Recent research on the mechanism underlying the interaction of bacterial pathogens with their host has shifted the focus to secreted microbial proteins affecting the physiology and innate immune response of the target cell. These proteins either traverse the plasma membrane via specific entry pathways involving host cell receptors or are directly injected via bacterial secretion systems into the host cell, where they frequently target mitochondria. The import routes of bacterial proteins are mostly unknown, whereas the effect of mitochondrial targeting by these proteins has been investigated in detail. For a number of them, classical leader sequences recognized by the mitochondrial protein import machinery have been identified. Bacterial outer membrane β-barrel proteins can also be recognized and imported by mitochondrial transporters. Besides an obvious importance in pathogenicity, understanding import of bacterial proteins into mitochondria has a highly relevant evolutionary aspect, considering the endosymbiotic, proteobacterial origin of mitochondria. The review covers the current knowledge on the mitochondrial targeting and import of bacterial pathogenicity factors.

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Introduction
It is generally accepted that mitochondria have arisen from an endosymbiotic alpha-proteobacterial ancestor [1,2]. This fact is reflected in the protein content of mitochondria, many of mitochondrial proteins having counterparts in modern-day bacteria [3]. During the establishment of endosymbiosis, mitochondria have transferred the information for the majority of their proteins to the host cell nucleus. As a consequence, the import of nuclear-encoded proteins into mitochondria made necessary the development of protein import machines. It is possible that one process was accelerated by the other, or that both processes happened simultaneously.

Mitochondria have been identified as the target of an increasing number of bacterial proteins, which are transferred to the cell during infection, a process that often plays a crucial role in bacterial pathogenicity [4]. Consistent with a central role of mitochondria in apoptosis regulation [5], nearly all identified bacterial proteins targeting mitochondria modulate host cell death. They frequently induce the release of pro-apoptotic factors like cytochrome c and cause a breakdown of the mitochondrial membrane potential (Δψm). Less is known about cell entry route and mitochondrial import, whereas mitochondrial phenotypes caused by bacterial effectors are well investigated.

Multiple traits indicate parallels between bacterial and mitochondrial protein transport systems. For example, a peptidase from Rickettsia prowazekii with structural homology to mitochondrial processing peptidase (MPP) can cleave MPP substrates, the authentic signal sequences of proteins targeting mitochondria [6]. Additionally, certain bacterial signal peptides are recognized by mitochondrial import receptors and mediate protein transport into mitochondria [7]. Moreover, bacterial pathogenicity factors have been shown to contain N-terminal mitochondrial targeting signals [8,9] capable of directing to mitochondria any protein fused to it. Bacterial porins can also be targeted to host mitochondria [10–12]. They assume β-barrel topology and are therefore similar to mitochondrial porin, or voltage-dependent anion-regulated channel (VDAC). Remarkably, β-barrel proteins are found exclusively in outer membranes of bacteria, mitochondria, and chloroplasts, pointing to a common evolutionary origin of this protein class. Besides proteins with N-terminal mitochondrial presequence or β-barrel structure, a heterogeneous class of bacterial proteins including many bacterial toxins target mitochondria by so far unknown import routes [13–20].

Here, we will summarize the current view on the mitochondrial targeting and import of an emerging class of bacterial pathogenicity factors.

Protein import into mitochondria
Protein import machines of mitochondria are complex and differ between organisms, though a core of each machine seems to be present in all eukaryotes [21*].
Most of the components have been first described and functionally studied in fungal systems, to be later identified in mammals [22]. In the outer membrane, practically all proteins are first recognized by the translocase of the outer mitochondrial membrane (TOM) complex, which consists of the core component Tom40, receptors Tom20, Tom22, and Tom70, and various numbers of small Tom proteins (Figure 1). Proteins are afterwards transferred either to the TIM complexes in the inner mitochondrial membrane or to the SAM/TOB complex in the outer mitochondrial membrane. The TIM23 complex, with the PAM complex, is responsible for the import of proteins containing an N-terminal presequence, which are usually matrix or inner-membrane proteins. Preproteins are processed by the MPP. The TIM22 complex is necessary for the membrane integration of proteins that contain internal targeting signals, as are carrier proteins of the inner mitochondrial membrane. The SAM/TOB complex, with Sam50 as the central component, facilitates membrane integration of β-barrel proteins [23–25]. Recently, metaxins have also been identified as functioning with Sam50 during integration of β-barrel proteins in mammalian mitochondria, though they probably exist in a protein complex separate from Sam50 [26].

