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Review

Mitochondrial Dynamics at the Interface of Immune Cell Metabolism and Function

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Immune cell differentiation and function are crucially dependent on specific metabolic programs dictated by mitochondria, including the generation of ATP from the oxidation of nutrients and supplying precursors for the synthesis of macromolecules and post-translational modifications. The many processes that occur in mitochondria are intimately linked to their morphology that is shaped by opposing fusion and fission events. Exciting evidence is now emerging that demonstrates reciprocal crosstalk between mitochondrial dynamics and metabolism. Metabolic cues can control the mitochondrial fission and fusion machinery to acquire specific morphologies that shape their activity. We review the dynamic properties of mitochondria and discuss how these organelles interlace with immune cell metabolism and function.

Trends

Immune cell function crucially depends on mitochondrial bioenergetics.

Mitochondrial function is controlled by their dynamics where coordinated forces of fission and fusion shape mitochondrial morphologies.

Genetic deletion of fission and fusion proteins impacts on immune cell metabolism and function.

The regulatory network of mitochondrial fusion/fusion responds rapidly to metabolic cues, supporting reciprocal crosstalk between mitochondrial dynamics and immunometabolism.

Given the additional non-mitochondrial functions of the fission/fusion machinery, and the formation of functional interactions between mitochondria and other organelles, experimental data on mitochondrial dynamics require careful interpretation.

Metabolic Shifts during Immune Responses

For an immune response to proceed, specialized cells of the immune system morph from a state of relative quiescence to one of high activity. A prime example of a cell type that undergoes this transformation is the T lymphocyte. Initially patrolling our body as quiescent naïve T cells, these cells become rapidly activated upon antigen detection to T effector (T_{eff}) cells that proliferate, secrete cytokines, and migrate to the sites of infection. Once the antigen load is reduced, and supportive signals wane, the vast majority of T_{eff} cells die, while a small number of long-lived memory T (T_{mem}) cells persist over time, maintaining a state of relative quiescence. It is now well established that T cells, as well as several other immune cell types such as B cells, macrophages, and dendritic cells, must reprogram their cellular metabolism to acquire their different phenotypic and functional states (reviewed in [1,2]). Cells such as T_{mem} cells, regulatory T cells, and alternatively activated (M2) macrophages rely on catabolic metabolism where nutrients are fully degraded and shuttled toward energy-generating pathways. As such, they rely on mitochondrial activity driven, for example, by pyruvate- or fatty acid-driven **oxidative phosphorylation** (OXPHOS; see Glossary). By contrast, the anabolic metabolism of activated cells is directed at balancing sufficient energy production with the synthesis of macromolecules that are necessary for cell division as well as DNA and protein synthesis. Metabolically this is often achieved by commitment to aerobic glycolysis, where high rates of glycolysis allow cells to sustain their ATP production. Under such conditions, mitochondrially generated **tricarboxylic acid (TCA) cycle** metabolites are used to build macromolecules, provide substrates for posttranslational modifications (PTMs), and change the epigenetic landscape. Anabolic states have been linked to the function of T_{eff} cells, the activation of dendritic cells and proinflammatory (M1) macrophages, and the degranulation of mast cells [1–3].

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While the bioenergetic role of mitochondria in regulating immune responses is widely appreciated [2,4], only recently has another aspect of mitochondrial biology taken center stage: the ability of mitochondria to fuse and divide, directly impacting on their functional capacity. Substantial evidence is emerging to demonstrate reciprocal crosstalk between mitochondrial dynamics and metabolism, where metabolic cues literally shape mitochondria via fission/fusion events, and these morphological changes dictate the bioenergetic capacity of the organelle. In this review we summarize recent exciting findings on how mitochondrial dynamics and metabolism are interlinked to shape immune cell metabolism and fate. We discuss these novel insights into immunometabolism in the light of our more detailed understanding of mitochondrial dynamics in non-immune cells.

Mitochondrial Metabolism and Its Cellular Function

Mitochondria are characterized by a complex architecture and high degree of compartmentalization that are crucial for their function. They are composed of an outer mitochondrial membrane (OMM), and a heavily folded inner mitochondrial membrane (IMM), the site of the **electron transport chain** (ETC). Historically, the major role of mitochondria was thought to be to the efficient coupling of substrate oxidation through the TCA cycle to ATP production by the ETC. In fact, mitochondria produce up to 36 ATP from one molecule of glucose, compared to two ATP from glycolysis [5], and are thus a more efficient source of cellular ATP. In addition to pyruvate derived from glucose, mitochondria can utilize fatty acids or amino acids and oxidize them in the TCA cycle. The substrate-driven fueling of the TCA cycle generates the reducing equivalents NADH and FADH2 that provide electrons to the ETC. By transferring electrons to molecular oxygen, the ETC pumps protons across the inner mitochondrial membrane, resulting in the generation of the proton-motive force that is utilized to produce ATP by the ATP synthase. While ATP production is the major role of the ETC, it can also produce **mitochondrial reactive oxygen species** (mtROS) that, depending on the generated amount, can either function as cellular signaling cue or lead to cell damage [2]. In addition to the production of ATP, an equally important function of mitochondria is the utilization of TCA cycle intermediates in anabolic or regulatory reactions. For example, citrate can be transported into the cytosol where acetyl-CoA is generated to drive fatty acid synthesis and protein acetylation [6]. Similarly, the TCA cycle intermediate α-ketoglutarate is required for function of the α-ketoglutarate-dependent dioxygenase family of proteins, which include prolyl hydroxylases (PHDs) and Jumonji domain-containing histone demethylase (JHDM), while fumarate and succinate are inhibitors of these proteins [7]. Given the widespread functions this organelle regulates through its metabolic modes, it is clear that mitochondrial activity needs to be finely tuned. In the following sections we discuss how the ability of mitochondria to fuse and divide contributes to this control by both integrating and instructing cell metabolism.

