Biochemical Pharmacology 94 (2015) 173–185

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

# Mitochondria: A target for bacteria

Elodie Lobet [a], Jean-Jacques Letesson [b], Thierry Arnould [a,*]

[a] Laboratory of Biochemistry and Cellular Biology (URBC), NAmur Research Institute for LIfe Science (NARILIS), University of Namur, 61 Rue de Bruxelles, 5000 Namur, Belgium
[b] Research Unit in Microorganisms Biology, University of Namur, 61 Rue de Bruxelles, 5000 Namur, Belgium

ARTICLE INFO

*Article history:*
Received 1 December 2014
Received in revised form 12 February 2015
Accepted 12 February 2015
Available online 20 February 2015

*Keywords:*
Mitochondria
Bacteria
Metabolism
Calcium
mtROS
Immunity

ABSTRACT

Eukaryotic cells developed strategies to detect and eradicate infections. The innate immune system, which is the first line of defence against invading pathogens, relies on the recognition of molecular patterns conserved among pathogens. Pathogen associated molecular pattern binding to pattern recognition receptor triggers the activation of several signalling pathways leading to the establishment of a pro-inflammatory state required to control the infection.

In addition, pathogens evolved to subvert those responses (with passive and active strategies) allowing their entry and persistence in the host cells and tissues. Indeed, several bacteria actively manipulate immune system or interfere with the cell fate for their own benefit. One can imagine that bacterial effectors can potentially manipulate every single organelle in the cell. However, the multiple functions fulfilled by mitochondria especially their involvement in the regulation of innate immune response, make mitochondria a target of choice for bacterial pathogens as they are not only a key component of the central metabolism through ATP production and synthesis of various biomolecules but they also take part to cell signalling through ROS production and control of calcium homeostasis as well as the control of cell survival/programmed cell death. Furthermore, considering that mitochondria derived from an ancestral bacterial endosymbiosis, it is not surprising that a special connection does exist between this organelle and bacteria. In this review, we will discuss different mitochondrial functions that are affected during bacterial infection as well as different strategies developed by bacterial pathogens to subvert functions related to calcium homeostasis, maintenance of redox status and mitochondrial morphology.



*Abbreviations:* A/A, ammonia/ammonium; AIM2, absent in melanoma 2; AMPK, AMP-activated protein kinase; APC, antigen presenting cell; CpG-ODN, CpG-oligodeoxynucleotide; DAMP, damages-associated molecular pattern; DC, dendritic cells; DRP1, dynamin-related protein 1; ECSIT, evolutionarily conserved signalling intermediate in Toll pathways; Eis, enhanced intracellular survival; ER, endoplasmic reticulum; ERRα, estrogen-related receptor alpha; ETC, electron transport chain; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPX, glutathione peroxidase; HIF-1α, hypoxia-inducible factor-1 alpha; IFNβ/IFNγ, interferon-beta/interferon-gamma; IL-4, interleukin-4; IRF, interferon regulatory factor; JNK, Jun N-terminal kinase; LLO, listeriolysin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAVS, mitochondrial antiviral signalling protein; MCU, mitochondrial calcium uniporter; MDA-5, melanoma differentiation-associated gene-5; mtDAMP, mitochondrial DAMP; mtDNA, mitochondrial DNA; mtROS, mitochondrial ROS; MyD88, myeloid differentiation primary response gene 88; NFAT5, nuclear factor of activated T-cells 5; NFκB, nuclear factor-kappa B; NRL, NOD-like receptor; NOX, NADPH oxidase; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PAMP, pathogen-associated molecular pattern; PGC-1β, PPAR gamma coactivator-1 beta; PI3K, phosphoinositide 3-kinase; PMN, polymorphonuclear neutrophil; PPA, propionic acid; PPAR, peroxisome proliferator-activated receptor; PPP, pentose phosphate pathway; PRR, pattern recognition receptor; PRX, peroxyredoxins; RLR, Rig-I like receptor; ROS, reactive oxygen species; SAM, sorting and assembly machinery; SERCA, sarco/endoplasmic reticulum calcium ATPase; SLO, streptolysin; SOD, superoxide dismutase; STAT6, signal transducer and activator of transcription 6; STING, stimulator of interferon genes; TXSS, type X secretion system; TACE, TNFα-converting enzyme; TCA, tricarboxylic acid; TcdB, *Clostridium difficile* toxin B; TCR, T-cell receptor; TFAM, mitochondrial transcription factor A; TIM, translocase of the inner membrane; TLR, Toll-like receptor; TNFα, tumour-necrosis factor alpha; TOM, translocase of the outer membrane; TRAF6, TNFα receptor-associated factor 6; UCP-2, uncoupling protein-2; VacA, vacuolating cytotoxin A; VDAC, voltage-dependent anion channel.

