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Roles of the Nfu Fe–S targeting factors in the trypanosome mitochondrion

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ABSTRACT

Iron–sulphur clusters (ISCs) are protein co-factors essential for a wide range of cellular functions. The core iron–sulphur cluster assembly machinery resides in the mitochondrion, yet due to export of an essential precursor from the organelle, it is also needed for cytosolic and nuclear iron–sulphur cluster assembly. In mitochondria all [4Fe–4S] iron–sulphur clusters are synthesised and transferred to specific apoproteins by so-called iron–sulphur cluster targeting factors. One of these factors is the universally present mitochondrial Nfu1, which in humans is required for the proper assembly of a subset of mitochondrial [4Fe–4S] proteins. Although most eukaryotes harbour a single Nfu1, the genomes of *Trypanosoma brucei* and related flagellates encode three Nfu genes. All three Nfu proteins localise to the mitochondrion in the procyclic form of *T. brucei*, and *Tb*Nfu2 and *Tb*Nfu3 are both individually essential for growth in bloodstream and procyclic forms, suggesting highly specific functions for each of these proteins in the trypanosome cell. Moreover, these two proteins are functional in the iron–sulphur cluster assembly in a heterologous system and rescue the growth defect of a yeast deletion mutant.

1. Introduction

Trypanosoma brucei and related flagellates are unicellular parasites that cause devastating diseases of humans and livestock and thus have a major impact on health and economy, mostly in sub-Saharan Africa but also in other tropical regions. The trypanosome life cycle is rather complex with different stages in mammalian and insect hosts that differ dramatically in their morphology and metabolic requirements (Matthews, 2005). These differences are generally necessitated by the different environments the parasites find themselves in. In the glucose-rich blood of their mammalian host, the bloodstream form (BSF) relies mainly on glycolysis for its energy generation, while the insect-dwelling procyclic form (PCF) needs a fully functional mitochondrion with active oxidative phosphorylation to meet its energetic demands (Tielens and van Hellemond, 2009). As a consequence, the BSF mitochondrion is much more reduced while its PCF counterpart is highly elaborate, extensively branched and metabolically active (Verner et al., 2015).

Despite these obvious differences, both BSF and PCF mitochondria harbour a similar cohort of proteins important for iron–sulphur cluster (ISC) biogenesis, although their abundance in the BSF is generally much lower (Lukeš and Basu, 2015). ISC biogenesis is the most fundamental process that defines a mitochondrion, and in fact the only known common denominator of all mitochondria and mitochondrion-derived organelles, since it is also found in the most reduced mitosomes of several anaerobic protists (Maguire and Richards, 2014; Makiuchi and Nozaki, 2014).

These evolutionarily ancient and highly important ISCs are cofactors of proteins involved in a variety of cellular functions such as metabolic catalysis, DNA replication and repair, translation and iron regulation to name the most prominent ones (Brzóska et al., 2006). With almost 20 well conserved proteins participating in the mitochondrial stage of ISC biogenesis, the process is rather complex and still not fully understood. We will now briefly describe what is known about the mitochondrial steps of ISC biogenesis in yeast and mammalian cells (using the yeast

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nomenclature for this well conserved process) and compare this with the situation in trypanosomes.

Mitochondrial ISC assembly takes place on the Isu1/Isu2 scaffold (Isu2 having arisen from a gene duplication event specific to yeast) with sulphur being provided by the reduction of cysteine to alanine which is catalysed by the desulfurase complex Nfs1-Isd11 (Mühlenhoff et al., 2003) (Fig. 1). The sulphur is reduced by a dedicated electron transport chain constituted of ferredoxin and ferredoxin reductase, with the iron possibly provided by a putative donor frataxin (Lill et al., 2012). The *T. brucei* scaffold protein Isu and the desulfurase Nfs are both indispensable for the PCF and their depletion negatively impacts on aconitase activity (used as a readout for the synthesis of [4Fe–4S] clusters) (Smíd et al., 2006). Moreover, Isu is also essential in the BSF, and both Isu and Nfs localise to the mitochondrion as well as to the nucleolus in both life stages, although the role these proteins might play there remains a matter of speculation (Kovářová et al., 2014). The desulfurase complex of *T. brucei* was also shown to be required for tRNA thiolation in the PCF, however it remains to be resolved whether this is due to direct involvement of this complex in the process or indirectly by the provision of an ISC for another component (Paris et al., 2010; Kovářová et al., 2014).

With the help of the heat shock protein 70 (Hsp70) chaperones Ssq1 and Jac1, the nascent [2Fe–2S] cluster is temporarily transferred to the monothiol glutaredoxin Grx5 from which it can be directly handed over to target [2Fe–2S] apoproteins (Uzarska et al., 2013). While the trypanosome mitochondrial Hsp70 is mostly involved in mitochondrial DNA maintenance, its presence is also required for ISC synthesis (Týč et al., 2015). The mitochondrial glutaredoxin Grx1 can bind a [2Fe–2S] cluster in vitro and plays an important role in iron metabolism of the parasite (Comini et al., 2008). The Isa1, Isa2 and Iba57 proteins participate in the conversion of [2Fe–2S] to [4Fe–4S] clusters, which are eventually transferred to distinct apoproteins with the help of ISC targeting factors such as Nfu1, BolA3 or Ind1 (Lill et al., 2012). Putative homologues of all these proteins are present in *T. brucei* but, with the exception of the Isa1 and Isa2 proteins (Long et al., 2011), experimental studies have not been published (Lukeš and Basu, 2015). The mitochondrial ISC biogenesis machinery is also essential for the synthesis of cytosolic and nuclear Fe–S proteins, since it depends on the export of a still unknown sulphur-containing compound to the cytosol. The so-called CIA (for cytosolic ISC assembly) pathway is outside the scope of this research and will hence not be discussed here.

