EMBO reports

review

Mitochondrial shape changes: orchestrating cell pathophysiology

Silvia Campello[1] *& Luca Scorrano*[1,2+]

[1]University of Geneva, Genève, Switzerland, and [2]Venetian Institute of Molecular Medicine, Padova, Italy

Mitochondria are highly dynamic organelles, the location, size and distribution of which are controlled by a family of proteins that modulate mitochondrial fusion and fission. Recent evidence indicates that mitochondrial morphology is crucial for cell physiology, as changes in mitochondrial shape have been linked to neurodegeneration, calcium signalling, lifespan and cell death. Because immune cells contain few mitochondria, these organelles have been considered to have only a marginal role in this physiological context—which is conversely well characterized from the point of view of signalling. Nevertheless, accumulating evidence shows that mitochondrial dynamics have an impact on the migration and activation of immune cells and on the innate immune response. Here, we discuss the roles of mitochondrial dynamics in cell pathophysiology and consider how studying dynamics in the context of the immune system could increase our knowledge about the role of dynamics in key signalling cascades.

Keywords: mitochondria; fusion/fission; T cell; migration; adaptive/innate immunity

EMBO *reports* (2010) **11,** 678–684. doi:10.1038/embor.2010.115

See Glossary for abbreviations used in this article.

Introduction

Mitochondria are dynamic and versatile organelles that produce most of the cellular ATP and are essential components of several signalling pathways crucial for the life and death of the cell (Ernster & Schatz, 1981; Rizzuto *et al*, 2000; Green & Kroemer, 2004). Their functional versatility is paralleled by their morphological complexity (Frey & Mannella, 2000), which is heterogeneous in living cells as a result of the balance between fusion and fission of mitochondrial membranes (Bereiter-Hahn & Voth, 1994). Mitochondrial morphology is controlled by a set of 'mitochondria-shaping' proteins that either share structural homology with the large GTPases—dynamins (Okamoto & Shaw, 2005)—or are members of a cohort of 'non-conventional' proteins, the molecular function of which is less characterized. Growing evidence indicates that morphology and the +regulation of mitochondrial shape are crucial for cellular physiology (Cereghetti & Scorrano, 2006) and new mitochondria-shaping proteins continue to be discovered. However, the field is young and many questions remain open (Sidebar A). In this review, we provide an overview of mitochondrial fusion and fission in mammals and consider recent findings on the role that morphological changes of mitochondria have in the immune system—a research area that shows great promise for understanding the multifaceted role of mitochondrial shape in cell signalling.

The fusion and fission machineries

The two dynamin-related GTPases MFN1 and MFN2 are the principal regulators of mitochondrial outer membrane fusion in mammals (Santel & Fuller, 2001). Although the two are highly homologous to one another, they have different roles in cell physiology. MFN2 is the more versatile and participates in cell metabolism—tethering the ER to mitochondria—and cell proliferation (Fig 1; de Brito & Scorrano, 2008a). Moreover, mutations in the *MFN2* gene are associated with the peripheral neuropathy Charcot–Marie–Tooth disease type 2A (Zuchner *et al*, 2004).

Another dynamin-like GTPase, OPA1, is a key pro-fusion player (Fig 1; Cipolat *et al*, 2004) that is anchored to the mitochondrial inner membrane and is mutated in the disease ADOA (Alexander *et al*, 2000), which results in the loss of some or most of the fibres of the optic nerve. Both OPA1 and the activities of other mitochondria-shaping proteins are tightly regulated by complex post-transcriptional mechanisms that include proteolytic processing (Ehses *et al*, 2009; Liesa *et al*, 2009). Interestingly, OPA1 has other functions independent of its fusogenic activity: heterocomplexes of different OPA1 forms regulate apoptosis by controlling the structure of cristae and the release of cytochrome *c* (Germain *et al*, 2005; Cipolat *et al*, 2006; Frezza *et al*, 2006; Yamaguchi *et al*, 2008). The list of proteins involved in the control of mitochondrial fusion recently grew to include LETM1 (Dimmer *et al*, 2008); the phospholipase PLD, which is associated with the outer membrane (Choi *et al*, 2006); and prohibitins that act as scaffolds for OPA1 processing or assembly (Merkwirth *et al*, 2008).

