School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science

Erv1 associated mitochondrial import-export pathway and the cytosolic iron-sulfur protein assembly machinery in *Trypanosoma brucei*

Ph.D. Thesis

Somsuvro Basu

2014

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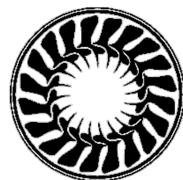

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ANOTATION

This thesis highlights a divergent mitochondrial intermembrane assembly pathway in the parasitic protist *Trypanosoma brucei.* A comparative genomic study reveals the connection of Erv1 with the cytosolic iron-sulfur protein assembly (CIA) pathway in trypanosomatids. Further, the CIA machinery of *T. brucei* has been described using RNAi interference and other biochemical and complementation assays. Finally, part of the divergent CIA machinery has been identified in the human intestinal pathogen *Giardia intestinalis* by means of complementation assays in *T. brucei.*

FINANCIAL SUPPORT

This work was supported by the Grant Agency of the Czech Republic, the Ministry of Education of the Czech Republic, and the Praemium Academiae award to Julius Lukeš.

DECLARATION

I hereby declare that I did all the work presented in this thesis by myself or in collaboration with co-authors of the presented papers and only using the cited literature.

České Budějovice, 17 August 2014 Somsuvro Basu

PROHLÁŠENÍ

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This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Parasitology, Biology Centre of the ASCR, supporting doctoral studies in the Molecular and cell biology and Genetics.

Statement regarding contribution

The thesis is structured based on the following papers (listed chronologically):

1. **Basu S.**, Leonard J.C., Desai N., Mavridou D.A., Ho Tang K., Goddard A.D., Ginger M.L., Lukeš J., Allen J.W. (2013) **Divergence of Erv1-associated mitochondrial import and export pathways in trypanosomes and anaerobic protists**. *Eukaryotic Cell* **12**: 343-355. (IF = 3.18)

Somsuvro Basu prepared the RNAi cell lines, did Western blot analysis for confirming RNAi and transmission electron microscopy, which is ca., 50% of the data accumulated. He also analyzed the data and help writing the manuscript.

2. **Basu S**., Netz D.J., Haindrich A.C, Herlerth N., Lagny T.J., Pierik A.J., Lill R., Lukeš J. (2014) **Cytosolic iron-sulphur protein assembly is functionally conserved and essential in procyclic and bloodstream *Trypanosoma brucei* (In Press**, *Molecular Microbiology*) (IF = 5.03)

Somsuvro Basu prepared almost all RNAi constructs, did electroporations, growth curve analyses, Western blot analyses, measured the enzymatic activities, in total about 60% of the data. Moreover, he analyzed the data and participated in writing the manuscript.

3. Lukeš J., **Basu S**. **Fe/S protein biogenesis in trypanosomes – a review** (Invited review article, **revision submitted**) *BBA Molecular Cell Research*. (IF = 5.29)

Somsuvro Basu wrote ca. 45% of the manuscript and prepared all figures. The results achieved during his PhD thesis are also included in this manuscript.

4. Verner Z., **Basu S**., Benz C., Dixit S., Dobáková E., Faktorová D., Hashimi H., Horáková E., Huang Z., Paris Z., Pena P., Ridlon L., Týč J., Wildridge D., Zíková A., Lukeš J. **The malleable mitochondrion of *Trypanosoma brucei*** (Invited review article, **submitted**) *International Review of Cell and Molecular Biology.* (IF = 4.52)

Somsuvro Basu wrote the 'Fe-S cluster and protein biogenesis' section and prepared the respective figure.

Julius Lukeš, the corresponding author of all mentioned papers, approves the contribution of Somsuvro Basu in these papers as described above.

…………………………………...

Prof. RNDr. Julius Lukeš, Csc.

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How should I start!!!.....this is the toughest nut to crack!!

The journey of the last four and a half years would have been disarrayed without the most influential person in my graduate life, my supervisor Julius Lukeš. Jula (though, the 'Sir → Jula' transformation was quite tough to achieve) proved his patience right from the beginning when the Skype-call got hampered for an hour during my interview. His guidance, passion to science, ability 'to remember each-and-every Western blot' I have produced, encouraged me to grow in every step of my PhD. The time he is devoting towards his students is more than commendable. I will never forget this advice from him: "always do controls-of-controls-of-controls." We also had pretty good time talking about politics over 'few beers.' Overall, the time spent in Jula's den was remarkable.

I should not forget the immense efforts Eva Horáková has provided during the last three years. I am going to miss our 'weekly-cluster-meetings' badly. Thank you Evička, for everything!!

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Abbreviations

ABCE1	ATP Binding Cassette E1
Atm1	ABC Transporter, Mitochondrial
BOLA3	bolA-like protein family
BS	Bloodstream Stage
Cfd1	Cytosolic Fe/S cluster Deficient
CIA	Cytosolic Iron-sulfur protein Assembly
Cox17/19	Cytochrome *c* Oxidase
Dre2	Derepressed for Ribosomal protein S14 Expression
EPR	Electron Paramagnetic Resonance
Erv1	Essential for Respiration and Viability
FAD	Flavin Adenine Dinucleotide
Fe/S	Iron-sulfur
FMN	Flavin Mononucleotide
GFP	Green Fluorescence Protein
Grx	Glutaredoxin
HA	Hemagglutinin
HCF101	High Chlorophyll Fluorescence
Hep1	mtHsp70 Escort Protein
Hot13	Helper Of Tim
Hsp	Heat Shock Protein
IBA57	Iron-sulfur cluster assembly factor for Biotin synthase and Aconitase-like mitochondrial proteins
IMS	Inter-membrane Space
IND1	Iron-sulfur protein required for NADH Dehydrogenase
IRP1/2	Iron Regulatory Protein
ISC	Iron-Sulfur Cluster
ISD11	Iron-Sulfur protein biogenesis, Desulfurase-interacting protein
kDNA	Kinetoplastid DNA
LGT	Lateral Gene Transfer
Mge1	Mitochondrial GrpE
MIA	Mitochondrial Intermembrane space Assembly
Mms19	Methyl-Methanesulfonate Sensitivity
mt	Mitochondrial
NADP(H)	Nicotinamide Adenine Dinucleotide Phosphate
Nar1	Nuclear Architecture Related
Nbp35	Nucleotide Binding Protein
Nfs1	NifS-like protein
NFU1	NifU-like protein
NIF	Nitrogen Fixation
NTP	Nucleoside Triphosphate
PNO	Pyruvate: NADP Oxidoreductase

PS	Procyclic Stage
PTP	ProtC-TEV-ProtA
RNAi	RNA interference
SAM	S-adenosylmethionine
SUF	Sulfur mobilization
Tah18	Top1T722A mutant Hypersensitive
TAP	Tandem Affinity Purification
Tb	*Trypanosoma brucei*
TET	Tetracycline
TIM	Translocase of the Inner Membrane
VSG	Variant Surface Glycoprotein

1. SUMMARY

Trypanosoma brucei, a unicellular protist and the model organism of this thesis, is the etiological agent of the human African sleeping sickness and nagana in livestocks. The available nuclear and mitochondrial genomes and an easy cultivation made this eukaryote a subject of many research studies, making use of several available tools of forward and reverse genetics.