* Corresponding author. Tel.: +32 81 72 41 25; fax: +32 81 72 53 06.
*E-mail addresses:* elodie.lobet@unamur.be (E. Lobet), jean-jacques.letesson@unamur.be (J.-J. Letesson), thierry.arnould@unamur.be (T. Arnould).

http://dx.doi.org/10.1016/j.bcp.2015.02.007
0006-2952/

## 1. Introduction

Mitochondria are dynamic organelles with a morphology controlled by fusion and fission events [1] that evolved from endosymbiotic α-proteobacteria belonging to *Rickettsia* gender [2]. They still present many similarities with prokaryotic cells such as a double membrane, the ability to produce ATP through the generation of a proton gradient generated across the inner membrane or the fact that they have their own genome and bacterial type ribosomes [2]. The bacterial origin of mitochondria is further supported by the fact that various antibiotics, especially bactericidal ones such as quinolones, aminoglycosides and β-lactams are also able to induce mitochondrial dysfunction and reactive oxygen species (ROS) production [3].

The mitochondrial DNA (mtDNA) encodes two ribosomal, 22 transfer RNA and only 13 peptides of the mitochondrial proteins involved in the oxidative phosphorylation (OXPHOS) system. Most of the mitochondrial proteome is thus encoded by the nuclear genome [4].

Mitochondrial double membrane results in the formation of four sub-compartments. Firstly, the outer mitochondrial membrane (OMM) contains numerous porins that make it passively permeable to small molecules (<5 kDa). Secondly, the inter-membrane space (IMS) sequesters numerous of proteins acting as damages-associated molecular patterns (DAMPs), such as cytochrome c, endonuclease G, apoptosis-inducing factor (AIF), and several pro-caspases, which are also recognised by pattern recognition receptors (PRR) [5]. Indeed, their release in the cytosol will induce inflammation and/or cell death. As subversion of mitochondrial death pathways has already been extensively reviewed [6], mechanisms related to apoptotic cell death will not be developed in this review. Thirdly, the inner mitochondrial membrane (IMM) contains the different complexes of the respiratory electron transport chain (complexes I–IV) as well as the Fo-F1 ATP synthase (complex V), which are responsible for ATP production by the OXPHOS. This membrane is however much more impermeable than the OMM. Furthermore, cardiolipin, a phospholipid found exclusively in inner mitochondrial membrane and bacterial plasma membrane, make the IMM less fluid [7]. Consequently, metabolites have to use a variety of selective transporters to cross the inner membrane. The surface of this membrane forms cristae to increase the ability to produce ATP in the matrix. In addition, many mitochondrial proteins encoded by the nucleus will need the import and sorting machinery present in both OMM and IMM to reach the different sub-compartments [8]. According to the compartment they reach, proteins will use different transport complexes: translocase of the outer membrane (TOM)/translocase of the inner membrane 23 (TIM23)/presequence translocase-associated motor (PAM) for the matrix, TOM/TIM23 or TOM/TIM22 for IMM, TOM/mitochondrial inter-membrane space assembly (MIA) for IMS and TOM/sorting and assembly machinery (SAM) for β-barrel proteins located in the OMM [8]. Finally, the matrix contains multiple copies of mtDNA organised into nucleoids as well as the machinery that is necessary to transcribe and translate mtDNA-encoded genes. Reducing agents (NADH and $FADH_2$) are also generated in the matrix by the tricarboxylic acid (TCA) cycle and the fatty acid β-oxidation (FAO) [9].

