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The role of Erv1 in the mitochondrial import machinery and iron sulphur cluster export machinery in *Trypanosoma brucei*

RNDr. Thesis

Bc. Michala Boudová

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Annotation

Sulfhydryl oxidase Erv1 is a ubiquitous conserved protein of the mitochondrial intermembrane space, which is implicated in mitochondrial protein import as well as in the biogenesis of iron sulphur cluster proteins. While the role of Erv1 is quite well established in higher eukaryotes, Erv1 orthologues of parasitic protists exhibit some structural peculiarities. The study presented in this thesis therefore attempted to elucidate the function of Erv1 in *Trypanosoma brucei*.

Declaration

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Author contributions

The co-author listed below fully acknowledges that Michala Boudová is the first author of the publication presented here. The article is based on the results of her bachelor thesis, which was supervised by Alexander Haindrich, who conceived and planned the experiments. Michala has maintained the cell culture and performed most of the experiments (immunofluorescence assay, aconitase activity measurement, co-immunoprecipitation, measurement of mitochondrial membrane potential, subcellular fractionation and western blotting) or prepared samples for the subsequent analysis (electron microscopy, mass spectrometry). She has also participated in drafting the manuscript. The co-author supports this statement with his signature.

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prof. RNDr. Julius Lukeš, CSc.

Introduction

During the course of evolution of endosymbiotic α-proteobacteria into semi-autonomous organelle, leading to the origin of eukaryotic cells, many genes were lost or transferred to the host nucleus, resulting in the mitochondria coding for as few as about 13 proteins (Taanman 1999, Timmis et al 2004). The development of organellar transport mechanisms therefore necessarily accompanied and followed this process. Whereas the mechanisms that mediated gene transfer are not yet fully understood (Berg & Kurland 2000), a growing body of evidence sheds light on the mitochondrial protein import and export pathways (Dudek et al 2013, Poyton et al 1999).

Mitochondria have long been considered indispensable for eukaryotic cells as they represent the "powerhouse" of the cell performing a pivotal role in cellular metabolism and thus energy production. However, it had been later established that processes such as ATP synthesis by oxidative phosphorylation, citric acid cycle or fatty acid oxidation, could be, under certain conditions, inactivated without affecting cell viability. Herein, it was demonstrated that the essential character of these organelles in fact stems from their involvement in the assembly of a key component required for the biogenesis of iron-sulphur cluster [Fe-S] proteins (Lill et al 2005).

This idea was further supported by findings of organisms that lack classical mitochondria and instead, harbour various reduced forms of the organelle, such as hydrogenosomes or mitosomes (Hjort et al 2010). These evolved by successive loss of mitochondrial genes and its typical functions, with the notable exception of [Fe-S] protein biogenesis (van der Giezen & Tovar 2005).

In eukaryotes there are about 20 components known so far to facilitate the maturation of [Fe-S] proteins and approximately half of them are essential for viability (Lill & Mühlenhoff 2005). The indispensability of [Fe-S] proteins lies in the wide array of cellular processes that they assist, such as enzyme catalysis, electron transport, and regulation of gene expression (Lill et al 2005).

Prokaryotes possess three distinct biosynthetic pathways to generate [Fe-S] proteins, two of which were inherited to eukaryotes in the process of endosymbiosis, namely the [Fe-S] assembly machinery (ISC) present in mitochondria and the sulphur-utilization factors system that was passed to plastids. Additionally, there are two pathways for [Fe-S] protein biogenesis exclusive to eukaryotes, these are the [Fe-S] export apparatus and the cytosolic [Fe-S] assembly (CIA). Hence, the components of mitochondrial ISC machinery display high sequence

similarity with bacterial homologues and, they are conserved among all eukaryotes (Lill & Mühlenhoff 2005).