The first part of the thesis deals with the characterization of the TbErv1-associated mitochondrial import-export machineries of *T. brucei.* We showed the divergence of the mitochondrial intermembrane space, which is likely a consequence of the absence of the key player Mia40. It was demonstrated that TbErv1 is capable of passing electrons to both oxygen and cytochrome *c*. Additionally, a comparative genomics analysis indicated the conservation of proteins engaged in the delivery and/or assembly of the cytosolic Fe/S proteins in trypanosomatids **(4. Published results, study # 1, Basu *et al.* 2013, *Euk. Cell.*).**

The second part highlights the cytosolic iron sulfur protein assembly (CIA) machinery in *T. brucei*. This essential biosynthetic process is remarkably conserved in the studied protist. An extensive reverse genetic approach has been taken to characterize the early and middle parts of the pathway, for which RNAi depletion of one or two genes have been performed. The utility of the CIA electron transfer chain module containing TbTah18-TbDre2 has been demonstrated using spectroscopic studies. Moreover, in several cases, we have been able to complement yeast mutants expressing *T. brucei* proteins **(4. Published results, study # 2, Basu *et al.* 2014 *Mol. Microbiol.*).**

In the following part we studied the CIA targeting complex including three proteins, TbCia2A, TbCia2B and TbMms19. Interestingly, both TbCia2 proteins are essential in the bloodstream stage but not in the procyclic stage *T. brucei.* Besides, only TbCia2B could complement yeast Cia2 depleted mutants **(5. Unpublished results, study # 1, Basu *et al.*, in progress).**

In the most current work of this thesis, we scrutinized the CIA pathway of *Giardia intestinalis* where some interesting deviation from the classical pathway have been observed. We expressed individually *G. intestinalis*-specific two Tah18-like proteins in *T. brucei* TbTah18-TbDre2 double RNAi knockdown cell line, which partially rescued its growth and cytosolic aconitase activity **(5. Unpublished results, study # 2, Pyrih *et al.,* in progress).**

Finally, we characterized the *T. brucei* mitochondrial Hsp70 (mtHsp70) and its co-chaperone Hep1 and the nucleotide exchange factor Mge1. Being bot essential for the parasite, TbHep1 and TbMge1 were shown to prevent aggregation of TbmtHsp70 **(5. Unpublished results, study # 3, Týč *et al.*, in progress).**

2. OVERVIEW

2.1. *Trypanosoma brucei* and African trypanosomiasis

Trypanosoma brucei, a protozoan parasite, belongs to the eukaryotic supergroup Excavata, kingdom Euglenozoa. Trypanosomes are placed under Kinetoplastea which obtained its name from kinetoplast, the mass of DNA located within the single mitochondrion of these parasitic protists (Lukeš et al., 2014).

T. brucei is the causative agent of the human African trypanosomiasis, colloquially known as sleeping sickness, and nagana in cattle (Barrett et al., 2003). There are three different *T. brucei* subspecies that cause disease in mammals. *T. b. gambiense* is the causative agent of West African Sleeping Sickness, a chronic disease prevalent in west and central Africa, whereas *T. b. rhodesiense* causes the East African Sleeping Sickness, an acute and virulent form prevalent in eastern parts of Africa (Gibson, 1986). The third subspecies, *T. b. brucei*, is prevalent throughout sub-Saharan Africa. It is susceptible to trypanosome lytic factor, and thus does not affect humans (Vanhamme et al., 2003), yet it is responsible for deadly nagana in cattle and other larger animals (http://www.who.int). Transmission of all subspecies occurs via the bite of an infected tsetse fly (*Glossina* spp.), a blood-sucking insect vector that principally populates woodland and savannah zones.

The human African trypanosomiases are traced mostly in poor populations existing in remote rural areas of sub-Saharan Africa. The disease, commonly fatal if untreated, is infrequent in urban regions of countries endemic for the disease. Before 2005, approximately 50,000 to 70,000 new infections occurred in Africa each year. However, the annual reported cases dropped below 10,000/year for the first time in 50 years, as a result of the persistent control endeavors by the WHO. Current control tools and strategies adopted by WHO have demonstrated to be effective in gradually reducing prevalence by 75% since 2000 (WHO report, Geneva 2012). Still, many cases remain undiagnosed or unreported, so the actual number of new cases is certainly significantly higher than the record.

2.1.1. Life cycle of *T. brucei*

Trypanosoma brucei undergoes an intricate life cycle comprised of many developmental stages while alternating between the mammalian and the insect hosts **(Fig. 1)**. Infectious non-proliferative metacyclic trypanosomes residing in the salivary gland of the tsetse fly are introduced into the mammalian bloodstream during a blood meal, where they transform into the proliferative long slender trypanosomes, which are commonly called as the bloodstream stage (BS). The metacyclic form is pre-adapted for survival in the mammalian host in that it already expresses the surface coat with variant surface glycoprotein (VSG), which is periodically altered by means of a process called antigenic variation. This phenomenon allows the flagellates to escape the mammalian host's immune system (Pays et al., 2004). When the parasites reach their maximum density, they are believed to secrete a Stumpy Induction Factor which stimulates differentiation into the non-proliferative short stumpy form (Vassella et al., 1997). The BS subsequently differentiates into a non-dividing stumpy form, which can be transmitted to the tsetse fly during a successive blood meal (Matthews, 1999). In the tsetse fly midgut, the stumpy form differentiates into the procyclic stage (PS), which multiplies expressing a protective protein surface coat consisting of procyclin (Stebeck and Pearson, 1994). Following the differentiation into epimastigotes, they multiply in the salivary gland of tsetse fly. Conclusively, the epimastigotes differentiate into the infective metacyclic form, thus completing the life cycle (Matthews, 2005).

