.* 1999; Glazner *et al.* 2000), the AMPAR-associated proteins post-synaptic density-95 (PSD-95) (Liu *et al.* 2010), CaMKII, calcineurin A, and the activity-regulated cytoskeleton-associated protein (Arc) (Fig. 2) (Lu *et al.* 2002; D'Amelio *et al.* 2011; Jo *et al.* 2011; Snigdha *et al.* 2012). LTD induction also requires caspase-3-mediated proteolysis of RAC-alpha serine/threonine-protein kinase (Akt1) (Li *et al.* 2010). Active Akt1 suppresses LTD by phosphorylating and inhibiting the glycogen synthase kinase-3β (GSK3β), which also regulates BAX translocation to the mitochondria (Peineau *et al.* 2007; Li *et al.* 2010). Although the same phosphatases responsible

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for BAD activation are implicated in both LTD and apoptosis, it is likely that distinct outcomes occur by varying its degree of activation. The extent to which Bcl-2 proteins change mitochondrial membrane permeability may control the direction of change in synaptic strength produced or, if more extreme, can trigger apoptosis. Neuronal treatment with different NMDA concentrations causes differential caspase-3 activation, indicating that Ca^{2+} levels appear to be one of the critical upstream triggers determining the extent of activation of the BAD–BAX–caspase-3 cascade (Jiao & Li, 2011). In this model, lower Ca^{2+} concentration localized to synaptic sites leads to lower levels of calcineurin activation, resulting in weak and brief activation of BAD. The spatiotemporal profile of caspase-3 activation is probably controlled by mitochondrial position relative to active synaptic sites, and is tightly regulated by mechanisms that provide its rapid removal. One such regulator is the X-linked inhibitor of apoptosis protein (XIAP), which targets caspase-3 removal through its ubiquitination and degradation by the proteasome (Suzuki *et al.* 2001; Ertürk *et al.* 2014). Further, morphological rearrangements occurring concurrently with functional plasticity may be a consequence of caspase cleavage of cytoskeleton-associated proteins, including Gas2, gelsolin, ROCK1, β-catenin, fodrin, actin, PAK-2, spectrin and tau (Brancolini *et al.* 1995; Cryns *et al.* 1996; Kothakota *et al.* 1997; Rudel & Bokoch, 1997; Wang *et al.* 1999; Gamblin *et al.* 2003; Williams *et al.* 2006).