Cytosolic DRP1 (Smirnova *et al*, 2001) and outer-membrane-associated FIS1 (Fig 1; James *et al*, 2003) have important roles in regulating mitochondrial division in mammalian cells. DRP1 needs to be activated and recruited to mitochondria to induce mitochondrial fission (Yoon *et al*, 2003) and its activity is highly regulated by post-translational modifications (Fig 1; Harder *et al*, 2004; Yonashiro *et al*,

[1]Department of Cell Physiology and Metabolism, University of Geneva, 1 Rue M. Servet, 1206 Genève, Switzerland
[2]Dulbecco–Telethon Institute, Venetian Institute of Molecular Medicine, Via Orus 2, 35129 Padova, Italy
[+]Corresponding author. Tel: +41 (0)22 3795235; Fax: +41 (0)22 3795338;
E-mail: luca.scorrano@unige.ch

Received 26 March 2010; accepted 15 July 2010, published online 20 August 2010

Sidebar A | In need of answers

(i) Which is the exact mechanism of fusion of both the outer and inner mitochondria membranes?
(ii) What happens to the inner membrane during fusion?
(iii) Which proteases are responsible for cleavage and processing of OPA1?
(iv) Is FIS1 the only partner of DRP1 in the fission machinery? How does recruited DRP1 induce fragmentation of mitochondria?
(v) Are mitochondria-shaping proteins crucial in the regulation/amplification of programmed cell death? Do they influence a physiological form of cell death?
(vi) How do mitochondria fragment during apoptosis? Do mitochondria-shaping proteins interact directly with members of the BCL2 family?
(vii) Do mitochondrial dynamics have a crucial role in key T-cell processes other than chemotaxis?

2006; Han *et al*, 2008). There is some evidence that FIS1 is the receptor on the outer membrane for DRP1 (Yoon *et al*, 2003), although evidence of its absolute requirement remains controversial. In addition to these two players, other molecules that were later identified as fission regulators are endophilin B1 (Karbowski *et al*, 2004), MTP18 (Tondera *et al*, 2005), MIB (Eura *et al*, 2006) and GDAP1, which is an outer membrane protein mutated in Charcot–Marie–Tooth disease type 4A (Pedrola *et al*, 2005).

Physiological roles for mitochondrial morphology

In recent years we have learnt that changes in mitochondrial shape influence crucial cellular functions, from Ca^{2+} signalling (Szabadkai *et al*, 2004) to the generation of reactive oxygen species (Yu *et al*, 2006), and from neuronal plasticity (Li *et al*, 2004) to muscle atrophy (Romanello *et al*, 2010) and even lifespan (Scheckhuber *et al*, 2007). Why is mitochondrial morphology and movement so important for cellular physiology? We doubt that there is a unique determinant for this; rather, we think a few key aspects should be taken into account: spatial compartmentalization achieved by specific mitochondrial localization, the effects of shape changes on mitochondrial function, and the downstream effects of mitochondrial shape on cellular viability.

The cytosolic localization of mitochondria is not random: these organelles accumulate where high amounts of ATP are required, or where Ca^{2+} signalling needs to be regulated. In this respect, one can regard mitochondria as movable tuners of signalling events that the cell can deploy where they are most needed. Not surprisingly, mitochondrial movement is highly coordinated with changes in organelle shape in order to produce mitochondria whose size is compatible with their movement (de Vos *et al*, 2005). For example, the expression of pro-fusion shaping proteins decreases mitochondrial movement along axons and dendrites and consequently reduces the number of dendritic spines and synapses (Li *et al*, 2004), the formation or maintenance of which require local levels of high mitochondrial ATP production. In fact, mitochondria cluster at many sites of high ATP demand in different cell types. As such, a possible direct, functional interaction between mitochondria and ATP-consuming cellular structures has been suggested in *Drosophila* neuromuscular junctions: synaptic mitochondria are required to fuel the myosin ATPase that mobilizes a reserve pool of vesicles (Verstreken *et al*, 2005).