Even if mitochondria still share some features with its bacterial ancestor, the organelle also acquired new characteristics such as a dynamic morphology of the mitochondrial network that affects both mitochondrial activity and function. According to cell types and functional status, mitochondria can thus shift from separated rounded/fragmented mitochondria into interconnected and elongated tubular network [1]. This very dynamic organelle thus continuously adapts the morphology and move to specific cellular sub-compartments using different components of the cytoskeleton [1]. The mitochondrial morphology is determined by the balance between two opposing processes that occur continually in the cell: the mitochondrial fission and fusion that are mediated by large GTPases related to the dynamin superfamily [10]. The fusion occurs in two steps: first the fusion of OMM mediated by the homo-/heterodimerisation of mitofusin1/2 (MFN1/2) and then optic atrophy 1 (OPA1) that forms homodimers leading to IMM fusion. Fission process requires the recruitment of dynamin-related protein 1 (DRP1) to the outer mitochondrial membrane, where it will assemble to form a constriction ring leading to the fission. Four different receptors for DRP1 located in the outer membrane have been identified so far: mitochondrial fission 1 (FIS1), mitochondrial fission factor (MFF) and mitochondrial dynamics protein of 49 and 51 kDa (MID49 and MID51) [1]. It is important to note that mitochondrial morphology influences the mitochondrial (dys)function while mitochondrial functional status also controls the dynamics and shape of the organelle [11]. Indeed, extremely depolarised and fragmented mitochondria are degraded by mitophagy, a specific form of autophagy [1]. The best-characterised mitophagy pathway involves the recruitment of Parkin (an ubiquitin ligase) from the cytosol to the OMM by PTEN-induced putative kinase 1 (PINK1). This relocation also allows Parkin to poly-ubiquitinate proteins located in the OMM, leading to their degradation by the 26S proteasome [12].

## 2. Mitochondrial targeting by bacteria

While the impact of the mitochondria functional status on the efficiency and persistence of infection and/or trafficking (for intracellular bacteria) is still poorly understood, the effects of bacteria infection on several parameters of the mitochondrial population start to be better delineated.

First, to impact mitochondria, bacterial effectors need to cross several barriers. They have first to be secreted out of the different layers of the bacterial envelope through dedicated secretion systems, then to reach (and pass through) the host plasma and organelle membranes. Several bacterial effectors, collectively called AB toxin, once secreted in the extracellular medium, are able to translocate inside the eukaryotic cell cytoplasm [13]. The B domain is responsible for the cellular tropism and often induces the receptor mediated-endocytosis [14] of the holotoxin followed by the translocation of the A domain into the cytoplasm of the targeted cell. The A domain has an activity responsible for the "toxic effect" such as the glycosyl transferase activity of the *Clostridium difficile* toxin B (TcdB) [14] or the pore forming activity of the *Helicobacter pylori* VacA toxin [15]. Moreover, in Gram-negative bacteria, some complex secretion systems are able to deliver effectors directly from the bacterial cytoplasm into host cytoplasm [16] as observed for EspF of the enteropathogenic *Escherichia coli* [6]. It is important to note that, to impact mitochondria, effectors do not necessarily have to enter host cells. Indeed, pore-forming toxins can induce mitochondrial dysfunction and organelle fragmentation just by inducing ion fluxes through the plasma membrane (e.g. listeriolysin (LLO) of *Listeria monocytogenes* [17]). Once in host cells, toxins that directly target mitochondria have to enter and reach the appropriate mitochondrial sub-compartment. They usually interact with mitochondrial translocase complex to be imported (e.g. OMM: *Neisseria* PorB [18], IMM: *H. pylori* VacA (*vacuolating cytotoxin A*) [15] and mitochondrial matrix: enteropathogenic *E. coli* MAP effector [19]).

As mitochondria evolved from an ancestral bacterium, some similarities are observed between proteins containing mitochondrial targeting sequence (MTS) and sequences targeting to bacterial cytoplasmic membrane such as the presence of a cleavable hydrophobic domain containing non-conserved amino