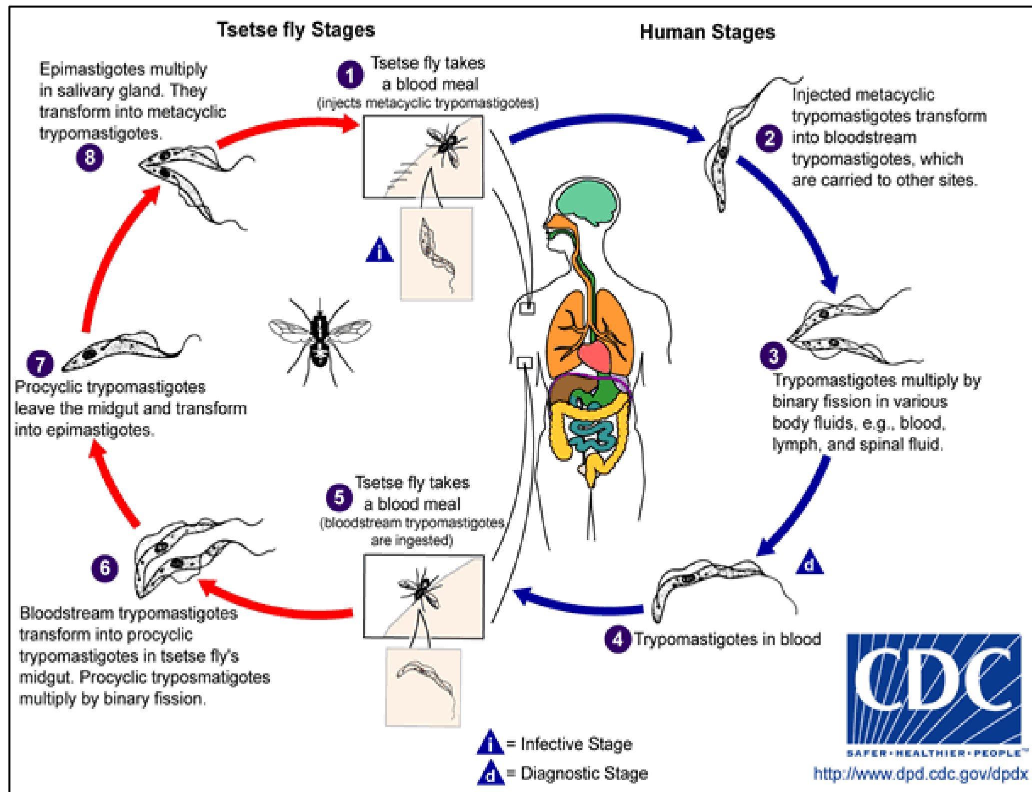

Figure 1: **Life cycle of *Trypanosoma brucei*.** The life cycle initiates through a blood meal when the tsetse fly injects the infectious metacyclic trypomastigotes into the mammalian host's (here human) bloodstream. Further it is transformed into the proliferative bloodstream trypomastigotes (BS). Bloodstream trypomastigotes then multiply in the host body fluids. Afterwards, they transformed into the non-dividing stumpy form which is later taken up by the tsetse fly in a successive blood meal. In the insect host's midgut they are transformed into the dividing procyclic trypomastigotes (PS). Subsequently, PS trypomastigotes alter into epimastigotes and enter the salivary gland of the vector. In the salivary gland epimastigotes multiply and give rise to the metacyclic trypomastigotes, prepared for the following blood meal to be injected into the host bloodstream. (Source: CDC)

2.1.2. Morphology of *T. brucei*

T. brucei contains all main organelles like a characteristic for a typical eukaryotic cell, such as a nucleus, endoplasmic reticulum, golgi apparatus and a single mitochondrion (Matthews, 2005) **(Fig. 2)**. The kinetoplast DNA which is a characteristic feature of this group of protists is situated within the mitochondrion near the basal body, which is part of the flagellum (Matthews, 2005). The parasite's motility is achieved by a single flagellum, most of which is attached to the cell body by an undulating membrane, which increases its motility in the mammalian

bloodstream. The flagellum is supported by the paraflagellar rod, another characteristic feature of kinetoplastid flagellates (Maga and LeBowitz, 1999). The cytoskeleton is composed of subpellicular microtubules, which run the length of the organism just under the cell surface (Vedrenne et al., 2002). Furthermore, *T. brucei* contains glycosomes, peroxisome-like organelles, which function in several cellular processes including glycolysis and purine salvage (Parsons et al., 2001).

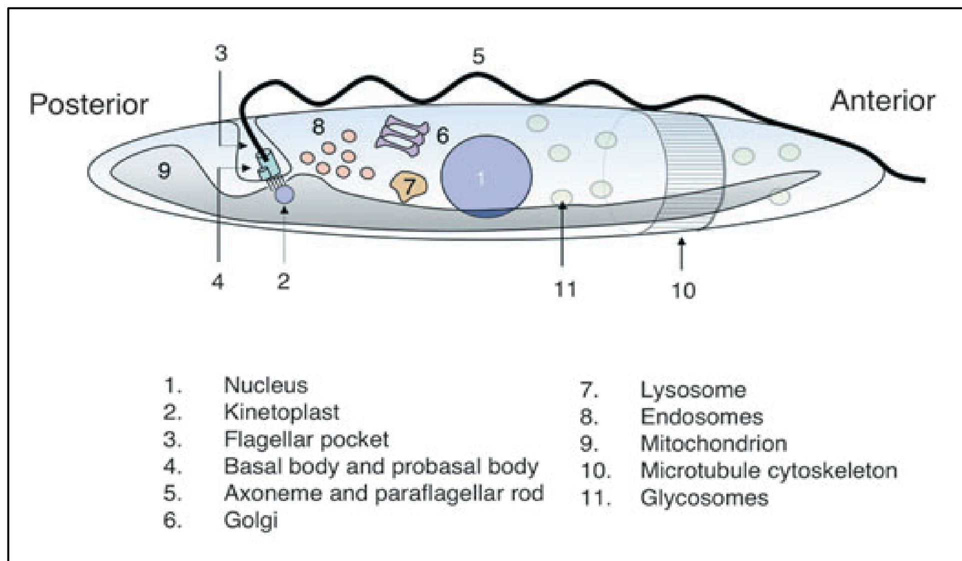

Figure 2: Morphology of *T. brucei* [Source: (Matthews K.R, 2005)]

2.1.3. *T. brucei*: a model organism, fascinating in its own right

Overall, protozoan model organisms are employed in various investigations, solving some key biological questions (Montagnes et al., 2012). The main reason to study trypanosomes lies in their medical and economical importance, which is also true for other protozoan pathogens, such as *Leishmania* and *Plasmodium*. Apart from that, *T. brucei* stands out to an unusually high number of unique features, like polycistronic transcription and trans-spliced maturation of mRNA, mitochondrial RNA-editing, antigenic variation, or the unique organelle glycosome (Johnson et al., 1987; Sutton and Boothroyd, 1986; Benne, 1994; Barry and McCulloch, 2001; Michels et al., 2006).

Along with two other related pathogenic trypanosomatids (*Leishmania major* and *Trypanosoma cruzi*), the genome of *T. brucei* has been sequenced almost a

decade ago and is now accessible as the TriTryp database (Aslett et al., 2010). The sequenced genome not only helps us to perform comparative studies among kinetoplastids but opens door to clarify broad biological queries involving proteomics and transcriptomics (Butter et al., 2013; Kolev et al., 2010).