Mitochondrial Fission and Fusion Dynamics

For a long time mitochondria were viewed as isolated and static organelles, until advances in live cell imaging and genetic screening revealed that mitochondria are highly dynamic. Not only can mitochondria, driven by cytoskeletal transport, change their position inside cells but their architecture is also continuously modulated by fission and fusion reactions. In recent years these mitochondrial shape-changes, referred here to as 'mitochondrial dynamics', have gained substantial attention because they are essential to ensure the segregation of **mitochondrial DNA** (mtDNA) and regulate mtROS levels, calcium homeostasis, and oxidative phosphorylation [8]. Of particular interest were studies highlighting that such mitochondrial dynamics and cellular metabolism are coupled. In fact, in particular metabolic states, mitochondria were shown to acquire specific morphologies. For example, in cultured cell lines, elongated mitochondria are observed in conditions associated with increased ATP requirements [9–11]. Similarly, in cultured cells grown under nutrient-restricted conditions or in livers of starved

Glossary

Apoptosis: programmed cell death.
Autophagy: a process that depends on the *de novo* formation of a double-membrane enclosed organelle, the autophagosome, that is able to engulf cytosolic material and target it for lysosomal degradation.
Cristae: the folds of the inner mitochondrial membrane.
Electron transport chain (ETC): at the inner mitochondrial membrane (IMM) four complexes (I–IV) transfer electrons from electron donors to acceptors to produce an electrochemical proton gradient across the IMM.
Endoplasmic reticulum (ER): a single-membrane enclosed organelle important for protein secretion, calcium homeostasis, and lipid metabolism.
Lipid droplet: a monolayer-confined organelle functioning as a storage site for intracellular fatty acids and cholesterol.
Lysosome: a single-membrane enclosed acidic organelle that drives the degradation of proteins, lipids, and carbohydrates in a pH-dependent manner.
Mitochondrial DNA (mtDNA): circular DNA localized in the mitochondrial matrix, encoding 37 mitochondrial genes, including two rRNAs, 22 tRNAs, and 13 polypeptides.
Mitochondrial ROS (mtROS): as a result of ETC leakage, electrons are transferred to oxygen, producing superoxides and eventually hydrogen peroxide.
Mitochondrial unfolded protein response (mtUPR): a conserved stress program involved in mitochondrial chaperone and protease gene expression, metabolic adaptation, and immune responses.
Mitophagy: the autophagosomal removal of damaged mitochondria. Several pathways exist, the most prominent being driven by Pink1/parkin. Loss of mitochondrial membrane potential induces Pink1 stabilization on the OMM and recruitment of the E3 ligase parkin, marking mitochondria as autophagosomal targets.
Oxidative phosphorylation (OXPHOS): at the inner mitochondrial membrane four complexes (I–IV) transfer electrons from electron donors to acceptors to

animals, unopposed fusion drives mitochondria into large interconnected networks [12–14]. These hyperfused states are essential to sustain intramitochondrial exchange of fatty acids, mitochondrial respiration, and cell survival [12–15]. By contrast, nutrient excess and cellular damage induce mitochondrial fragmentation [16] and promote their degradation through **mitophagy** (reviewed in [17]). Together, these data reveal a picture that mitochondrial dynamics represent a metabolic nodal point bidirectionally integrating metabolic supply and output (Figure 1, Key Figure). In the following section we first introduce the basic principles of the mitochondrial fission and fusion machinery (Figure 2) before discussing how their functions are intertwined with immune cell metabolism and function.

The Mitochondrial Fission Machinery

Mitochondrial fission is regulated by the GTPase dynamin-related protein 1 (Drp1) that drives division at specific points along mitochondria. These sites are pre-marked by the **endoplasmic reticulum** (ER) [18] and actin [19,20], allowing Drp1 to assemble into oligomeric spirals that constrict and finally pinch the mitochondrion apart. The final separation step proceeds via cooperation between Drp1 and dynamin 2 [21]. Because most cellular Drp1 is not constitutively associated with mitochondrial membranes, its recruitment requires specific adaptor proteins. To date, the mitochondrial fission factor (Mff), Fis1, and Mid49-51 [22] have been described to regulate Drp1 recruitment, with partially overlapping functions.

In addition to influencing mitochondrial morphology, fission has been ascribed multiple other functions including facilitating mitochondrial transport along the cytoskeletal network, mitophagy, **apoptosis**, and calcium homeostasis [23,24]. Furthermore, Drp1 also regulates fission of the **peroxisome** [25]. In line with these multiple effects, loss of Drp1 function leads to severe physiological consequences. Two clinical studies have linked human Drp1 mutations to microcephaly, neonatal lethality [26], and refractory epilepsy [27], and another has linked Mff mutation to two cases of developmental delay with neuromuscular dysfunction [28–31].