Mitochondria-shaping proteins could alternatively influence morphogenesis by having an impact on organellar function. In the 1960s, Hackebrock described the classical transition of mitochondria

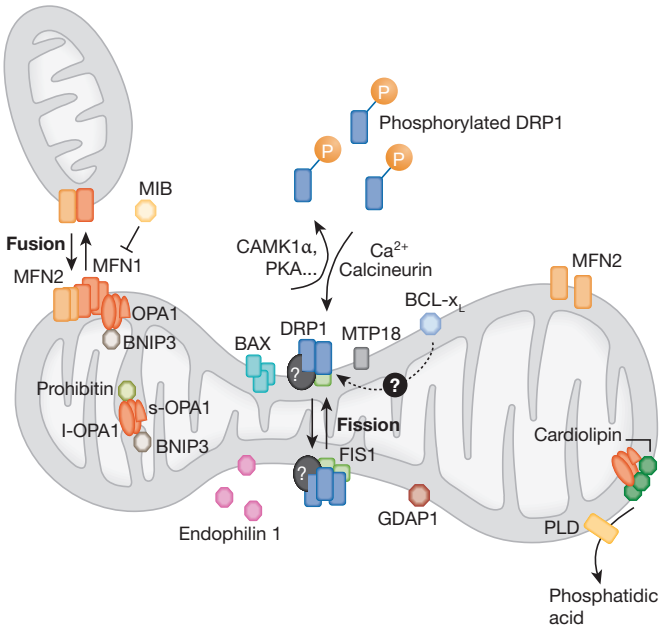

Fig 1 | The dynamics of mitochondrial fission and fusion. The localization, as well as some interaction and modification of the principal proteins involved in the two processes are shown. Once dephosphorylated, DRP1 is recruited to the outer membrane by FIS1 or by another, unknown, component. The oligomerization of DRP1 is followed by constriction of the membrane and mitochondrial fission. The pro-fusion proteins (MFNs on the outer membrane and OPA1 on the inner membrane) oligomerize to induce fusion of the membranes. Other additional components of the machinery are shown. BAX, BCL2-associated X protein; BNIP3, BCL2/E1B 19 kDa-interacting protein 3; CAMK1a, calcium/calmodulin-dependent protein kinase 1a; DRP1, dynamin-related protein 1; FIS1, fission protein 1; GDAP1, ganglioside-induced differentiation-associated protein 1; l-OPA1, long form of OPA1; MFN, mitofusin; MIB, mitofusin-binding protein; MTP18, mitochondrial protein 18 kDa; OPA1, optic atrophy 1; PKA, protein kinase A; PLD, phospholipase D; s-OPA1: short form of OPA1.

from the orthodox morphology of quiescent "state 4" to the condensed one of respiring "state 3" (Hackenbrock, 1966, 1972), demonstrating a relationship between mitochondrial structure and function. However, these studies were performed with isolated organelles and in non-physiological, sugar-based incubation media. Recently, an association between different phosphorylation abilities and mitochondrial morphology has been unveiled; however, an unequivocal relationship between P/O ratios (the number of ATP molecules produced per oxygen molecule oxidized) and mitochondrial morphology is lacking, raising the question of whether the two are linked (Benard *et al*, 2007; Benard & Rossignol, 2008; Rossignol & Karbowski, 2009).

Mitochondria-shaping proteins have been reported to have a crucial role in apoptosis. There is intense debate about whether and how they modulate the complete release of cytochrome *c* and other pro-apoptotic cofactors (Wasilewski & Scorrano, 2009). At an early stage during apoptosis, mitochondria undergo marked structural changes, including fragmentation and so-called cristae remodelling that ultimately mobilizes the cristae pool of cytochrome *c* (Frank *et al*, 2001; Scorrano *et al*, 2002). The mechanism of the massive and reversible fragmentation is still unclear, but DRP1 is involved (Martinou *et al*,