The only one of these complexes with a bacterial counterpart is the SAM complex. Its core component, Sam50, shares sequence homology with Omp85, a protein of the outer membrane of Neisseria sp. Similar to Sam50 in mitochondria, Omp85 facilitates the integration of Neisseria porins into the outer bacterial membrane [27]. Omp85 homologs have since been identified in other bacteria, as well as in mitochondria and chloroplasts of all other organisms [28–30]. However, the main entry point of practically all proteins into mitochondria, the TOM complex, has no homologous machinery in bacteria [21].

**Bacterial proteins with N-terminal mitochondrial targeting sequence**

Mitochondrial-associated protein (Map, former Orf19) and EspF from enteropathogenic E. coli (EPEC) are the best studied bacterial proteins that possess an N-terminal mitochondrial presequence with putative cleavage signal. Both proteins are injected into host cells via the type 3 secretion system (TTSS) and colocalize with host mitochondria [8,9,31].

Mitochondria-associated Map causes the dissipation of ∆Ψm and mitochondrial fragmentation, but appears to be dispensable for EPEC-induced host cell apoptosis [8,31**]. A smaller form of Map can be found, possibly representing a mitochondrial imported form cleaved by the host MPP [8]. In vitro import assays with mitochondria from temperature-sensitive yeast mutants have shown that Map import depends on Tom22, Tom40, and...
Bacterial proteins imported into mitochondria

Kozjak-Pavlovic, Ross and Rudel

Mitochondria-targeted bacterial proteins with β-barrel structure

Neisserial PorB proteins and Omp38 from Acinetobacter baumannii belong to this group [4,10–12]. These proteins are not secreted by classical bacterial secretion systems and the details of how they reach the host cell mitochondria during infection are still unknown. Omp38 of A. baumannii is shown to be targeted to mitochondria of Hep2 cells, where it induces the release of cytochrome c and apoptosis inducing factor (AIF), promoting apoptosis [12]. How Omp38 is recognized and taken up by mitochondria has not been investigated. The import of PorB from Neisseria gonorrhoeae has been studied in yeast mitochondria, where it was shown that this protein follows the import pathway similar to that of VDAC, being recognized by Tom20 and transported through the Tom40 pore [33]. In the light of the SAM complex discovery, it remains to be determined whether Sam50, a homolog of neisserial Omp85, plays a role in mitochondrial import of PorB. Gonoococcal PorB expressed in human cells localizes to mitochondria and causes the dissipation of ΔΨm, but not the release of cytochrome c. The previously determined outer membrane localization, however, does not explain the decoupling effect of PorB, leaving the possibility that some of the PorB molecules spontaneously integrate into the inner mitochondrial membrane where they dissipate ΔΨm.

Proteins that do not belong to either category

This large group of proteins consists mainly of bacterial toxins, which are secreted by the bacteria and enter the host cell by endocytosis. Two exceptions are the EPEC-

mtHsp70. Moreover, import of Map requires an intact ΔΨm, typical for proteins imported into the mitochondrial matrix via the TIM23 complex [31**]. It is not known whether homologs of Map, EPEC TrcA/TrcP and Shi-gella IpgB, have similar characteristics [8].

EPEC EspF has also a canonical N-terminal mitochondrial targeting signal and, upon infection, localizes to these organelles. EspF triggers apoptosis during EPEC infection by dissipating ΔΨm, causing cytochrome c release and activating caspases 9 and 3. The N-terminal 34 amino acids of EspF fused to eGFP are sufficient to guide this construct to mitochondria. As for Map, a smaller form of EspF, possibly cleaved by the host MPP, can be detected in infected cells [9]. Recently, Nagai et al. demonstrated that the loss of ΔΨm inhibited the mitochondrial import of EspF, suggesting a role of the inner membrane translocation machinery [32**]. Results from our group indicate that EspFu, an EspF homologue from enterohaemorrhagic E. coli (EHEC), contains an N-terminal mitochondrial targeting sequence, which guides its translocation to mitochondria upon overexpression (V.K.-P., John Leong, T.R., unpublished data).