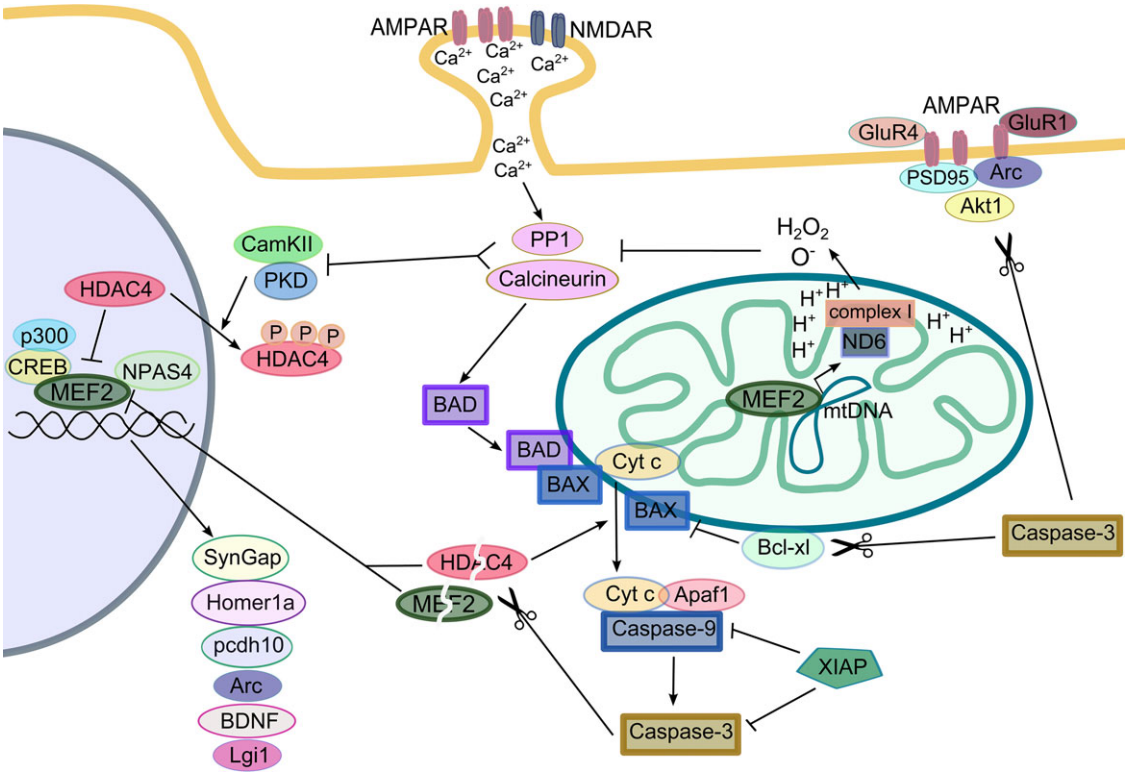

Figure 2. Interactions between mitochondria and MEF2 regulate neuronal structural and functional plasticity
Postsynaptic Ca^{2+} activates the protein phosphatases PP1 and calcineurin, which dephosphorylate and induce translocation of BAD to the mitochondrial membrane to activate BAX. The BAD–BAX complex increases mitochondrial outer membrane permeability, resulting in the release of cytochrome *c* into the cytosol. In association with Apaf-1, cytochrome *c* forms the apoptosome and activates the initiator caspase-9 that activates caspase-3. Both caspases-3 and -9 can be inhibited by XIAPs. Active caspase-3 cleaves and degrades AMPAR subunits GluR1 and GluR4, PSD95, Arc and Akt1, reducing synaptic strength. To regulate gene expression, caspase-3 cleaves HDAC4 and MEF2, degradation products of which can inhibit MEF2-dependent transcription. HDAC4 cleavage products can also increase mitochondrial outer-membrane permeability and promote cytochrome *c* release. Among other synaptic proteins, MEF2 promotes the transcription of Arc, Homer1a, pcdh10 and SynGap to promote synaptic weakening, and of BNDF and Lgi1 to promote synaptic strength. In the mitochondrion, MEF2 promotes the expression of the gene *ND6*. *ND6* is essential for the formation and function of complex I of the mitochondrial electron transport chain, regulating mitochondrial production of ROS. Mitochondrial release of superoxidase and hydrogen peroxide inactivates the protein phosphatases PP1 and calcineurin. Phosphatase inhibition leads to the indirect activation of CaMKII and PKD, which phosphorylates HDAC4 and promotes its shuttling to the cytoplasm. Furthermore, PP1 and calcineurin inhibition by ROS enables MEF2-dependent transcription.

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MEF2, mitochondria and plasticity

The transcription factor MEF2 was first identified as a regulator of muscle cell differentiation and development (Olson *et al.* 1995; Shalizi & Bonni, 2005; Potthoff & Olson 2007). This family of transcription factors (MEF2 A–D) is characterized by a highly conserved MADS box (MCM1, agamous, deficiens and serum response factor) domain at the N terminus, and an isoform-specific C-terminal transcriptional activation domain (Martin *et al.* 1994). Immediately adjacent to the MADS box is the MEF2 domain, which mediates DNA binding, dimerization and cofactor interactions (Black & Olson 1998; McKinsey *et al.* 2002).

Recent studies have found that MEF2 function is sensitive to plasticity-inducing stimuli (Shalizi *et al.* 2006; Fiore *et al.* 2009; Pfeiffer *et al.* 2010; Cole *et al.* 2012; Rashid *et al.* 2014). MEF2 is highly expressed throughout the developing brain during periods of heightened structural and functional plasticity underlying neural circuit formation. In both immature and mature neurons, MEF2 regulates functional plasticity and activity-dependent dendritic morphogenesis (Shalizi *et al.* 2006; Fiore *et al.* 2009; Pfeiffer *et al.* 2010). In adult mice, fear conditioning and spatial memory tasks decrease MEF2 levels and increase its phosphorylation, both of which inhibit MEF2-mediated transcription. Further, increasing MEF2 function prevents the formation of spatial memory and associated increase in spine density. Together, these findings suggest that MEF2-mediated transcription constrains memory formation by interfering with structural plasticity (Cole *et al.* 2012; Rashid *et al.* 2014).