Glossary

ADOA	autosomal dominant optic atrophy
AP1	activator protein 1
APC	antigen-presenting cell
BAX	BCL2-associated X protein
BAK	BCL2-antagonist/killer
BCL2	B-cell lymphoma protein 2
BH3	Bcl-2 homology domain 3
BNIP3	Bcl2/E1B 19 kDa-interacting protein 3
CXCL12	CXC (chemokine) ligand 12
CRAC	Ca^{2+} release-activated Ca^{2+} channel
DRP1	dynamin-related protein 1
ER	endoplasmic reticulum
FIS1	fission protein 1
FOXO3	forkhead box O3
GDAP1	ganglioside-induced differentiation-associated protein 1
IKK	IκB kinase
LETM1	leucine zipper-EF-hand-containing transmembrane protein 1
MAVS	mitochondrial antiviral signalling
MFN1/2	mitofusin 1/2
MHC	major histocompatibility complex
MIB	mitofusin-binding protein
MTP18	mitochondrial protein 18 kDa
MyD88	myeloid differentiation primary response gene 88
NIX	Nip3-like protein X
NFAT	nuclear factor of activated T cells
OPA1	optic atrophy 1
ORAI1	calcium-release-activated calcium modulator 1
PARL	presenilin-associated rhimboid-like protease
PLD	phospholipase D
RLH	retinoic acid-inducible gene I-like helicase
SRC	abbreviation of Sarcoma
STIM1	stromal interacting molecule 1
STING	stimulator of interferon genes
TBK1	TANK-binding kinase 1
TCR	T-cell receptor
TLR	Toll-like receptor
vMIA	viral mitochondria-localized inhibitor of apoptosis
ZAP70	zeta-chain-associated protein kinase 70

1999; Frank *et al*, 2001). When the proapoptotic BCL2 family member BAX translocates to mitochondria, it co-localizes with DRP1 and MFN2 on the induction of stress-dependent apoptosis (Karbowski *et al*, 2002). Thus, BAX might be targeting subdomains of mitochondria that are most prone to dynamic membrane rearrangements. Similarly, the consequences of fragmentation are unclear: most laboratories have reported a role for fragmentation in the progression of apoptosis (Lee *et al*, 2004) and in the survival of cells in clonogenic assays (Cereghetti *et al*, 2010), but others have shown that fragmentation has no role in cell death (Autret & Martin, 2009).

Cristae remodelling is a process by which cristae junctions are widened, leading to the mobilization of the cytochrome *c* pool from the cristae to the inter-membrane space (IMS) for its complete release (Scorrano *et al*, 2002). A complex comprising a longer membrane-bound fraction and a shorter soluble IMS fraction of OPA1 generated by the rhomboid protease Parl controls the cristae junction size (Frezza *et al*, 2006; Cipolat *et al*, 2006). During apoptosis this complex is disrupted—even before the activation of multidomain pro-apoptotic BCL2 proteins (Yamaguchi *et al*, 2008)—leading to complete cytochrome *c* release. Changes in cristae structure have been reported to be caspase-dependent (Sun *et al*, 2007) or very subtle (Yamaguchi *et al*, 2008). It is beyond the scope of this review to address the detailed experimental differences leading to these results. Here, it suffices to mention that (i) the disassembly of the OPA1 complex has been found in all the apoptotic paradigms in which it has been tested; (ii) cristae remodelling was originally identified (and is still studied) by using an *in vitro* system in which caspases are absent, ruling out a role for any post-mitochondrial pathway; and (iii) differences in the experimental conditions and in the way that measurements in electron tomograms are performed could blunt changes in the cristae junction. Mounting evidence substantiates a model in which changes in mitochondrial ultrastructure occur during apoptosis, are regulated by the OPA1 complex, and are required for complete cytochrome *c* release. Whether or not the OPA1 complex is a direct target for pro-apoptotic members of the BCL2 family remains unclear. The BH3-only member of the BCL2 family, BNIP3, was recently found to interact with OPA1, inhibiting its fusogenic function and inducing OPA1 de-oligomerization in a BAX/BAK and BH3-domain-dependent manner (Landes *et al*, 2010). Interestingly, BNIP3 is positioned at the crossroad between apoptosis and autophagy and is believed to participate in the selective autophagy of mitochondria, called mitophagy.