Mitochondria preform a prime role in [Fe-S] protein biogenesis, thus the function of ISC is central not only to the maturation of mitochondrial, but all cellular [Fe-S] proteins. In particular, to orchestrate the cytosolic [Fe-S] assembly and its transfer to the recipient apoproteins, a sulphur-containing compound (X-S) generated by the ISC machinery is required. Whereas the components of ISC as well as CIA machineries were studied thoroughly, the exact nature of X-S still remains enigmatic, likewise the components that facilitate its translocation across the membranes, members of the [Fe-S] export pathway (Lill & Mühlenhoff 2005).

The first protein identified to take a part in the export process is an ABC transporter (ScAtm1), localized to mitochondrial inner membrane (MIM). ScAtm1 is believed to play a role in the export process, as its depletion results in decreased activity of a cytosolic [Fe-S]-carrying enzyme accompanied by mitochondrial iron accumulation, while the ISC remains unaffected (Kispal et al 1999). An identical phenotype was reported to follow the depletion of a sulfhydryl oxidase (ScErv1), that operates in the mitochondrial intermembrane space (IMS) (Lange et al 2001). Similarly, in the absence of glutathione tripeptide (GSH), defective assembly of [Fe-S]s into cytosolic and nuclear, but not mitochondrial proteins, is observed (Sipos et al 2002). GSH is known to be able to coordinate an [Fe-S] (Qi et al 2012) and, ScAtm1 can interact with GSH, its substrate binding pocket however, provides much more space than for GSH alone, which is thus probably a part of a larger substrate (Srinivasan et al 2014). ScErv1 was suggested to be involved in the export of X-S, upstream of ScAtm1, and may in theory facilitate stabilization of the compound by the introduction of disulphide bridges, prior to its export to cytosol (Lill et al 2014). Although a later study challenged the role for ScErv1 in cytosolic [Fe-S] protein maturation as well as in iron homeostasis (Ozer et al 2015).

Additional to its role in the ISC export apparatus, Erv1 is also implicated in mitochondrial protein import. Specifically, Erv1 is a part of the mitochondrial IMS assembly (MIA), that is responsible for the import of IMS-targeted protein precursors of small size (>20 kDa) into the IMS, these include many proteins of outstanding importance such as components of the translocase of MIM. The substrate protein precursors of MIA are recognized by their conserved patterns of cysteine residues, which are upon the import involved in the formation of disulphide bonds and thus, stable folding into mature proteins in the process of oxidative folding (Mesecke et al 2005).

In yeast, MIA is mediated by the action of two proteins; ScMia40, anchored to the MIM, functions as a receptor to recognize incoming substrates and, as a chaperone to facilitate their

oxidative folding. Subsequent to completing the reaction, ScMia40 is left in its reduced form, which is then re-oxidized by ScErv1, that is recycled by shuttling the electrons to cytochrome c (Stojanovski et al 2012). In the absence of ScErv1, ScMia40 accumulates in its reduced form, leading to the inhibition of protein import into the IMS (Bihlmaier et al 2007, Stojanovski et al 2012).

The MIA pathway known from Opisthokonts is however not conserved in several protozoan lineages, including Kinetoplastida, which lack an obvious Mia40 homologue. On the contrary, Erv1 is conserved in nearly all eukaryotes, raising the question about the import of MIA-substrates and the exact role of Erv1 in this process (Allen et al 2008). Interestingly, kinetoplastid Erv1 exhibits some structural peculiarities such as lack of N-terminal domain otherwise essential for ScErv1/ScMia40 interaction, an extended C-terminal domain and an unusual arrangement of cysteine pair motifs (Eckers et al 2013). This makes it structurally most similar to Erv1 homologue from plants (AtErv1). Curiously, AtMia40, in contrast to ScMia40, is not essential and furthermore, AtErv1 was shown to mediate protein import and oxidative folding in the IMS independently of Mia40 (Carrie et al 2010, Peleh et al 2017).