An interesting complexity of the role of MEF2 in regulating neural plasticity has recently been discovered. Studies using direct *in vivo* imaging of structural and functional plasticity during brain circuit formation within awake developing *Xenopus laevis* tadpoles revealed that MEF2 does not directly induce synaptic plasticity, but functions as a master regulator of plasticity thresholds (Chen *et al.* 2012). Rather than directly inducing changes to synaptic strength or dendritic morphology itself, MEF2 levels determine how growing neurons respond to plasticity-inducing stimuli. Such regulation of plasticity thresholds is termed 'metaplasticity'. Metaplasticity, or the 'plasticity of plasticity', is triggered by periods of high or low neural activity and is essential for maintaining synaptic strength and neuronal firing within a functional dynamic range. In the developing tadpole visual system, it was found that unpatterned white noise visual stimulation, which drives strong neural firing, yet without the statistical repetition at individual synapses required for plasticity induction, does not induce lasting structural or functional changes, but shifts subsequent responses to plasticity-inducing visual input. Intriguingly, this metaplasticity-inducing unpatterned stimulus elicited the rapid loss of MEF2 in visual brain neurons, and knocking down MEF2 by itself induces a metaplastic shift in plasticity thresholds (Chen *et al.* 2012). This MEF2 loss is dependent on NMDAR-mediated synaptic transmission and the activation of caspases-9 and -3, thereby linking MEF2 regulation of metaplasticity to mitochondrial protein pathways.

The regulation of MEF2-dependent transcription is complex. Numerous cofactors influence MEF2 function, including GATA, GRIP1, histone deacetylases (HDACs), MyoD, NFAT and p300 (Sartorelli *et al.* 1997; Miska *et al.* 1999; Blaeser *et al.* 2000; Morin *et al.* 2000; Lazaro *et al.* 2002). Additionally, MEF2 transcriptional activity is intricately regulated through its phosphorylation at multiple sites. In general, hypo-phosphorylated MEF2 binds DNA with higher affinity and exhibits more efficient transcriptional activation than its hyper-phosphorylated form. However, phosphorylation of the MEF2 transcription activation domain by p38 mitogen-activated protein kinase (MAPK) increases expression of MEF2 targets (Mao, 1999; Zhao *et al.* 1999). Membrane depolarization, Ca^{2+} influx and activation of the major histocompatibility complex I (MHCI) lead to calcineurin-mediated dephosphorylation of multiple serine residues on MEF2 (Mao, 1999; Flavell *et al.* 2006, Shalizi *et al.* 2006; Elmer & McAllister, 2013). Cyclin-dependent kinase 5 (CDK5) and the extracellular signal regulated kinase 5 (ERK5) can phosphorylate MEF2 and also regulate MEF2 transcriptional activity (Kato *et al.* 2000; Gong *et al.* 2003).

Links between MEF2 and mitochondrial apoptotic pathways were first apparent when it was discovered that caspase-3 degrades MEF2 (Okamoto *et al.* 2002; Chen *et al.* 2012). Caspase-3 cleaves MEF2 in the trans-activation domain, and the cleavage product blocks remaining transcriptional activity of uncleaved MEF2 via dominant interference (Fig. 2) (Okamoto *et al.* 2002). Caspases selectively target phosphorylated MEF2 (Li *et al.* 2001), with digestion blocked by calcineurin dephosphorylation (McKinsey *et al.* 2002). Caspase-3 also regulates MEF2 function by cleaving its cofactor HDAC4, which produces a carboxy-terminal fragment localized to the cytoplasm, and an amino-terminal fragment that accumulates in the nucleus and acts as a strong repressor of MEF2 transcription. Also, both caspase-generated fragments of HDAC4 are able to trigger cytochrome *c* release from mitochondria (Paroni *et al.* 2004). Upon membrane depolarization and Ca^{2+} influx, HDACs are phosphorylated by PKD and CaMKII and exported from the nucleus, relieving MEF2 repression and allowing activation of MEF2-dependent transcription (Fig.2) (McKinsey *et al.* 2002; Chawla *et al.* 2003).