Several molecular tools are available to experimentally define trypanosome biology **(Table 1)**. Specifically, *T. b. brucei* strain is commonly used as a model organism in laboratory. Inducible RNAi ablates target mRNA (Djikeng et al., 2004), whereas GFP-fusion techniques are instrumental for subcellular localization of proteins (Kelly et al., 2007). The cell lines that are generally used for RNAi generation and ectopic copy expression are the strain 29-13 of PS and the strain Lister 427 (cell line 90-13) of BS, expressing T7 polymerase for transcription and the TET repressor for regulation (Wirtz et al., 1999). Established affinity purification methods by means of TAP or PTP tag followed by mass-spec analysis have been utilised to identify protein-protein interactions (Panigrahi et al., 2009; Schimanski et al., 2005).

Molecular tools	References
Epitope-tagging (e.g. TAP, PTP, FLAG, GFP and YFP) and gene deletion using homologous recombination	(Kelly et al., 2007), (Schimanski et al., 2005), (Lee and Van der Ploeg, 1990), (ten Asbroek et al., 1990), (Bastin et al., 1996) and (Arhin et al., 2004)
Regulated gene expression using Tetracyclin repressor and T7 RNA polymerase	(Wirtz et al., 1999) and (Wirtz and Clayton, 1995)
RNAi, genome-wide RNAi screening	(Ngô et al., 1998), (Englund et al., 2005) and (Alsford et al., 2011)
Fluorescence in situ hybridization	(Ersfeld and Gull, 1997)
Affinity purification	(Schimanski et al., 2005), (Acestor et al., 2011) and (Morriswood et al., 2013)
Chromatin immunoprecipitation (ChIP)	(Siegel et al., 2009) and (Tiengwe et al., 2012)
Live-cell imaging	(Price et al., 2010) and (Huang et al., 2014)

Table 1: Molecular tool-kit available for *T. brucei* [Adapted from: (Akiyoshi and Gull, 2013)]

2.2. Biochemical attributes of mitochondrial intermembrane space

Almost all eukaryotic proteins are synthesized in the cytosol and subsequently targeted to specific organelles. Mitochondria contain four different sub-compartments; outer membrane, intermembrane space, inner membrane space (IMS) and mitochondrial matrix. Consequently, a dedicated import system ensuring the accurate mitochondrial protein translocation is required. The IMS organizes multiple crucial cellular functions, including the exportation of ATP (produced by oxidative phosphorylation), or the enigmatic compound 'X' to cytoplasm (see **section 2.3.1.A**); IMS also contains cytochrome *c*, an essential component of the electron transport chain. Furthermore, IMS accommodates small conserved cysteine-rich proteins with signature twin CX3C or twin CX9C motifs (here, X represents non-cysteine amino acid residues) (Stojanovski et al., 2008b). Proteins of this family include Cox17 and Cox19 (cytochrome *c* oxidase accessory factors) or small TIM (translocase of the inner membrane) chaperone family.

Import of the bulk of soluble IMS proteins including the above mentioned twin CX3C or twin CX9C family proteins is assisted by the mitochondrial intermembrane space assembly (MIA) pathway **(Fig. 3)**, a process associated with the oxidative folding of the proteins. The key players involved in this process are Mia40 and the sulfhydryl oxidase Erv1 (Allen et al., 2005).

2.2.1. The mitochondrial intermembrane space assembly pathway

The Mia40 protein possesses a structural helix-loop-helix motif with two stabilizing disulfide bonds and a redox-active CPC motif (Kawano et al., 2009). First, the incoming IMS precursors communicate with Mia40 via a transient intermolecular disulfide bond **(Fig. 3)**. MIA substrates of twin CX3C and twin CX9C family contain a MISS/ITS motif, which is recognized by the Mia40 followed by the formation of the disulfide bonded Mia40-substrate intermediate via the CPC motif (Stojanovski et al., 2008b). The formation of the disulfide bonded intermediate prevents the backsliding of the immature substrate back to the cytosol ensuring the following steps (Banci et al., 2010). Erv1 liaises with Mia40 in the transfer of disulfide bonds to the substrates. Erv1 associates with Mia40 and the substrate forming a transient ternary complex,

establishing multiple disulfide bonds (Stojanovski et al., 2008a). Upon accomplishment of disulfide exchange, substrates are released in an oxidized state qualified of assembly into mature complexes (Müller et al., 2008). The oxidation of mitochondrial intermembrane space substrate proteins causes the reduction of cysteine residues in the CPC motif of Mia40. For the reoxidization of cysteine residues, Erv1 receives electrons from Mia40 by guaranteeing its activation for a subsequent round of the substrate-importing assembly pathway (Grumbt et al., 2007). Erv1 allocates electrons via cytochrome *c* to the respiratory chain, and hence to oxygen (Dabir et al., 2007). In addition to key components Mia40 and Erv1, another IMS protein called Hot13, a cysteine-rich protein competent of chelating zinc ions, has been proposed to play a role in the biogenesis of the IMS proteins (Mesecke et al., 2008).

Remarkably, *T. brucei* lacks Mia40 in its genome (Basu et al., 2013). This divergence from the prototypical situation leads to a hypothesis that in trypanosomatids import and oxidative folding of cysteine-rich proteins in the mitochondrial IMS of the primordial eukaryotes occurred in the absence of a Mia40-type protein, but was reliant on a functional Erv1 orthologue (Allen et al., 2008).