Autophagy is a form of recycling that reprocesses cytosolic constituents, from bulk cytoplasm to entire organelles (Glick *et al*, 2010). The importance of this mechanism is emerging in the context of crucial physiological as well as pathological processes (Cecconi & Levine, 2008). Hypoxia-induced autophagy depends on both BNIP3 and NIX (Bellot *et al*, 2009; Zhang & Ney, 2009; Novak *et al*, 2010) and the fragmentation of mitochondria occurs before being engulfed by autophagosomes (Twig *et al*, 2008). The disruption of the mitochondrial network similarly constitutes an amplificatory loop during autophagy in muscular atrophy (Romanello *et al*, 2010). During atrophy, conserved atrophic factors, such as FOXO3, trigger BNIP3-dependent mitochondrial fragmentation, which causes mitochondrial dysfunction and is required for organelle removal. Interestingly, mitochondrial fission associated with dysfunction is able to induce muscle atrophy, elucidating a feed-forward loop that stems from mitochondria to activate the conserved autophagic pathways (Romanello *et al*, 2010).

Mitochondrial dynamics in the immune system

As we have outlined above, mitochondrial dynamics have a role in the regulation of several key cellular processes. One of the emerging areas in which mitochondria and mitochondrial dynamics are increasingly recognized as crucial is the immune system. The immune response can be broadly divided into innate and adaptive immunity: the first is thought to constitute an evolutionarily older defence strategy and provides immediate and nonspecific defence against infection (by recognizing components that are conserved among broad groups of microorganisms), whereas the second is composed of highly specialized, systemic cells that provide the vertebrate immune system with the ability to recognize and remember specific pathogens, and to mount stronger attacks each time the pathogen is encountered. The signalling pathways involved are fairly well characterized in both innate and adaptive immunity, making these systems useful to elucidate whether mitochondrial shape has any role.

Mitochondrial dynamics in innate immunity

When an organism senses a viral infection, it directly activates immune cells through a complex signalling pathway. The recognition of viral material is performed mainly by two groups of receptor:

the TLRs and the RLH family. These specific receptors interact with cytoplasmic adaptor molecules such as MyD88 (Loiarro *et al*, 2009) and MAVS (Seth *et al*, 2005), which in turn activate two cytosolic protein kinase complexes, TBK1 and IKK, leading to the production of type I interferons and pro-inflammatory cytokines (Moore & Ting, 2008). Mitochondria were thought to have a role when the specific mitochondrial localization of MAVS was discovered (Seth *et al*, 2005). More recently, mitochondrial morphology has been found to have a crucial role in modulating downstream signalling of MAVS (Castanier *et al*, 2010). After specific RLH activation, mitochondria elongate and enhance antiviral signalling. Accordingly, MAVS and the pro-mitochondrial fusion protein MFN1 interact under normal conditions. Mechanistically, this could be explained by the fact that mitochondrial elongation regulates the association of MAVS with STING, an ER molecule crucial for the antiviral cell response (Ishikawa & Barber, 2008). The activation of RLH induces the degradation of one MAVS isoform with the consequent disruption of a MAVS–MFN1–STING complex located at sites of ER–mitochondrial tethering. This frees MFN1 to orchestrate mitochondrial fusion. Fusion is required to enhance the STING–MAVS—that is, the remaining isoform—interaction and the downstream signalling propagation. The expression of the viral protein vMIA—used by viruses to enhance infectivity—fragments mitochondria, reduces STING–MAVS association and delays the degradation of the MAVS isoform.

Similarly, MFN2 inhibits mitochondrial antiviral immunity: MFN2 overexpression blocks antiviral signalling induction and its silencing decreases viral replication (Yasukawa *et al*, 2009). Interestingly, MFN2 is a physical tether of ER to mitochondria (de Brito & Scorrano, 2008b), pointing to a role of ER–mitochondria tethering in the modulation of the innate immune response.

A link between mitochondrial dynamics and signalling?