Considering that unlike Mia40, Erv1 is ubiquitously present and essential in most eukaryotes, and the dependence of Mia40 on Erv1 for its function, it seems plausible, that the evolution of MIA took place in a stepwise manner, proceeding from Erv1-only system that perhaps still operates in early branching eukaryotes such as *Trypanosoma brucei*, to that of Erv1/Mia40 known in Opisthokonts (Deponte et al 2009, Carrie & Soll 2017). This hypothesis is further supported by the fact that in organisms which lost Erv1 from their genome, also the substrates of MIA are absent. Alternatively, in eukaryotes lacking Mia40 homologue, Erv1 may also cooperate with other, yet unidentified oxidoreductase (Allen et al 2008).

Additionally, while some protozoans lost Mia40 homologue as well as MIA substrates, Erv1 is still conserved and presumably functions in the [Fe-S] protein biogenesis. Furthermore, the absence of Erv1 from some organisms correlates not only with the absence of MIA substrates, but also Atm1 orthologue (Allen et al 2008).

Hence, the study presented in this thesis addressed the aforementioned questions using *T. brucei* as a model organism; here we show that Erv1 of *T. brucei* (TbErv1) localizes to the IMS of mitochondria like in other eukaryotes. To test whether TbErv1 has a role in the ISC export machinery, we determined [Fe-S]-enzyme activity in both cytosol and mitochondria, our results suggest that while TbErv1 may be involved in the assembly of mitochondrial [Fe-S]s, it does not participate in the export of X-S into cytosol. Furthermore, affinity purification of the protein combined with mass spectrometry analysis did not detect any interaction with TbAtm1,

neither any protein possibly capable of performing the function of Mia40. Moreover, upon depletion of TbErv1 we observed changes in mitochondrial morphology accompanied by an accumulation of a precursor of the respiratory chain component trCOIV. Our data point to the involvement of TbErv1 in mitochondrial protein import, independent of any interaction partner. The phenotype triggered by the ablation of TbErv1 can thus be explained by the disrupted mitochondrial protein import of either subunits of the translocase of MIM and/or other cysteine-rich proteins, which represent the source of sulphur for the ISC (Haindrich et al 2017).

Subsequently, the role for TbErv1 in mitochondrial protein import was supported by a proteomic study which showed that downregulation of TbErv1 results in impaired import into the IMS, as reflected by the depletion of MIA substrates in the compartment, including subunits of the translocase of the MIM (Peikert et al 2017).

Another recent publication reported the identification of a member of the mitochondrial site and cristae organizing system (MICOS) complex, Mic20, presumably a functional analogue of Mia40. TbMic20 interacts with the MIM, while its extension is present in the IMS and, displays a structural motif similar to the active site of Mia40. Furthermore, the depletion of TbMic20 resulted in reduced IMS protein abundance, with about 25% of proteins downregulated overlapping with those of the aforementioned TbErv1 depletome. While it is feasible that TbMic20 represents the alternative oxidoreductase of the MIA pathway, no interaction between TbMic20 and TbErv1 was detected. However, it is still possible that the interaction between the proteins is very transient and therefore could not be detected by the methods employed, this hypothesis is currently awaiting further validation (Kaurov et al 2018).

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Abstract

Sulfhydryl oxidase Erv1 is a ubiquitous and conserved protein of the mitochondrial intermembrane space that plays a role in the transport of small sulfur-containing proteins. In higher eukaryotes, Erv1 interacts with the mitochondrial import protein Mia40. However, Trypanosoma brucei lacks an obvious Mia40 homologue in its genome. Here we show by tandem affinity purification and mass spectrometry that in this excavate protist, Erv1 functions without a Mia40 homologue and most likely any other interaction partner. Down-regulation of TbErv1 caused a reduction of the mitochondrial membrane potential already within 24h to less than 50% when compared with control cells. The depletion of TbErv1 was accompanied by accumulation of trCOIV precursor, with a concomitant reduction of aconitase activity both in the cytosol and mitochondrion. Overall, TbErv1 seems to have a role in the mitochondrial translocation and Fe-S cluster assembly in the organelle.