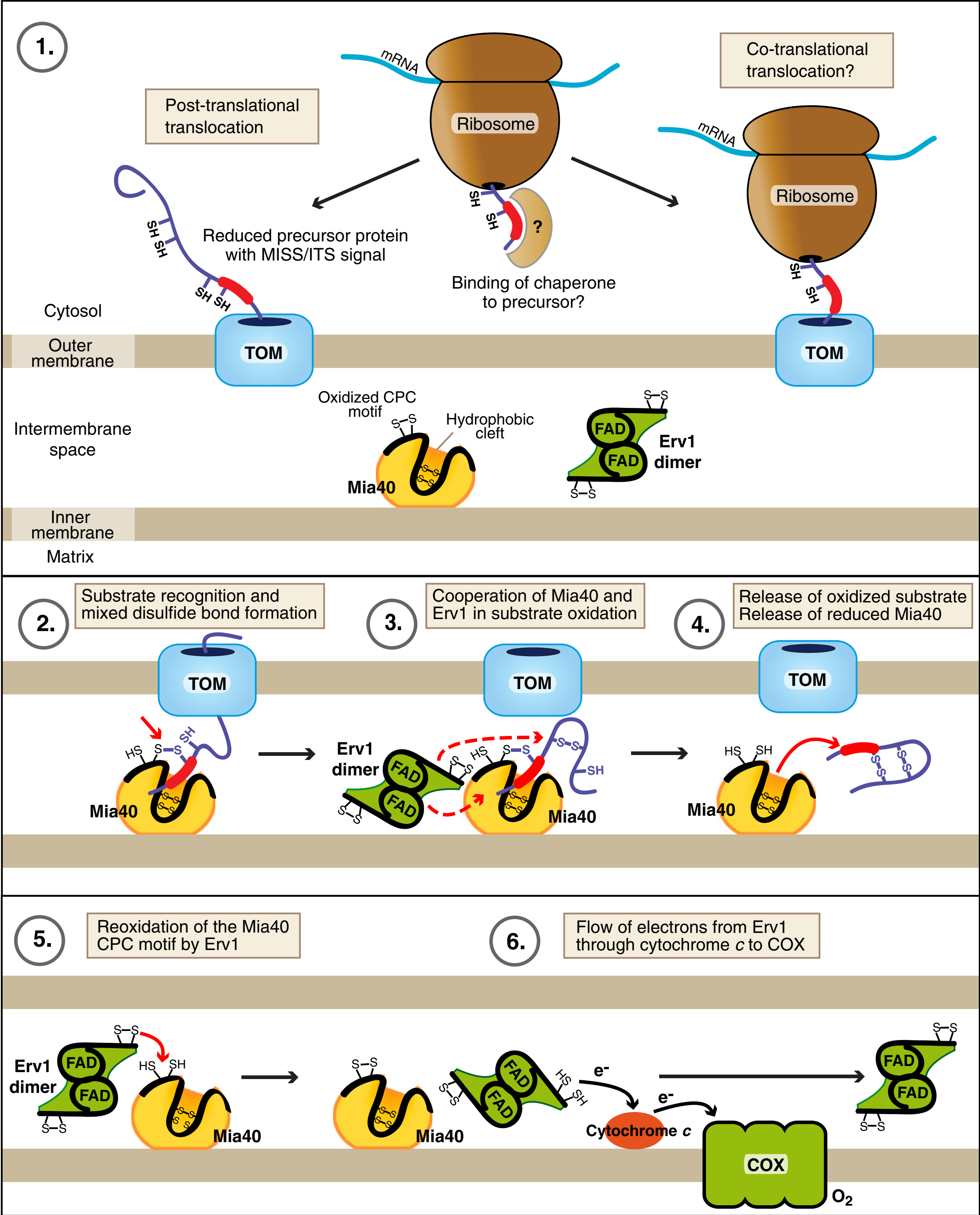

Figure 3: MIA pathway facilitated oxidative biogenesis of the IMS substrate proteins [Source: (Stojanovski et al., 2012)]

2.3. Iron-sulfur clusters

Iron-sulfur (Fe/S) clusters are simple and evolutionary ancient inorganic cofactors accomplishing versatile functions in all three realms of life, i.e. archaea, bacteria and eukaryotes (Lill and Mühlenhoff, 2005). The dependence of numerous crucial cell functions on Fe/S clusters fosters the 'Fe/S world theory', where Fe/S mineral surface was hypothesized to be the catalyzer of maiden biochemical reactions (Wächtershäuser, 2000).

The Fe/S clusters were discovered by Helmut Beinert *et. al* in the 1960s by means of spectroscopic and analytical approaches (Beinert et al., 1997). In addition to their fundamental role as electron transporters in respiration, they are engaged in several essential cellular processes that include cofactor biosynthesis, amino acid synthesis, tRNA modification, iron homeostasis, gene expression, ribosome biogenesis and nucleic acid metabolism (**Fig. 4**) (Lill and Mühlenhoff, 2008).

Basic structures of Fe/S clusters are the rhombic [2Fe-2S] cluster and the cubane [4Fe-4S] cluster (Beinert et al., 1997). Upon the loss of an iron ion, the less common [3Fe-4S] cluster is also viable. In proteins, the cluster is usually coordinated by cysteine thiol groups, although other amino acids like histidine, arginine and serine also could assist the same function. Apart from these rudimentary forms of Fe/S clusters, there are other more complex structures, for example P-cluster of nitrogenase (Rees et al., 2005).

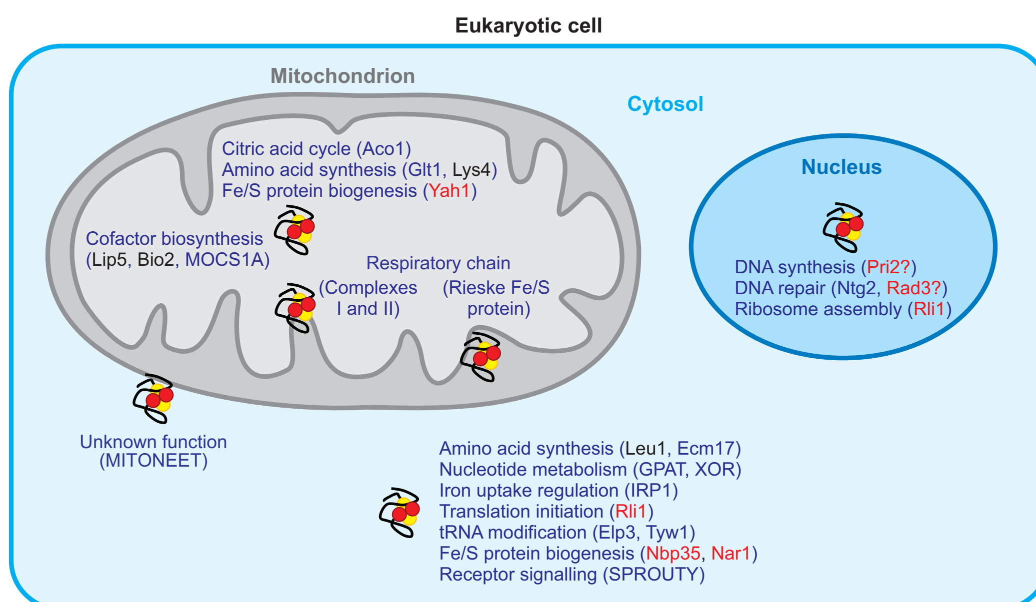

Figure 4: Fe/S cluster coordinating eukaryotic proteins and there roles [Source: (Lill and Mühlenhoff, 2008)**]**