The Mitochondrial Fusion Machinery

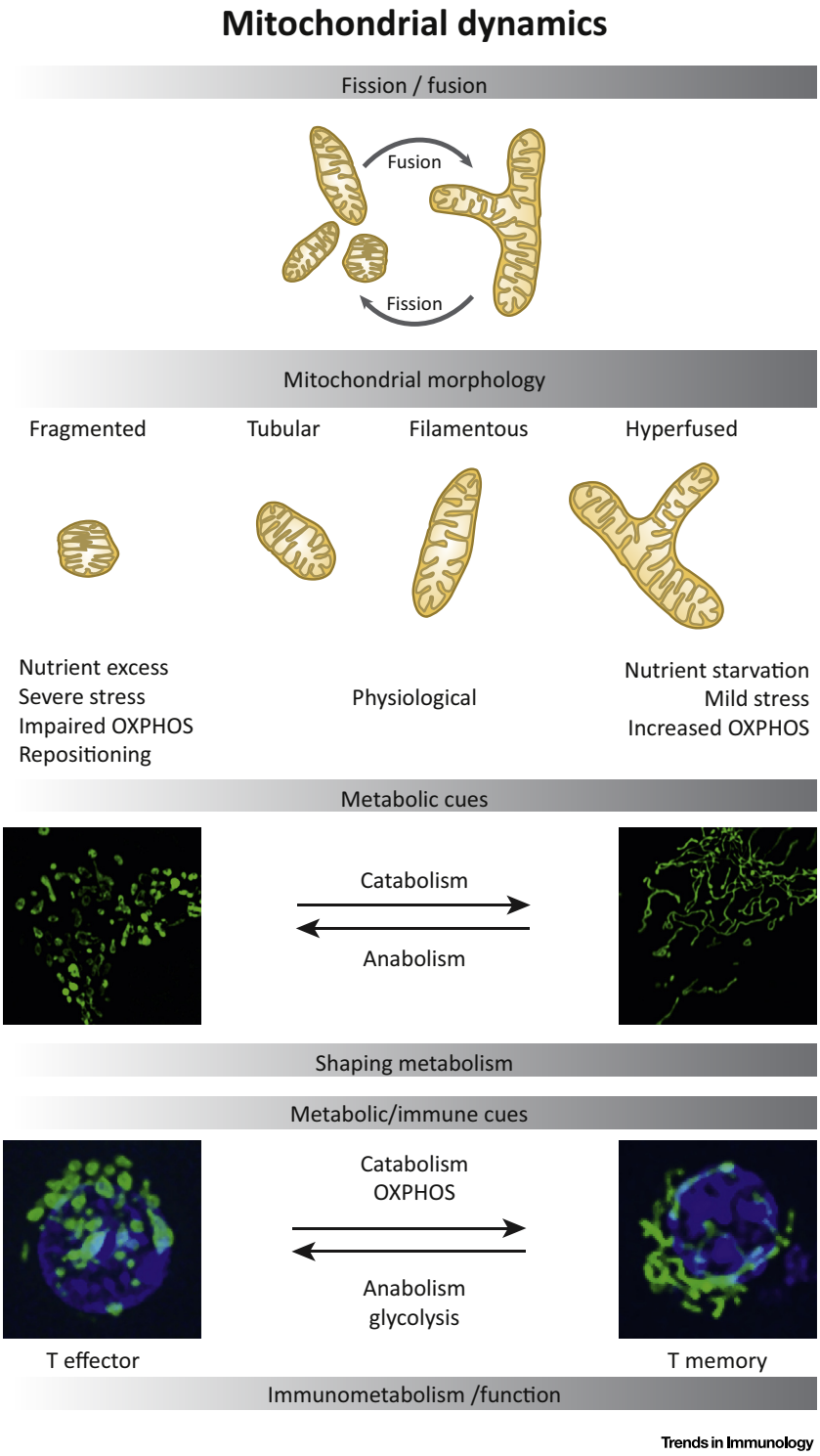
Mitochondrial fusion is a two-step process where OMM fusion is followed by fusion of the IMM. The mitochondrial fusion machinery consists of three dynamin family GTPases, mitofusin (Mfn) 1 and 2 on the OMM and optic atrophy protein 1 (Opa1) on the IMM [29–31]. The exact mechanism of how Mfns mediate the fusion of the OMM is not clear, but several lines of evidence support a mechanism whereby Mfn1 and Mfn2 interact with their C-termini in *trans* between neighboring mitochondria, thus promoting tethering and subsequent fusion of the OMM [32]. In addition to its localization at the OMM, Mfn2 has also been found at the ER where it promotes ER–mitochondria interactions and Ca^{2+} transfer between the two organelles [33]. Very recent data further implicate Mfn2 as a mediator of mitochondria and **lipid droplet** interactions, influencing lipolytic processes in brown adipose tissue [34].

Cooperating with Mfn1, Opa1 drives the fusion of the IMM [35]. Opa1 is localized to the mitochondrial intermembrane space and the mitochondrial inner membrane. There are different forms of Opa1, and long forms (L-Opa1) are proteolytically cleaved by two IMM peptidases to generate S-Opa1 [36,37]. While the exact mechanism of how Opa1 mediates mitochondrial fusion is still unknown, it is clear that the relative levels of the long and short forms dictate the fusion-competence of mitochondria. In fact, a recent study elegantly demonstrated that L-Opa1 is sufficient to mediate fusion, while S-Opa1 induces mitochondrial fission [37]. Beyond regulating mitochondrial fusion, Opa1 also controls **cristae** morphology, mtDNA maintenance, and **supercomplex** assembly [23,38]. For this reason, in many Opa1-depletion or -overexpression models it remains to be determined whether the fusion activity or other functions of Opa1 are responsible for the reported effects.

produce an electrochemical proton gradient across the IMM. This is used by the ATP synthase (complex V) to synthesize ATP from ADP and inorganic phosphate.
Peroxisome: a single-membrane enclosed organelle driving non-mitochondrial fatty acid oxidation, lipid synthesis, and ROS production.
Reactive oxygen species (ROS): superoxides and hydrogen peroxide that are produced from different cellular sources.
Spare respiratory capacity: the difference between OXPHOS at basal and at maximal activity.
Supercomplexes: supramolecular structures comprising OXPHOS enzymes in the IMM.
Tricarboxylic acid (TCA) cycle: the stepwise oxidation of acetyl-CoA in the mitochondrial matrix, generating reducing equivalents NADH and FADH2.
Vacuole: the equivalent of the lysosome in *Saccharomyces cerevisiae.*