There is still a certain lack of knowledge about how discrete subsets of Fe–S cluster apoproteins are recognised by specific targeting factors such as Nfu1. This protein shows homology to the C-terminal domain of NifU, which is a scaffold in ISC biogenesis in nitrogen-fixing bacteria (Fig. 2A) (Smith et al., 2005). In humans and yeast, Nfu1 is responsible for the transfer of [4Fe–4S] clusters to a small subset of mitochondrial proteins which include components of respiratory complexes I and II and lipoic acid synthase (LipA) (Cameron et al., 2011; Navarro-Sastre et al., 2011). What makes Nfu1 particularly compelling to study is its involvement in human disease. Point mutations in, or deficiencies of, the protein cause a fatal mitochondrial disease called Multiple mitochondrial dysfunction syndrome with functional Nfu1 deficiency (MMDS1), which is characterised by symptoms such as lactic acidosis, hyperglycinemia and reduced activity of respiratory chain complexes I and II (Cameron et al., 2011; Navarro-Sastre et al., 2011). Somewhat surprisingly, given the severity of the human phenotype, depletion or deletion of Nfu1 from HeLa cells (Navarro-Sastre et al., 2011) and yeast *Saccharomyces cerevisiae* (Schilke et al., 1999), respectively, causes only a very mild growth phenotype in culture. However, a specific impact on several enzymatic activities has been detected. The levels of lipoic acid-bound enzymes (E2

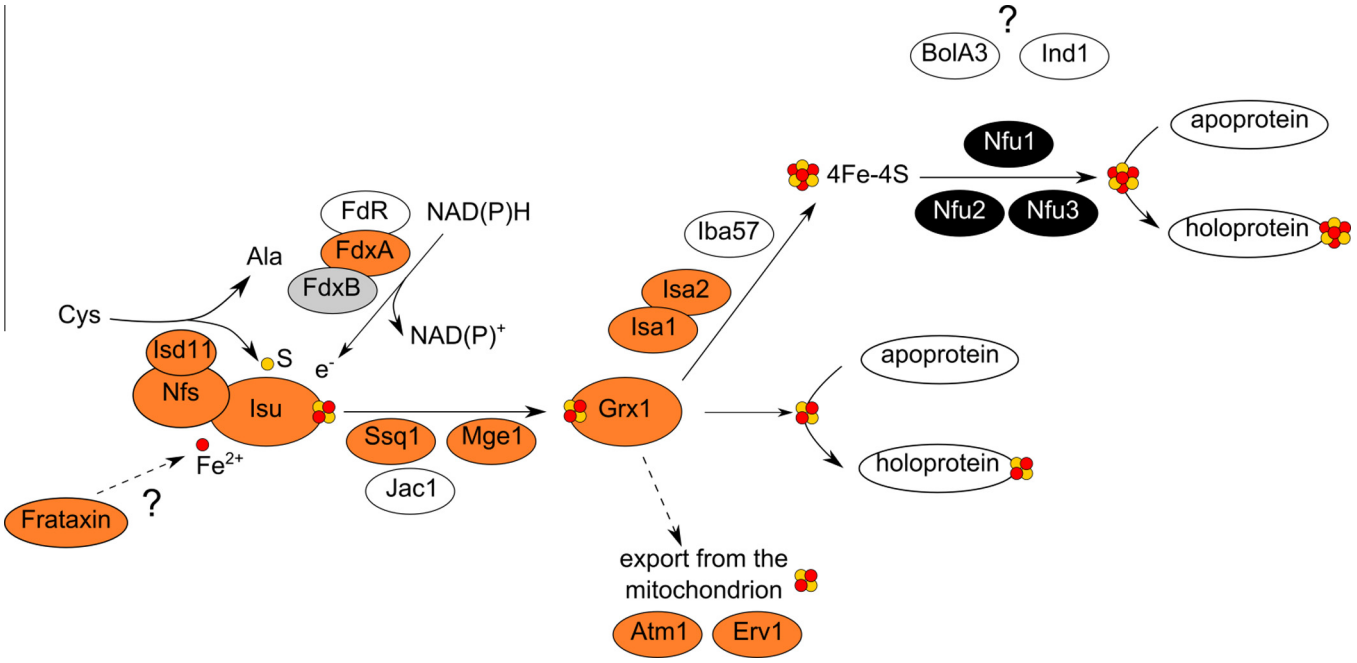

Fig. 1. Scheme of the mitochondrial iron–sulphur clusters assembly pathway in *Trypanosoma brucei*. Components essential in procyclic form are indicated in orange (dark grey), dispensable ones are in light grey, Nfu proteins are in black, and proteins present in the genome but not yet assayed are in white. *Tb*Grx1 is homologous to yeast Grx5. The Nfs-Isd11 desulfurase complex (Paris et al., 2010) provides sulphur on the Isu scaffold (Smíd et al., 2006), while ferredoxins A and B facilitate its reduction (Changmai et al., 2013), and frataxin probably provides iron (Long et al., 2008). Heat shock proteins (Týč et al., 2015) facilitate transfer of newly created [2Fe–2S] on the Grx1 glutaredoxin (Comini et al., 2008). Isa1/2 and Iba 57 proteins enable formation of [4Fe–4S] clusters (Long et al., 2011). A still unknown S-containing component is exported into the cytosol via the inner membrane transporter Atm1 (Horáková et al., 2015), the sulfhydryl oxidase Erv1 of the intermembrane space and glutathione (Basu et al., 2013), and is utilised in the cytosolic iron–sulphur cluster assembly pathway. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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