Among the bacterial toxins associating with mitochondria, the vacuolating cytotoxin (VacA) from Helicobacter pylori has been extensively studied. Both purified and overexpressed VacA localized to mitochondria of mammalian cells and induced the loss of ΔΨm and the release of cytochrome c [13,35]. Radiolabeled VacA was shown to be rapidly imported over the mitochondrial outer membrane, though it seems to lack a mitochondrial targeting sequence [13]. Cytotoxic activity of VacA may come from its tendency to form anion-selective channels in mitochondrial membranes [35]. However, in another report, VacA was found in late endosomes and it was demonstrated that H. pylori-induced cytotoxicity does not require a direct contact between VacA and mitochondria [36].

Secretory and autocrine toxins that require a direct contact with the host cell, such as EPEC EspF and VacA, also interact with mitochondria during infection. Although these toxins are not secreted by classical bacterial secretion systems, their mitochondrial targeting is achieved by both protein sequence and localization of the host cell mitochondria. Among the bacterial toxins associating with mitochondria, the vacuolating cytotoxin (VacA) from Helicobacter pylori has been extensively studied. Both purified and overexpressed VacA localized to mitochondria of mammalian cells and induced the loss of ΔΨm and the release of cytochrome c [13,35]. Radiolabeled VacA was shown to be rapidly imported over the mitochondrial outer membrane, though it seems to lack a mitochondrial targeting sequence [13]. Cytotoxic activity of VacA may come from its tendency to form anion-selective channels in mitochondrial membranes [35]. However, in another report, VacA was found in late endosomes and it was demonstrated that H. pylori-induced cytotoxicity does not require a direct contact between VacA and mitochondria [36].

Conclusions

Bacterial proteins targeting mitochondria comprise an emerging group of pathogenicity factors involved in the regulation of host cell death. The few examples investigated in detail unveiled an involvement of mitochondrial import machines in their uptake (Figure 2). Several proteins for inner-membrane transport and outer-membrane assembly (Sam50) have structural and functional bacterial counterparts, opening the possibility of a co-

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Import of bacterial proteins into mitochondria. EPEC proteins EspF and Map enter mitochondria via the TOM complex and are subsequently sorted to the mitochondrial matrix. Uptake of PorB from N. gonorrhoeae by mitochondria requires the TOM complex. Consistent with the β-barrel topology, PorB may integrate into the outer membrane. A localization to the mitochondrial inner membrane would be consistent with the strong uncoupling activity observed for PorB. The entry routes for VacA from H. pylori and Omp38 from A. baumannii are still date unknown.