Key Figure

Mitochondrial Morphology Is Linked to the Cellular Metabolic State



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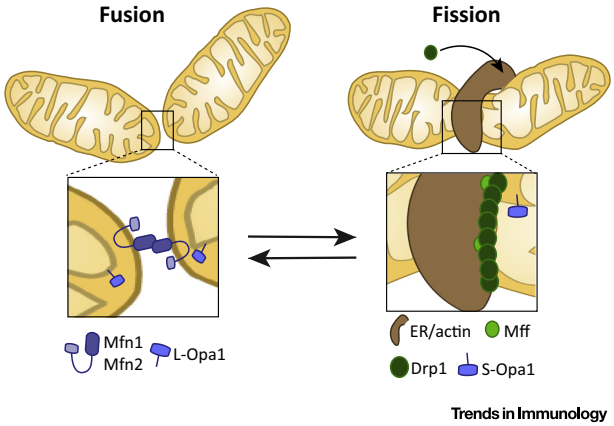

Figure 2. Regulation of Fission and Fusion. (Left) Mitochondrial fusion on the OMM is driven by homotypic and heterotypic interactions of Mfn1 and Mfn2, thus tethering and subsequently fusing two opposing mitochondrial membranes. Fusion of the IMM is driven by L-Opa1. (Right) Mitochondrial fission is induced at sites marked by the ER and actin. The mitochondrial adaptor protein Mff recruits Drp1 to the mitochondrial membrane, where it forms spirals that contract and finally pinch the mitochondrion apart. Recruitment of Drp1 can further be mediated by MiD49/51 and Fis1 (not depicted). Abbreviations: Drp1, dynamin-related protein1; ER, endoplasmic reticulum; IMM, inner mitochondrial membrane; Mfn, mitofusin; Mff, mitochondrial fission factor; OMM, outer mitochondrial membrane; Opa1, optic atrophy 1.

These core components of the fission/fusion machinery are ubiquitously expressed across cell types. Furthermore, research over the past decade has directly established that alterations in this machinery alter mitochondrial dynamics and with it function, leading to defects ranging from slower dynamics to complete mitochondrial dysfunction [29,30]. This observation holds true across various tissues, including the immune system. Nevertheless, there are cell type-specific differences in expression levels or splice variants. For example, immune cells primarily contain a specific Drp1 splice variant that associates with the cytoskeleton and is selectively mobilized by cyclin-dependent kinase signaling [39]. Cell type-specific differences need to be kept in mind when addressing the impact of fission/fusion proteins.

Mitochondrial Dynamics as a Driver for Metabolic Cell States

In several immune cells, including T cells, macrophages, and mast cells, mitochondria have been shown to adapt specific mitochondrial morphologies according to the cellular activation state [40–42]. Emerging evidence now also highlights that the ablation of fission and fusion proteins impacts on immune cell metabolism and function. These findings complement studies in metabolic tissues, such as the liver and the nervous system, where altered fission/fusion dynamics cause defects in cell metabolism (Box 1), supporting the close relationship between mitochondrial dynamics and metabolism.

Mitochondrial Dynamics Drive T Cell Metabolism and Function

Morphometric analysis revealed that mitochondria adopt specific shapes during the differentiation of T cells, reaching fragmented and hyperfused end-stages in T_{eff} and T_{mem} cells, respectively [40] (Figure 1). Inhibiting mitochondrial fusion by the T cell-specific ablation of

Figure 1. Driven by fission and fusion reactions, mitochondria can display a wide spectrum of morphologies. Cultured cells acquire specific mitochondrial morphologies in response to changes in nutrient availability and energy requirements (e.g., in mouse embryonic fibroblasts; see also [14]). Similar adaptations have been found to occur in T cells with selective metabolic states (left, glycolytic $CD8^+$ T_{eff} cells; right, OXPHOS-dependent $CD8^+$ T_{mem} cells), as recently shown [40]. The figure contains original images contributed by Angelika Rambold that are representative of the phenotypes observed in the studies cited. Abbreviation, OXPHOS, oxidative phosphorylation.

Box 1. Mitochondrial Dynamics Control Metabolism Outside the Immune System

Several studies performed outside the immune system have linked defects in mitochondrial fission/fusion dynamics to tissue dysfunction and altered metabolic homeostasis. Liver specific-ablation of Mfn2 induced mitochondrial fragmentation, elevated ROS levels, glucose intolerance, and impaired insulin signaling [88]. While limiting ROS production ameliorated the observed metabolic defects, mitochondrial fragmentation could not be reversed [89]. Thus, it remains unclear whether the observed metabolic changes in Mfn2-depleted hepatocytes result from impaired OMM fusion or from Mfn2-driven ER–mitochondria tethering. Supporting evidence for the latter stems from several studies demonstrating that changes in ER–mitochondria interactions regulate glucose homeostasis and insulin signaling [90]. However, a recent study also supports a role for mitochondrial dynamics in regulating liver metabolism. Deletion of *Drp1* in hepatocytes impairs mitochondrial fission and increases the expression of ATF4, CHOP, and FGF21 [91]. Importantly, FGF21 functions as a hepatokine that regulates the intake of both sugar and non-caloric sweeteners [92], thus providing an attractive explanation for the metabolic protection conferred by *Drp1* ablation.

Another important tissue regulated by mitochondrial fission/fusion dynamics is the nervous system. Dramatic changes in mitochondrial shape were reported in specialized neurons that orchestrate appetite in mice [93]. Agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons regulate feeding behavior in a positive and negative manner, respectively [93]. During feeding AgRP neuronal activity normally increases. However, altering mitochondrial size by depletion of Mfn1 or Mfn2 impaired their neuronal activity and mice gained less weight. Conversely, in POMC neurons, ablation of *Mfn2* caused severe obesity characterized by overeating, reduced energy expenditure, and endocrine dysregulation [94]. This was specific to *Mfn2* because *Mfn1* POMC deletion did not disrupt energy homeostasis. While it remains unclear which Mfn2 function underlies the feeding behavior directed by those neurons, a recent study also supports a role for mitochondrial fission in this process. Drp1 depletion in POMC neurons, and with it mitochondrial elongation, improves leptin sensitivity and glucose responsiveness during feeding [95], thus supporting a role of mitochondrial fusion in driving whole-body energy homeostasis.