There are over 180 known neuronal activity-regulated MEF2 target genes (Flavell *et al.* 2008). Many of these

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genes encode synaptic proteins with diverse and at times opposing functions (Flavell *et al.* 2006; Pfeiffer *et al.* 2010; Yamada *et al.* 2013). Some MEF2 target genes contribute to the weakening or loss of excitatory synapses, including Homer1a, which is involved in the disassembly of post-synaptic scaffolding complexes (Sala *et al.* 2003), the synaptic Ras GTPase-activating protein (SynGAP), Arc and c-*jun* N-terminal kinase (Jnk), which all promote AMPAR removal from the postsynaptic membrane (Zhu *et al.* 2005; Chowdhury *et al.* 2006). MEF2 and the fragile X mental retardation protein (FMRP) cooperatively regulate the expression of protocadherin 10 (Pcdh10), which is required for MEF2-induced synapse elimination, and functions to deliver ubiquitinated PSD-95 to the proteasome (Tsai *et al.* 2012). Reductions in PSD-95 can lead to reduced synaptic strength through the diffusion and endocytosis of AMPARs (Colledge *et al.* 2003; Haas *et al.* 2007; Keith & El-Husseini, 2008; Woods *et al.* 2011; Opazo *et al.* 2012). While these MEF2-target genes promote synaptic elimination, other MEF2 targets promote synaptic strengthening. The leucine-rich glioma-inactivated 1 protein (Lgi1) promotes AMPAR insertion (Fukata *et al.* 2006), adenylyl cyclase 8 mediates Ca^{2+}-dependent cAMP production necessary for Ca^{2+}-dependent synaptic strengthening (Wang et al., 2003) and the brain-derived neurotrophic factor (BDNF) enhances excitatory synaptic strength (Zhang & Poo, 2002). One possible explanation for such antagonistic function is that MEF2 may orchestrate the simultaneous disassembly and strengthening of distinct populations of synapses. Such synapse elimination and potentiation occur concurrently within individual cells during neuronal development, and are critical for refinement of neuronal connections underlying improvement of receptive field encoding (Chen & Regehr, 2000).

A further connection between MEF2 and mitochondria comes from the finding that MEF2 not only regulates transcription of nuclear DNA, but also directly affects mitochondrial function as a transcription factor of the mitochondrial genome (mtDNA) (Naya *et al.* 2002; She *et al.* 2011). Translocation of MEF2 into mitochondria is regulated by the chaperone protein mitochondrial heat shock protein 70 (mtHsp70). In mitochondria, MEF2 activates the transcription of a single mtDNA gene, the NADH dehydrogenase 6 (*ND6*) (Fig. 2). The MEF2-dependent transcriptional regulation of *ND6* is crucial for function of complex I of the mitochondrial electron transport chain (Bai & Attardi, 1998; She *et al.* 2011). Blocking MEF2 function in mitochondria decreases complex I activity, increases cellular hydrogen peroxidase levels and reduces ATP production (She *et al.* 2011).

A complex model is emerging of an intimate and dynamic relationship between mitochondrial function and neuronal plasticity. Mitochondrial effects on synaptic strength can be mediated through traditional homeostatic regulation of ATP, Ca^{2+} and ROS, which are influenced by activity-dependent regulation of mitochondrial motility, localization and fusion/fission events. Yet it is now clear that a host of mitochondrial pathways, best known for involvement in apoptotic cell death, play physiological roles in healthy neurons to mediate plasticity and metaplasticity, and that these pathways are linked to the transcription factor MEF2. NMDAR-mediated Ca^{2+} influx triggers mitochondrial release of cytochrome *c* leading to caspase-3 activation, which inhibits MEF2 by direct degradation, and through enhancing HDAC4 inhibition of MEF2-mediated transcription. Synaptic activity also modulates MEF2 function and its sensitivity to caspase degradation through complex phosphorylation regulation. As a transcription factor regulating both nuclear genes encoding proteins regulating synaptic strength, and a mitochondrial gene critical to cellular respiration and production of superoxidase and hydrogen peroxidase, MEF2 is in a key position to influence global and local neuronal plasticity. Future experiments are required to elaborate this model. More refined measures of the spatiotemporal dynamics of mitochondrial proteins, ATP, Ca^{2+}, and ROS, MEF2 activation states and their correlation to local functional and structural plasticity are required to decipher precise pathways regulating plasticity and metaplasticity.

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Additional information

Competing interests

None declared.

Author contributions

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