Eukaryotic Fe/S cluster-coordinating proteins are localized in the mitochondria, plastid, cytosol and nucleus. Unequalled to their structural simplicity, Fe/S clusters are assembled and inserted into apo-proteins in an exceptionally complex biosynthetic process. There is no central Fe/S cluster biosynthetic machinery, although different specialized machineries have evolved depending on the cellular compartment. At least four different biosynthetic systems are involved in Fe/S cluster synthesis and assembly, namely, NIF (nitrogen fixation), SUF (sulfur mobilization), ISC (iron-sulfur cluster assembly) and CIA (Cytosolic iron-sulfur protein assembly) machinery. The biogenetic process is remarkably complex and the ascertained participation of some 30 proteins in eukaryotes is a result of extensive research (Lill and Mühlenhoff, 2008; Lill, 2009). Mitochondrial Fe/S proteins require the ISC assembly machinery, inherited from an α-proteobacterial endosymbiotic ancestor; on the other hand the SUF system is confined to plastids (Balk and Pilon, 2011). The indispensability of the ISC machinery is demonstrated by the presence of this system in species with mitochondrion-derived organelles, such as hydrogenosomes and mitosomes of *Trichomonas vaginalis* and *Giardia intestinalis*, respectively (Tachezy et al., 2001; Tovar et al., 2003). However, several eukaryotes have been shown to feature divergent status quo. *Entamoeba histolytica* and *Mastigamoeba balamuthi* are entirely devoid of the ISC system, although they possess a NIF-related Fe/S biogenesis machinery acquired from ε-proteobacteria via lateral gene transfer (LGT) (van der Giezen et al., 2004; Nývltová et al., 2013). More recently, *Pygsuia biforma*, a free-living anaerobic amoeboid, was demonstrated to express duplicated methanomicrobiales/*Blastocystis*-like SUF system acquired via LGT (Stairs et al., 2014).

The maturation of cytosolic and nuclear Fe/S proteins also depends on the function of the ISC machinery, although it also requires the dedicated mitochondrial ISC export apparatus and the CIA machinery (Netz et al., 2013). The connection between these two systems is the mitochondrial ISC export machinery (Kispal et al., 1999; Lill, 2009).

2.3.1. Fe/S protein biogenesis

This dissertation is focused primarily on the *T. brucei* ISC export machinery and CIA machinery. Keeping that in mind, a brief introduction to these two machineries follows. *T. brucei*-specific ISC, ISC-export and CIA machineries have been elucidated exclusively in the **section 3. Review articles.**

2.3.1.A. ISC export machinery

The nucleus and cytosol accommodate numerous Fe/S proteins **(Fig. 4 and table 3)**, which are indispensable for viability and are connected to crucial metabolic functions. Alongside the eukaryote-only CIA machinery dedicated to the maturation of extra-mitochondrial Fe/S proteins, they require an unknown sulfur-moiety 'X' from mitochondria **(X-S** in **Fig. 5)**. CIA functionality closely depends on Nfs1 (cysteine desulfurase) localized in the mitochondria (Kispal et al., 1999). The involvement on Nfs1 advocates that the unknown compound is sulfur containing, but it remains to be elucidated whether a simple persulfide, a cluster or a dedicated co-factor is exported for CIA functionality. The enigmatic compound 'X' is transported to the cytosol by the mitochondrial inner-membrane transporter Atm1, an ATP-binding cassette transporter (Kispal et al., 1999). Recently, the crystal structures of free and glutathione-bound yeast Atm1 have been reported (Srinivasan et al., 2014). This would certainly boost the investigation to identify the substrate 'X' and the precise transport mechanism. Other components involved in the export machinery are glutathione and the intermembrane space sulfhydryl oxidase Erv1 (Sipos et al., 2002; Lange et al., 2001).

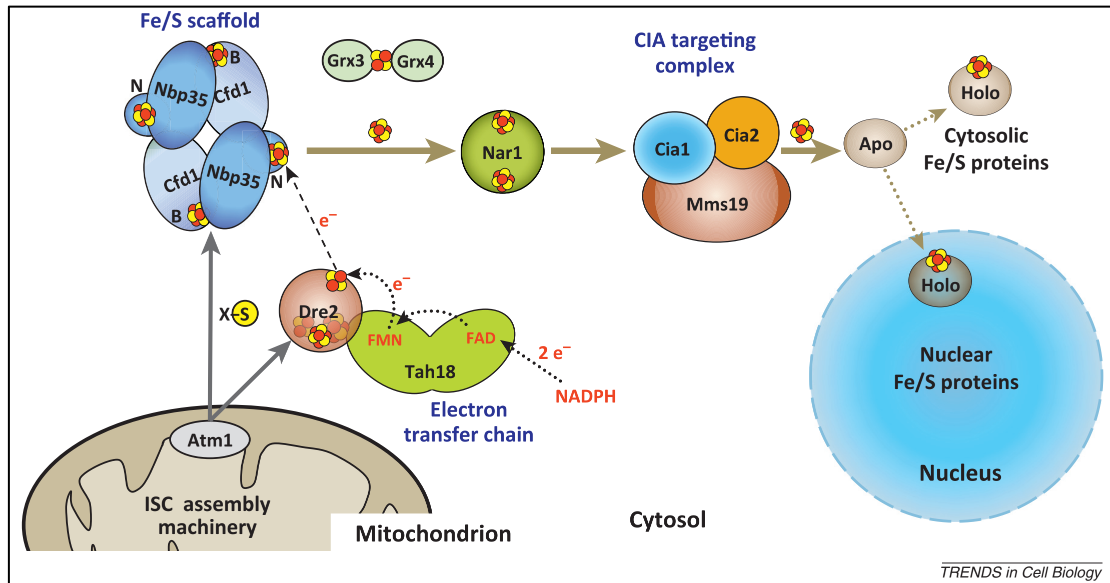

Figure 5: CIA machinery [Source: (Netz et al., 2013)]

The CIA machinery is essential for the biogenesis of cytosolic and nuclear Fe/S proteins. The figure depicts the yeast CIA machinery which consists at least 8 proteins. The assembly progression is divided into two separate phases. Initially, a bridging [4Fe-4S] cluster is assembled on the Cfd1-Nbp35 scaffold complex. This step requires a sulfur source (X-S) produced by the mitochondrial ISC assembly machinery and subsequently exported by the ISC export machinery. The NADPH-dependent electron transfer chain involving a flavoprotein Tah18 and the Fe/S protein Dre2 is essential for the generation of the functionally crucial N-terminal Fe/S cluster of Nbp35. Second, the bridging Fe/S cluster is released from the Cfd1-Nbp35 scaffold complex. This step involves the Fe/S protein Nar1 and the CIA targeting complex Cia1-Cia2-Mms19. The CIA targeting complex interacts with target apo-proteins ensuring incorporation of specific Fe/S cluster. The cytosolic monothiol glutaredoxins Grx3-Grx4 bridged by a [2Fe-2S] cluster might serve as the iron donor.

2.3.1.B. CIA machinery

The eukaryotic CIA machinery consists of at least eight proteins in yeast which are conserved throughout eukaryotes (Netz et al., 2013; Sharma et al., 2011) **(Fig. 5 and table 2)**.