Opa1 led to reduced mitochondrial respiration and altered cristae structure. This proved detrimental to the survival and differentiation of T_{mem} cells, while T_{eff} cell differentiation was not compromised. In contrast to T_{eff} cells, T_{mem} cells heavily rely on fatty acid-driven mitochondrial respiration and contain increased **spare respiratory capacity** and mitochondrial content [40,43,44], suggesting that Opa1 might be particularly important in cells with a high mitochondrial demand. Given that depletion of either Mfn1 or Mfn2 does not have the same consequences for T_{mem} cells, it is likely that non-fusion functions of Opa1 are necessary to reach the T_{mem} state. However, it cannot be ruled out that mitochondrial fusion additionally benefits mitochondrial respiration, a process mainly driven by fatty acid oxidation in T_{mem} cells. Elongated mitochondrial states have been suggested to facilitate the distribution of fatty acids across the mitochondrial network, and with it their oxidation, in cultured cell lines [13]. Similarly, fusion could alter the mitochondrial membrane composition or fluidity [45], and with it the activity of nutrient transporters or enzymes. Additional studies manipulating Drp1 activity or using Mfn1/2 double-knockout cells to prevent potential compensatory functions between these OMM fusion proteins will be necessary to provide more insight into which mitochondrial functions are required for the differentiation of T_{mem} cells.

In contrast to T_{mem} cells, T_{eff} cells are generally in an anabolic state driven by increased nutrient uptake of amino acids and glucose and engagement of glycolysis [46,47]. They also have been shown to mainly contain fragmented mitochondria [40]. While it remains unclear if this fragmentation benefits the anabolic state, separated mitochondria might regulate other central T_{eff} cell functions. In Jurkat T cells, fission facilitates mitochondrial transport to the immunological synapse (IS) where the organelle functions as a calcium buffer to regulate T cell receptor signal strength [48]. The localized uptake of Ca^{2+} at the IS prevents the premature closure of the calcium channel CRAC/ORAI, and thus sustains Ca^{2+} influx and increases signaling strength [49]. Furthermore, Drp1-driven positioning has also been shown to locally control ATP fueling in Jurkat T cells. One example of this is the promotion of myosin-driven cell contraction at the rear end of the cell [50]. Similarly, Baixauli *et al.* showed that fragmented mitochondria are essential to fuel the actomyosin-driven formation of the supramolecular activation cluster (cSMAC). Given the role of mitochondrial fission in facilitating apoptosis [24], retaining mitochondria in a

fragmented state might also enhance the elimination of T_{eff} cells when limited antigen induces the resolution phase of the immune response. Such a mechanism could also be involved during positive and negative selection in the thymus.

Mitochondrial Dynamics Drive Macrophage Metabolism and Function

Part of the innate immune system, macrophages respond to a multitude of local immune- and/or pathogen-derived signals. Macrophages reprogram their metabolism to acquire particular activation states. For example, macrophages fully commit to glycolysis to achieve polarization into a proinflammatory M1 state, while M2 macrophages, which are important for tissue repair and wound healing, rely on a mixed state driven by enhanced glucose utilization and OXPHOS [51–53]. Inhibition of mitochondrial fission through the Drp1 inhibitor Mdivi-1 was recently shown to reduce glycolytic reprogramming of lipopolysaccharide (LPS)-activated macrophages [40]. While this provides an intriguing link between mitochondrial dynamics and metabolism, Mdivi-1 might also accomplish this by altering complex I activity and mtROS production independently of preventing fission [54]. The role of mitochondrial fission/fusion for the metabolic reprogramming of macrophages clearly awaits further research.

Mitochondrial Dynamics Shape a Cellular Signaling Platform

Apart from its impact on metabolism, most evidence supporting a role for mitochondrial dynamics in innate immunity stems from studies highlighting mitochondrial membranes as an assembly and signaling platform [55]. The mitochondria antiviral signaling protein, MAVS, is located on the OMM and undergoes a prion-like aggregation step that induces downstream antiviral signaling by inducing type I interferons [55,56]. Depletion of Mfn1/2 or Opa1 reduced MAVS-driven innate antiviral signaling in a mitochondrial membrane potential-dependent manner [55,57,58]. Because only a limited number of MAVS molecules are present in each mitochondrion, it was suggested that fusion facilitates MAVS aggregation by supplying new molecules from different mitochondria. Mitochondria also serve as a crucial signaling platform for the NLRP3 inflammasome by altering mtROS, lipids, and membrane potential [42,59]. Mitochondrial fragmentation, by depletion of Mfn2, reduces NLRP3 activity [60]. By contrast, Drp1 ablation can increase or decrease NLRP3 activity, depending on its initiating stimulus [61,62]. With the overall emerging picture that mitochondria are vital signaling platforms tuned by mitochondrial dynamics, this raises the question of whether mitochondria are used in a