<table>
<thead>
<tr>
<th>Bacterial protein</th>
<th>Organism</th>
<th>Mitochondrial phenotype</th>
<th>Biological effect</th>
<th>Targeting signal/Required mitochondrial import proteins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EspF</td>
<td>EPEC</td>
<td>Loss of Δψₘₙ</td>
<td>Induction of apoptosis</td>
<td>N-terminal leader/n.d.</td>
<td>[9,32]</td>
</tr>
<tr>
<td>Map</td>
<td>EPEC</td>
<td>Mitochondrial fragmentation, loss of Δψₘₙ</td>
<td>Induction of apoptosis</td>
<td>Unknown N-terminal leader/Tom40, Hsp70</td>
<td>[8,31]</td>
</tr>
<tr>
<td>Tir</td>
<td>EPEC</td>
<td>Unknown</td>
<td>Induction of apoptosis</td>
<td>Unknown</td>
<td>[34]</td>
</tr>
<tr>
<td>SipB</td>
<td>Salmonella</td>
<td>Membrane fusion</td>
<td>Induction of autophagy</td>
<td>Unknown</td>
<td>[17]</td>
</tr>
<tr>
<td>PorB</td>
<td>N. gonorrhoeae</td>
<td>Release of cytochrome c, loss of Δψₘₙ</td>
<td>Induction of apoptosis</td>
<td>β-Barrel protein/Tom20, Tom40</td>
<td>[11,33]</td>
</tr>
<tr>
<td>PorB</td>
<td>N. meningitidis</td>
<td>Protection from Δψₘₙ loss</td>
<td>Inhibition of apoptosis</td>
<td>β-Barrel protein/n.d.</td>
<td>[10]</td>
</tr>
<tr>
<td>Omp38</td>
<td>A. baumanii</td>
<td>Release of cytochrome c, AIF</td>
<td>Induction of apoptosis</td>
<td>β-Barrel protein/n.d.</td>
<td>[12]</td>
</tr>
<tr>
<td>VacA</td>
<td>H. pylori</td>
<td>Release of cytochrome c</td>
<td>Induction of apoptosis</td>
<td>Unknown/n.d.</td>
<td>[13]</td>
</tr>
<tr>
<td>PVL</td>
<td>S. aureus</td>
<td>Release of cytochrome c</td>
<td>Induction of apoptosis</td>
<td>Unknown/n.d.</td>
<td>[18]</td>
</tr>
<tr>
<td>Lethal toxin</td>
<td>C. sordellii</td>
<td>Release of cytochrome c</td>
<td>Induction of apoptosis</td>
<td>Unknown/n.d.</td>
<td>[16]</td>
</tr>
<tr>
<td>Toxin A</td>
<td>C. difficile</td>
<td>Release of cytochrome c</td>
<td>Induction of apoptosis</td>
<td>Unknown/n.d.</td>
<td>[14]</td>
</tr>
<tr>
<td>Toxin B</td>
<td>C. difficile</td>
<td>Swelling, release of cytochrome c</td>
<td>Induction of apoptosis</td>
<td>Unknown/n.d.</td>
<td>[19]</td>
</tr>
</tbody>
</table>
In silico analyses supported by limited experimental evidence predicted a large number of mitochondria-targeting bacterial proteins [37]. Among these, bacterial pathogenicity factors may constitute a coherent group of proteins involved in the cross-talk between host and pathogen, awaiting their detection also in other bacteria. Such proteins may be identified in phenotypic screens. For example, a connection was established between chlamydial infection and mitochondria in a recent cytotoxicity screen in yeast. One of the proteins significantly impairing yeast growth was shown to target mitochondria [38].

So far, nearly all bacterial proteins identified to target mitochondria appear to play a role in apoptosis regulation (see Table 1). However, the few examples which could not be linked to cell death regulation indicate that there may be a broader function in pathogenicity, still to be discovered. For instance, several of the mitochondria-targeting bacterial proteins like Tir, Map, and EspF also interact with cytosolic host signaling factors involved in cytoskeleton regulation. Cytosolic depletion of such factors by mitochondrial import may influence their function on the host cell cytoskeleton. Modulation of the mitochondrial physiology by bacterial proteins, for example, to adjust the host cell to metabolite or energy source requirements of the pathogen is another possibility. The later assumption may be supported by a recent genome-wide RNA interference screen performed to identify host factors that, when depleted, inhibit the genome of the obligate intracellular bacterium Chlamydia caviae. Of these, members of TIM and TOM import machineries found in mitochondria. The specific chlamydial protein responsible for this effect remains to be identified. It is therefore a safe assumption that bacterial pathogens and host mitochondria as their distant relatives are still communicating in a much closer way than previously anticipated.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The paper reviews the current opinion on the evolution of protein import machineries found in mitochondria.


Using a genetic system, the authors demonstrate the mitochondrial import route of the EPEC Map protein. This is one of the few studies where import has been linked to factors of the mitochondrial protein import machineries.


The authors demonstrate that an EspF point mutant deficient in mitochondrial targeting prevents mitochondrial damage during infection of cell cultures. This same mutant is attenuated in an animal model for EPEC infection. This is the first study to demonstrate that mitochondrial import of a bacterial protein affects pathogenicity in an in vivo animal model.


A genome-wide RNA interference screen identified 54 factors that, when depleted, inhibit C. caviae infection. Among these factors were Tim and Tom proteins suggesting a role for mitochondrial import in C. caviae infection. This is the first screen that identified mitochondrial import as crucial for bacterial infection.