Lymphocytes are key cells of the immune system that are involved mainly in adaptive immunity and in the effective clearance of infections from the organism. They circulate in the blood until they are activated in response to an infection, at which point they sense and respond to chemo-attractant gradients that recruit them to the site of infection with asymmetrical changes in cell morphology (polarization) and mobility (chemotaxis). To achieve directed movement, cells organize and maintain spatial and functional asymmetry with a defined anterior (leading edge) and posterior (uropod; Lauffenburger & Horwitz, 1996). Thus, T-cell migration or chemotaxis is required to maintain homeostasis and to achieve appropriate immunological reactions. Once at the site of infection, T cells deploy an array of responses that lead to the activation and proliferation of specific effector T cells and an effective immune response. The activation of T cells occurs through the simultaneous engagement of the TCR and the co-stimulatory receptor CD28 on the T cell by the MHC peptide and B7 family members on the APC, respectively (Krammer *et al*, 2007). One of the earliest events in T-cell activation is the induction of the tyrosine kinase activity of members of the SRC and ZAP70 kinase families. Downstream from these, activation of the transcription factors NF-κB and AP1, as well as Ca^{2+}/calcineurin-dependent activation of the transcription factor NFAT, all induce the transcription of a pleiotropic set of genes that promote the long-term proliferation of activated T cells. Any of these steps requires energy or depends on Ca^{2+}, and is therefore likely to involve some degree of control by mitochondria.

Cells of the immune system have few mitochondria and are usually regarded as glycolytic; however, emerging evidence indicates a role for mitochondria in the response of T cells to external cues. Thus, T cells could be a good and 'simplified' system with which to characterize the interplay between mitochondrial morphology and signalling cascades, the latter of which are well characterized in T cells.

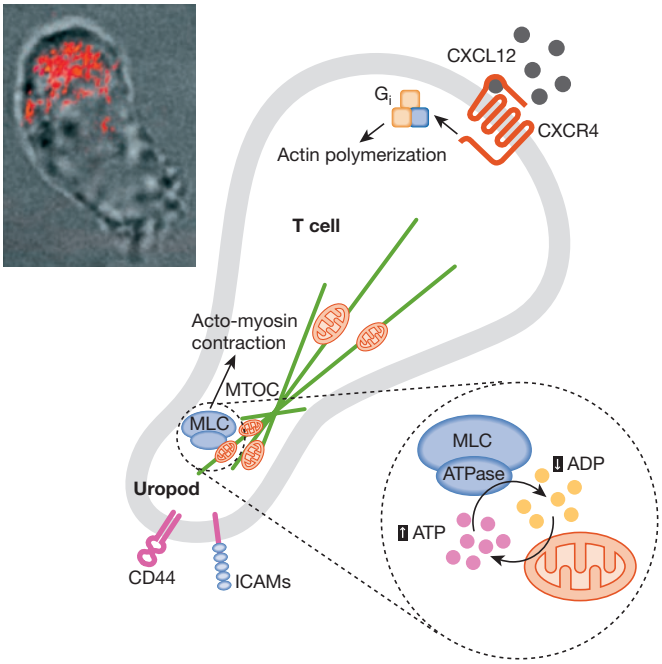

Fig 2 | A model for mitochondrial dynamics in the orchestration of T-cell chemotaxis. To achieve directed movement, lymphoid cells organize and maintain spatial and functional asymmetry, with a leading edge containing the machinery for actin polymerization and gradient sensing and a uropod containing the adhesion molecules and the MTOC. At the uropod, myosin filaments contract actin filaments to provide the tension required for cell movement. Myosin II activity is controlled mainly through the phosphorylation of MLC, which induces a conformational change allowing actin–myosin interactions and activating its ATPase activity. Myosin II ATPase depends on availability of the substrate and clearance of the product. Even in the presence of optimal ATP concentration, accumulation of ADP near the enzyme will slow down its activity and block migration. However, during lymphocyte migration, mitochondria are transported to the uropod along microtubules, in a process requiring G_i protein signalling and mitochondrial fission. The position of mitochondria is strategic for myosin ATPase activity: mitochondria not only supply ATP, but also withdraw ADP, thus creating optimal conditions for MLC. Inset: reconstruction of a confocal z-stack of images of a polarized Jurkat cell expressing mitochondrially targeted dsRED merged with a bright field image of the same cell showing polarization of mitochondria at the uropod. CXCL12, CXC ligand 12; CXCR4, CXC chemokine receptor 4; ICAM, intercellular adhesion molecule 1; MLC, myosin light chain; MTOC, microtubule organizing centre.

Mitochondria and mitochondrial dynamics have thus far been linked to at least two aspects of the function of T cells: chemotaxis and the regulation of the formation and activity of the immunological synapse. We recently demonstrated that mitochondria specifically redistribute to and accumulate at the uropod during directed leukocyte migration to provide ATP, thus regulating the cell motor of migrating lymphocytes (Fig 2; Campello *et al*, 2006).