Box 2. Signal Integration Through Mitochondrial Proteins with Nuclear Function

An increasing number of proteins with dual localization in the mitochondrion and nucleus have been reported over the years, providing a striking functional mito-nuclear integration point. Because this has been comprehensively reviewed elsewhere [96], we only highlight two pathways with known immunomodulatory function. The mammalian nuclear factor erythroid 2-related factor 2 (Nrf2) is critical for optimal NF-κB activation and regulates the expression of genes associated with a proinflammatory innate immune response [97]. Nrf2 is normally sequestered in a protein complex on the OMM. In response to mtROS and other mitochondrial stress, it translocates to the nucleus, inducing a transcriptional program regulating cellular redox balance [96]. Given that mtROS is controlled by mitochondrial dynamics [8], Nrf2 might serve as nodal point in the coordination of mitochondrial dynamics, metabolism, and immune function. Another example for the functional integration between mitochondria and the nucleus is the *Caenorhabditis elegans* transcription factor ATFS-1 (activating transcription factor associated with stress). ATFS-1 regulates the **mitochondrial unfolded protein response** (mtUPR), a retrograde nuclear signaling response that is also conserved in higher eukaryotes [98,99]. ATFS-1 is normally imported into mitochondria, where it undergoes proteolytic degradation. However, in response to mitochondrial dysfunction or infection, *de novo* import is reduced and ATFS-1 translocates to the nucleus. Here it induces a transcriptional response, promoting glycolysis [100] and regulating the expression of innate immune genes [101]. Given that mitochondrial fusion is required to sustain mitochondrial membrane potential, this mechanism might provide an intriguing immunometabolic integration. To date the direct homolog for ATFS-1 in mammals has not been identified, but several proteins have been linked to the mammalian mtUPR, including SIRT3, CHOP, and ATF5 [99,102,103]. In addition, members of the STAT (signal transducer and activator of transcription) family, STAT3 and STAT6, that are implicated in both immune regulation and metabolism [104], have been shown to localize to both the nucleus and mitochondria. As such, they might provide another example integrating immunometabolic regulation under the control of mitochondrial dynamics and function.

similar manner by other signaling molecules. Of particular interest are proteins that localize to both mitochondria and the nucleus, and that drive both metabolic and immune-regulatory processes (Box 2). Coordinated communication between the nucleus and mitochondria would serve as a clear adaptive advantage when cells need to integrate immune signals and metabolic reprogramming to rapidly achieve full effector functions.

Metabolism as a Driver for Mitochondrial Fission and Fusion Dynamics

While genetic studies are beginning to reveal mitochondrial dynamics as a crucial regulator of immune metabolism and function, our insights into the mechanistic regulation of this process are still developing. In non-immune cells, several regulatory layers control the fission/fusion core machinery from the transcriptional to the post-translational level [63]. This includes orchestration of fission/fusion activities by metabolic cues [64,65]. In the next paragraphs we summarize the evidence for such metabolic regulation of the fission and fusion machinery (Figure 3) and discuss their relevance for immune cell responses.

Metabolic Cues Driving Mitochondrial Fusion

Several metabolic sensor kinases have been shown to drive mitochondrial elongation during low nutrient supply [12,14]. Mechanistically this is mostly achieved by inhibiting Drp1 through phosphorylation at Ser637, close to its GTPase domain. Among the kinases controlling this site is PKA, a cAMP-dependent enzyme that indirectly responds to alterations in the levels of

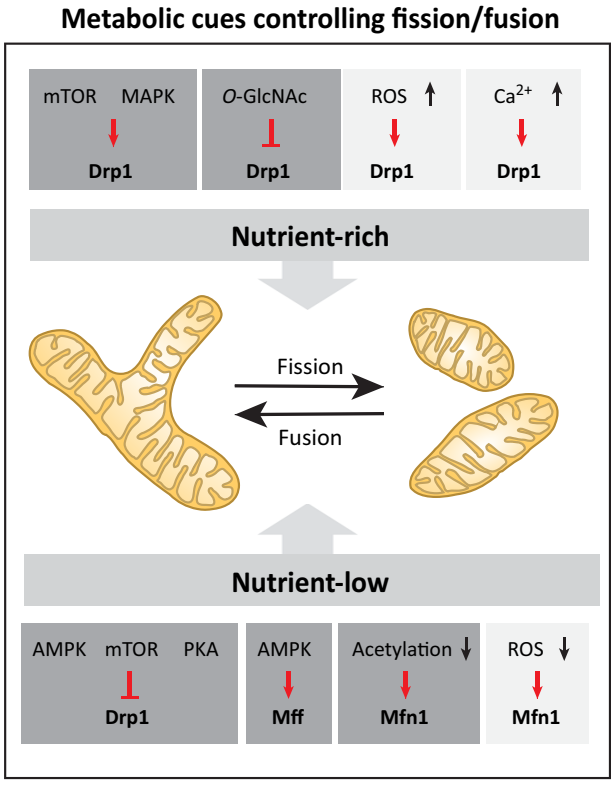

Figure 3. Mitochondrial Dynamics Are Regulated by Metabolic Cues. Mitochondrial morphology can adopt specific morphologies in response to different metabolic cues. (Dark-grey boxes) Metabolic cues directly impacted by nutrient availability. (Light-grey boxes) Metabolic cues not directly linked to nutrient availability. Abbreviations: AMPK, 5'-AMP-activated protein kinase; Drp1, dynamin-related protein1; Mff, mitochondrial fission factor; Mfn1, mitofusin 1; mTOR, mammalian target of rapamycin; ROS, reactive oxygen species.

cAMP/ATP [12,14,66]. Similarly, AMPK activation and mTOR inhibition, two major energy and nutrient sensors, have been shown to induce mitochondrial fusion [12,67] and drive mitochondria into a hypertubular state during nutrient depletion in cultured cells [12]. Mitochondrial elongation can also be induced through Mfn1. In mouse embryonic fibroblasts, low glucose availability leads to the deacetylation of Mfn1, thus promoting its stability and fusion activity [68]. Mutations that impair this process predispose cells to oxidative damage, albeit without affecting ATP production. While it is currently unclear whether these mechanisms are used to shape mitochondrial dynamics in immune cells, it is intriguing that both AMPK and mTOR activities shape the differentiation and metabolic reprogramming of several immune cell types [69–71], including T_{mem} cells that contain fused mitochondria [40].