The assembly process can be divided into two distinct phases. Firstly, a bridging [4Fe-4S] cluster is transiently assembled on the heterotetrameric P-loop NTPases Cfd1 and Nbp35 serving as the scaffold (Netz et al., 2007; Netz et al., 2012). This step requires the mitochondrial ISC machinery (Mühlenhoff et al.,

2004), as the sulfur source (compound 'X') is generated by the ISC cysteine desulfurase module Nfs1-Isd11 and exported by Atm1. However, *Arabidopsis thaliana* and the whole kingdom Plantae do not have a Cfd1 homolog in their genome, hence Nbp35 functions as a homodimer (Bych et al., 2008). Generation of the functionally essential N-terminal Fe/S cluster of Nbp35 depends on the flavoprotein Tah18, and the Fe/S protein Dre2 serving as a NADPH-dependent electron transfer chain (Netz et al., 2010) (**Fig. 5**).

Secondly, the bridging [4Fe-4S] cluster coordinated by the Cfd1-Nbp35 complex is released (Netz et al., 2012), facilitated by the Fe/S protein Nar1 (Balk et al., 2004) and the CIA targeting complex Cia1-Cia2-Mms19 (Gari et al., 2012; Stehling et al., 2012; Stehling et al., 2013a; Srinivasan et al., 2007). The later three proteins facilitate both Fe/S cluster transfer and target-specific cluster insertion into the apo-forms of various cytosolic and nuclear Fe/S proteins. In contrast to yeast, humans possess two isoforms of Cia2, CIA2A and CIA2B. CIA2B is the functional orthologue of yeast Cia2, and is involved in the biogenesis of canonical cytosolic and nuclear Fe/S proteins, while CIA2A is specific for the maturation of IRP1, a protein guiding cellular iron homeostasis. Additionally, CIA2A firmly binds IRP2 (Stehling et al., 2013a), which is a non-Fe/S protein that also plays a crucial role in cellular iron metabolism. Compared to the yeast counterpart, the human CIA targeting complex CIA1-CIA2B-MMS19 displays a salient specificity for target apo-proteins, because depletion of each one of these CIA proteins only affects a subset of target Fe/S proteins (Stehling *et al.*, 2013).

Apart from the afore-mentioned CIA components, the cytosolic monothiol glutaredoxins Grx3-Grx4 (termed Grx3 or PICOT in humans) are critical for cytosolic and nuclear Fe/S protein biogenesis (Mühlenhoff et al., 2010; Haunhorst et al., 2013). They act as general iron donors for the cell and hence are not regarded as members of the CIA machinery.

Human CIA proteins	Yeast CIA proteins	Important functional groups	Proposed main function
CFD1 (NUBP2)	**Cfd1**	Bridging [4Fe–4S] cluster with Nbp35	Scaffold complex, assembly, and transient binding of [4Fe–4S] cluster
NBP35 (NUBP1)	**Nbp35**	Bridging [4Fe–4S] cluster with Cfd1; N-terminal [4Fe–4S] cluster	Scaffold complex, assembly, and transient binding of [4Fe–4S] cluster
CIAPIN1 (Anamorsin)	**Dre2**	[2Fe–2S] and [4Fe–4S] clusters	Electron acceptor from NADPH–Tah18
NDOR1	**Tah18**	FAD, FMN	Electron transfer from NADPH to Dre2
IOP1 (NARFL)	**Nar1**	2× [4Fe–4S] clusters	CIA adaptor protein, mediates contact between early and late parts of CIA machinery
CIA1 (CIAO1)	**Cia1**	-	Docking site of the CIA targeting complex
CIA2B (FAM96B, MIP18)	**Cia2**	Hyper-reactive Cys	Insertion of Fe/S clusters into target apoproteins
CIA2A (FAM96A)	**-**	Hyper-reactive Cys?	Insertion of Fe/S clusters into IRP1
MMS19	**Mms19** (Met18)	-	Insertion of Fe/S clusters into target apoproteins

[a]Known yeast and human CIA proteins are listed. Note that in this review we mainly use the yeast nomenclature (bold face). Alternative names are provided in parentheses.

Table 2: Components of the CIA machinery [Source: (Netz et al., 2013)]

2.3.1.B.i. The scaffold module: Cfd1 and Nbp35

Cfd1 and Nbp35 are P-loop NTPases that belong to the Mrp/MinD sub-branch of the family and function as scaffolds for the Fe/S cluster assembly in the CIA system. Members of the Mrp/MinD family have the ability to coordinate Fe/S cluster and readily transfer them to apo-proteins. They could be found in all spheres of life; Ind1 in the mitochondria of mammals (Sheftel et al., 2009), ApbC in bacteria (Mrp in *E. coli*) and archaea (Boyd et al., 2008; Boyd et al., 2009), HCF101 in chloroplasts (Schwenkert et al., 2010) and AtNBP35 in plants (*Arabidopsis thaliana*) (Bych et al., 2008), where they were shown to coordinate and transfer Fe/S clusters *in vitro*. Neither *A. thaliana* along with other plants, nor bacteria possess a Cfd1 homolog in their genome.

Nbp35 and Cfd1 are highly similar at the amino acid level (49% identity) but are not functionally redundant (Hausmann et al., 2005). Nbp35 has an extension of 52 residues at the N-terminus, including four conserved cysteine residues that bind an Fe/S cluster. Nbp35 requires the electron transfer chain provided by Tah18-Dre2 for the maturation of this Fe/S cluster (Netz et al., 2010). In the C-terminal parts both Cfd1 and Nbp35 have a highly conserved motif predicted to bind Fe/S clusters, which bridges these two scaffold proteins (Netz et al., 2012). In yeast, Cfd1 and Nbp35 form a heterotetrameric complex and can bind Fe/S clusters *in vivo* and *in vitro*, with the absence of either of them leading to cell death (Netz et al., 2010). Both of them

contain a Walker A nucleotide binding motif for ATP hydrolysis, which is essential for the Fe/S protein assembly because the mutated version of this motif resulted in cell death in yeast (Netz et al., 2012).

2.3.1.B.ii. The electron transfer chain module: Tah18 and Dre2

The Dre2-Tah18 complex acts in the initial steps of CIA machinery. This particular module reduces the transient clusters assembled on the N-terminus of Nbp35 (Netz et al., 2010). Tah18 and Dre2 were first identified in a genetic screen for synthetic lethality with mutated pol3-13 allele encoding the catalytic subunit of polymerase δ (Chanet and Heude, 2003). Initial analysis had suggested Tah18 to be controlling mitochondrial integrity and cell death under oxidative stress together in a complex with Dre2. Under oxidative stress, Tah18 was shown to be relocalized into the mitochondria (Vernis et al., 2009). Dre2, on the other hand, was also identified by its synthetic lethality with two mitochondrial iron importers, Mrs3 and Mrs4 (Zhang et al., 2008). Electron paramagnetic resonance (EPR) spectroscopy revealed that Dre2 bears a mixture of [2Fe–2S] and [4Fe–4S] clusters in the C-terminus (Netz et al., 2010). In addition to that Dre2 carries a N-terminal S-adenosylmethionine (SAM) methyltransferase-like domain (Soler et al., 2012). Interestingly, a localization study revealed the presence of minute amounts of Dre2 within the intermembrane space of mitochondria (Zhang et al., 2008). Moreover, Mia40-dependent import (see **section 2.2.1.** MIA pathway) of CIAPIN1/anamorsin (human Dre2) into mitochondria was shown to involve a C-terminal cysteine motif (Banci et al., 2011).