More interestingly, the accumulation of mitochondria at the uropod requires their fission. Genetic manoeuvres that elongate mitochondria were found to block mitochondrial and cell polarization in response to chemokines and, more importantly, prevented the migration of T lymphocytes towards CXCL12 gradients. This inhibition of mitochondrial transport could reflect an inability of the cytoskeleton to transport organelles that are too large. Alternatively, altered mitochondria-shaping proteins could be unable to interact with the components of the mitochondria transport machinery, such as the kinesin motors or the adaptor Miro–Milton complex (Liu & Hajnoczky, 2009). Kinesin and dynein motors control the movement of mitochondria along microtubules, which in turn regulates the distribution of the organelles in the cell. To move, the extensive mitochondrial network must be divided into smaller organelles that can be moved readily by the motors (Hollenbeck & Saxton, 2005). To this end, the machinery that transports mitochondria is probably coordinated with mitochondria-shaping proteins, as substantiated by the finding that disruption of the dynein complex results in mitochondrial elongation caused by DRP1 blockage (Varadi *et al*, 2004). It remains to be seen whether changes in mitochondrial transport can reproduce the effects of unopposed fusion, *in vitro* and *in vivo*. Irrespective of the mechanism, when mitochondria do not relocate properly, T-cell polarization and migration are impeded, revealing a previously unexpected role in the immune system for morphological adaptations of mitochondria.

The second key physiological process in which mitochondrial shape has been implicated is the regulation of gene expression after the activation of T cells. The role of global Ca^{2+} signals for gene expression regulation (Feske *et al*, 2001), cell activation and proliferation (Schwarz *et al*, 2007) in T cells is already well established. An indispensable step during T-cell activation is Ca^{2+} entry across the plasma membrane through the opening of CRAC/ORAI1 channels, which are in turn opened by STIM1 as a consequence of the depletion of intracellular Ca^{2+} stores (Cahalan & Chandy, 2009). To prevent Ca^{2+}-dependent inactivation of CRAC/ORAI1 channels, mitochondria act efficiently as inflowing Ca^{2+} buffers (Parekh & Putney, 2005). It has been shown recently that mitochondria translocate towards the plasma membrane and the immunological synapse, which is the contact site between activator and effector cells, during activation of at least certain T-cell subpopulations (Abarca-Rojano *et al*, 2009; Kummerow *et al*, 2009). Mitochondrial fragmentation is implicated in this relocation, therefore adding another role for the fission machinery in immunological synapse formation and maintenance. However, mitochondrial accumulation at the immunological synapse seems to be more a consequence of cellular, rather than organellar, shape changes (Quintana *et al*, 2009). It therefore remains to be investigated whether the modulation of mitochondrial fusion, fission and transport influences the formation and stability of the immunological synapse, as well as the ability of mitochondria to regulate Ca^{2+} influx through store-operated channels.

Conclusions

The machinery controlling mitochondria shape and dynamics are becoming well established and defined. Emerging evidence increasingly suggests that these processes are crucial for the physiology of the cell, as well as for the pathology of the organism. Conversely, our knowledge of the role of mitochondria dynamics in the immune system is still rather scarce. Nevertheless, appealing yet preliminary data have shed light on the potential importance of mitochondrial dynamics in T-cell physiology. In addition, the signalling cascades that lead to immune cell activation are reasonably well understood, and genetic models make it possible to isolate the key steps of these cascades. Thus, T cells are an appealing system in which to place mitochondrial dynamics in the context of known elements of signalling. In the long term, mitochondria and mitochondrial dynamics could be unveiled as an important therapeutic target to modulate T-cell function in common autoimmune diseases, graft-versus-host diseases and infections, as well as to modulate viral infections in innate immunity.

ACKNOWLEDGEMENTS

L.S. is a senior telethon scientist of the Dulbecco-Telethon Institute and is supported by Swiss National Foundation Grant 31-118171, OncoSuisse and Telethon Italy. S.C. was supported by a Roche Foundation Postdoctoral Fellowship and is supported by a FP7-PEOPLE-IEF-2008 grant (Grant Agreement 235595).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Silvia Campello

Luca Scorrano