Metabolic Cues Driving Mitochondrial Fragmentation

Similarly to mitochondrial elongation, several metabolic cues drive mitochondrial fission by activating Drp1. For example, in cultured cell lines the calcium-regulated kinase calcineurin dephosphorylates Drp1 at Ser673, thus driving mitochondrial fragmentation [72]. The pro-fission activity of Drp1 can also be regulated by phosphorylation at Ser616. CDK1/cyclin and ERK1/2 target this site to ensure proper organelle segregation during mitosis [73], promote fission in tumor models [74], and regulate induced pluripotent stem cell reprogramming [75]. It is noteworthy that there is evidence for regulation by ERK1/2 in effector T cells. Dephosphorylation of Ser673 has been shown to mediate mitochondrial fission and positioning of mitochondria at the immunological synapse in Jurkat T cells [48]. In addition, Drp1 can be phosphorylated at Ser616 in primary T_{eff} cells [40]. Interestingly, it was found that Ser616 modification was lost upon further differentiation into T_{mem} cells, thus coinciding with metabolic reprogramming to OXPHOS and mitochondrial fusion. Which kinase targets this site during this differentiation process remains unclear. A further mechanism that might be relevant for immune cells is the regulation of Drp1 by *O*-GlcNAcylation [76], a process that is heavily dependent on sufficient nutrient availability [77]. In cardiac myocytes, Drp1 *O*-GlcNAcylation led to increased mitochondrial fission and decreased mitochondrial membrane potential. Importantly, *O*-GlcNAcylation levels have been shown to be upregulated in highly glycolytic immune cells, including T_{eff} cells and activated macrophages [52,78]. Whether *O*-GlcNAcylation impacts on mitochondrial dynamics in these cells remains unclear.

Fine-Tuning of Mitochondrial Dynamics by Metabolic Cues

Although the overall emerging picture links mitochondrial fusion and fission to catabolic and anabolic states, respectively, it must be mentioned that opposing mechanisms have also been reported. Limited glucose availability was shown to enhance Drp1 activity through AMPK-induced phosphorylation of Mff, thus leading to enhanced mitochondrial Drp1 recruitment and mitochondrial fragmentation in U2Os cells and mouse embryonic fibroblasts [79]. Furthermore, in cultured cells mild oxidative stress has been shown to promote disulfide-mediated dimerization of Mfn molecules, thus driving organelle tethering and subsequently fusion [80], while excessive ROS signaling leads to mitochondrial fission [81]. It is likely that targeting multiple steps of the fission/fusion process provides cells with an exquisite level of control to shape their morphology to the exact metabolic state. In this respect it is worth noting that metabolically active molecules have also been implicated in other aspects of mitochondrial biology. For example, in neurons increased GlcNAcylation of milton, part of the mitochondrial adaptor complex miro/milton associating with the motor protein kinesin-1, reduces mitochondrial transport in response to high glucose levels [82]. Similarly, high cytosolic Ca^{2+} levels shave been shown to halt mitochondrial movement in T cells [49]. At the molecular level this is mediated by Ca^{2+} binding to the EF hands of miro. This results in an altered interaction with kinesin-1, blocking its interaction with microtubules and thus transport [83]. Interestingly, Ca^{2+} also activates several mitochondrial enzymes, including the pyruvate, α-ketoglutarate, and isocitrate dehydrogenases [84], and it is thus clear that Ca^{2+} serves as a mitochondrial master-

Box 3. Mitochondria as Part of a Functional Organelle Network

Mitochondria are functionally coupled to most other organelles inside the cell (Figure I). Driven by changes in mitochondrial dynamics, they are known to regulate autophagosomal substrate selectivity. While damaged or fragmented mitochondria can be degraded by mitophagy, mitochondrial hyperfusion has been shown to spare them from autophagosomal degradation [12,14,105]. Beyond mitochondrial dynamics, direct membrane contact sites (MCSs) represent another form of interorganelle communication. The close proximity between the membranes of two organelles facilitates the exchange of ions and metabolites such as calcium, fatty acids, and amino acids, often coupling organelle function in a bidirectional manner. Many MCSs between mitochondria and other organelles have been identified. In yeast these include the **vacuole**–mitochondria patches (vCLAMPS) [106,107] and the ER–mitochondria encounter sites (ERMES) [108], while in mammals Mfn2 functions as a mitochondrion–ER tether [33], and Plin5 connects mitochondria with lipid droplets [109]. MCSs have recently received increased attention because they are linked to several human diseases and their functionality affects apoptosis, immune response, organelle dynamics/function, and ion or lipid homeostasis [55,87,110]. In addition to such direct contact modes, mitochondria are also indirectly coupled to many other organelles. For example, in aging yeast, decreased vacuolar acidity and amino acid storage subsequently leads to loss of mitochondrial membrane potential and mitochondrial fragmentation [111]. Large-scale remodeling of the mitochondrial proteome accompanies this process [112]. Functional coupling between the **lysosome** and mitochondria also occurs in mammalian cells. In T cells, defects in mitochondrial respiration induce lysosomal sphingomyelin accumulation and dysfunction, disrupting the endolysosomal trafficking pathway and **autophagy** [113]. The impaired lysosomal function drives T cells towards a proinflammatory state and exacerbates the *in vivo* inflammatory response. This study exemplifies the detrimental capacity of this organelle crosstalk, where defects in one subsystem lead to spreading effects and thus systemic cell failure.

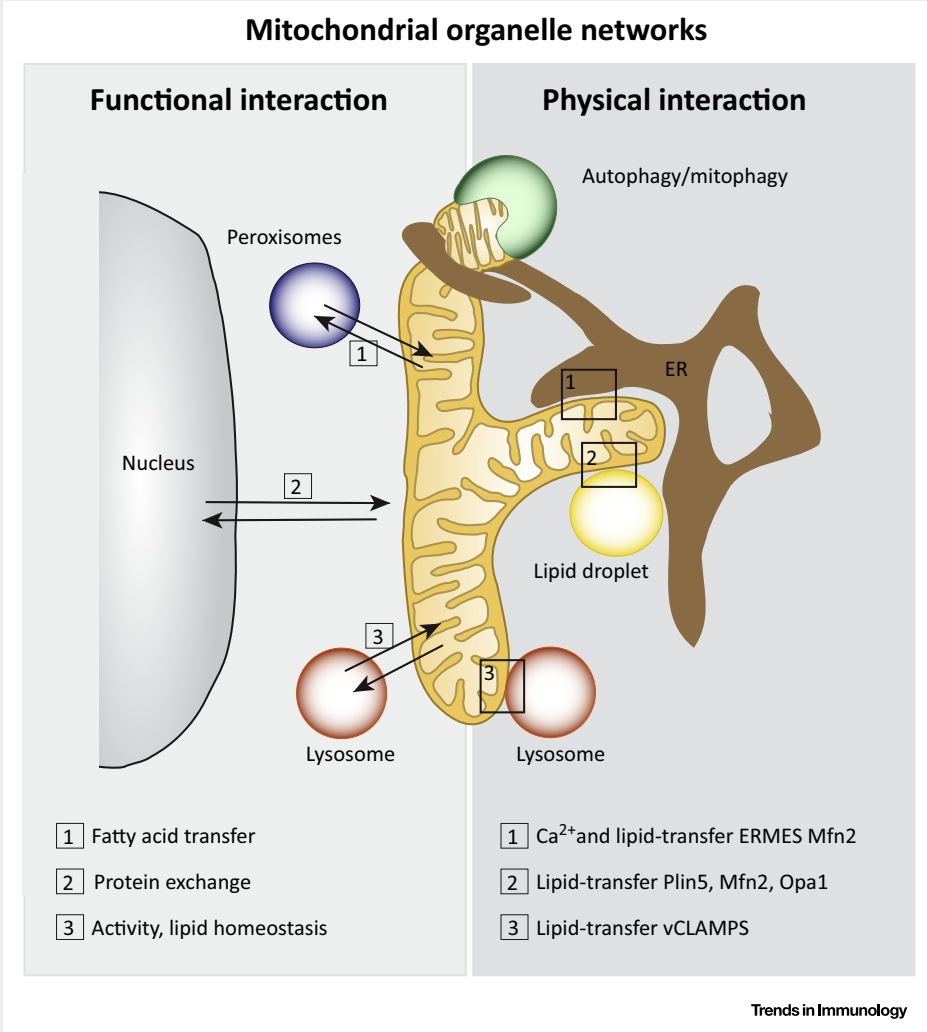

Figure I. Mitochondria are Functionally Coupled to Other Organelles. Mitochondria functionally interact with a multitude of other cellular organelles, leading to often bidirectional and widespread functional interactions. Such interactions can be of an indirect (left) or direct nature, driven by membrane contact sites (MCSs) (right), and can be shaped by mitochondrial dynamics.

regulator [85]. Insights into the integrative potency of Ca^{2+} are revealed by studies in Jurkat T cells, where Ca^{2+}-driven fission and positioning of mitochondria to the IS contributes to the signaling strength of the T cell receptor in a Ca^{2+}-dependent manner [49]. Further research is warranted to dissect how Ca^{2+} and other metabolic cues are used to fine-tune mitochondrial biology and immune cell function.

Concluding Remarks and Future Directions

In this review we have summarized recent exciting findings on how mitochondrial dynamics and metabolism are interlinked to shape immune cell function and fate. In recent years a growing number of studies have highlighted that mitochondria can acquire immune-stage specific morphologies. Preventing immune cells from reaching such states had dramatic consequences, impairing their metabolic reprogramming and/or the acquisition of specific functions. While our insight is still limited to only a few immune cell types, these data provide an exciting model in which mitochondrial dynamics are used as nodal point to integrate and shape immunometabolism and function. Together with additional studies on other immune cell types, this now awaits further questions that are inherent to a physiological immune response, such as how do differences in the cellular environment, substrate availability, and crosstalk with other cell types affect mitochondrial dynamics and cell function? While this will help us to gain more insight into the physiological relevance of mitochondrial dynamics, one of the most challenging tasks ahead will be to identify the molecular mechanisms underlying such processes. Such difficulties will mostly result from the functional redundancy of the fission/fusion machinery, where their depletion will not only alter mitochondrial morphology and function but also impact on the shape or function of other organelles [25,33,86]. Further complicating this issue is the fact that mitochondria in general are functionally coupled to a multitude of other organelles [87] (Box 3). It is therefore becoming increasingly clear that mitochondria must be viewed as functional organelle-networks rather than autonomous entities. Such networks are likely to be advantageous when immune cells are required to quickly adapt to new bioenergetic states across multiple organelle compartments. However, networks also harbor the danger that, once one of the compartments fails and cannot be repaired, defects can spread throughout the organelle interactome, eventually leading to systemic cell failures. Given these facts, it will be important to not only gain better tools to manipulate specific aspects of mitochondrial dynamics (without affecting the function of mitochondria), but also to invest in novel systems approaches directed at identifying mitochondria-driven organelle-communications pathways. Together, these advances might provide us with more tractable models to decipher mito-metabolic crosstalk and help to pave the way toward targeting mitochondria (or other organelles) as part of an immunopathological response.

Outstanding Questions

Do different immune cell types respond similarly when mitochondrial fission and fusion dynamics are altered, and how do they shape their metabolism and function?

How are mitochondrial dynamics and immune metabolism modulated in response to different inflammatory microenvironments or infectious settings *in vivo*?

What are the signals that shape mitochondrial dynamics in such different milieus?

How do mitochondrial interactions with other organelles contribute to the regulation of immune cell metabolism and function?

Can the modulation of mitochondrial dynamics serve as therapeutic strategy to manipulate immunometabolism and thus immune cell function?

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