On the other hand, Tah18 is a diflavin oxidoreductase with binding sites for flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADP), which is able to transfer electrons to Fe/S clusters of Dre2 (Vernis et al., 2009). EPR studies demonstrated that electrons are conveyed from NADPH via the FMN and FAD centers of Tah18 to the [2Fe–2S] cluster of Dre2 (Netz et al., 2010).

The Tah18-Dre2 electron transfer chain module is evolutionary conserved. Individual human Dre2 (CIAPIN1) and Tah18 (NDOR1) homologs and a combination of both homologs of *A. thaliana* or *T. brucei* can rescue cell growth and

Fe/S cluster assembly of yeast cells depleted for Dre2 and Tah18 (Netz et al., 2010; Bernard et al., 2013; Basu et al., 2014).

2.3.1.B.iii. Nar1

Nar1 belongs to the hydrogenase-related protein family, acts late in the pathway facilitating the transfer of Fe/S clusters to target proteins. All eukaryotic Nar1 homologs retain a N-terminal ferredoxin-like domain with four cysteine residues and a C-terminal domain, which is similar to the active site of Fe-only hydrogenases (Balk et al., 2004). Yeast or plant Nar1 could bind two [4Fe-4S] clusters (Balk et al., 2004; Cavazza et al., 2008). Yeast Nar1 interacts with Nbp35 *in vivo* (Balk et al., 2004) and also with the CIA targeting complex Cia1, Cia2, and Mms19 (Balk et al., 2005; Stehling et al., 2013a). Nar1, acting as a connector between early and late segments of the CIA pathway, is both a target and a CIA component which makes its maturation process an exciting issue to be dissected in the future. Humans possess two homologs of Nar1, namely IOP1 and IOP2. IOP1 has a role in the CIA machinery (Song and Lee, 2011), while IOP2 is localized in the nucleus and interacts with prelamin A, but its precise function is still unspecified (Barton, 1999).

2.3.1.B.iv. The CIA targeting complex: Cia1, Cia2 and Mms19

The recently classified CIA components Cia1, Cia2, and Mms19 form the so-called 'CIA targeting complex' which enables both Fe/S cluster transfer and target-specific cluster insertion into the various apoproteins (Gari et al., 2012; Srinivasan et al., 2007; Stehling et al., 2012; Stehling et al., 2013a; Weerapana et al., 2010). These reactions involve the direct physical interaction of the CIA targeting complex components with the target Fe/S apo-proteins. The direct contact between late-acting CIA and Fe/S proteins became apparent from affinity pull-down experiments in human cells where the various CIA targeting factors interact with a great number of cytosolic and nuclear Fe/S proteins (Gari et al., 2012; Seki et al., 2013; Stehling et al., 2013; Stehling et al., 2012; van Wietmarschen et al., 2012). The interacting Fe/S proteins includes DNA polymerases and primases, ATP-dependent DNA helicases, DNA glycosylases and the ABC protein ABCE1.

2.3.2. Fe/S protein biogenesis and human diseases

Several disorders (currently 11 diseases) with miscellaneous clinical phenotypes have been reported as a consequence of mutations in mitochondrial ISC factors (Sheftel et al., 2010; Rouault, 2012; Stehling and Lill, 2013). The neurodegenerative disease Friedreich's ataxia, where the early-acting ISC protein frataxin is functionally anomalous, occurs in 1 out of 50,000 Caucasians (Schmucker and Puccio, 2010). A splice mutation of the mitochondrial glutaredoxin GRX5 causes sideroblastic anemia of the erythroblasts (Camaschella *et al.*, 2007). Furthermore, a muscular myopathy is caused by the mutation of the ISC scaffold protein ISCU (Crooks *et al.*, 2012). Mutations of four late-reacting ISC proteins, IND1, NFU1, BOLA3 and IBA57, lead to diseases associated with abnormal respiratory complexes and defects in the lipoylation of proteins due to a maturation defect in the Fe/S protein lipoate synthase (Stehling and Lill, 2013). Last but not least, mutations in the ABC transporter ABCB7 cause X-linked sideroblastic anemia and cerebellar ataxia (Allikmets et al., 1999; Bekri et al., 2000).

Many Fe/S proteins are essential for life, in particular those in the cytosol and nucleus involved in genome maintenance and protein translation **(Table 3)**. Their indispensable function readily explains why numerous components of the mitochondrial ISC and CIA machineries are encoded by essential genes, and why mitochondria are indispensable for the eukaryotic cell. Some cytosolic and nuclear Fe/S proteins are linked to human diseases such as the DNA helicases involved in Fanconi anemia (FANCJ), Xeroderma pigmentosum (XPD) or Trichothiodystrophy (XPD) (Lehmann, 2003). To date, no disease has yet been reported to be associated with the defects in the CIA proteins.

Fe/S protein (human)	Yeast homolog	Fe/S cluster type	Proposed main function
DNA maintenance			
PRIM2	Pri2	[4Fe–4S]	Primase, synthesis of RNA primers for DNA replication
POLA	Pol1	[4Fe–4S]	Catalytic subunit of polymerase α, DNA replication
POLE1	Pol2	[4Fe–4S]	Catalytic subunit of polymerase ε, DNA replication
POLD1	Pol3	[4Fe–4S]	Catalytic subunit of polymerase δ, DNA replication
REV3L	Rev3	[4Fe–4S]	Catalytic subunit of polymerase ζ, DNA repair
FANCJ	absent	[4Fe–4S]	Helicase, DNA repair
NTHL1	Ntg2	[4Fe–4S]	DNA glycosylase, DNA repair
XPD	Rad3	[4Fe–4S]	Helicase, nucleotide excision repair
MUTYH	absent	[4Fe–4S]	DNA glycosylase, DNA repair
RTEL1	absent	[4Fe–4S]	Helicase, telomere stability, anti-recombinase
CHLR1	Chl1	[4Fe–4S]	Helicase, chromosome segregation
DNA2	Dna2	[4Fe–4S]	Helicase/nuclease, DNA repair Okazaki fragment processing,
Amino acid and nucleotide metabolism			
Absent	Leu1	[4Fe–4S]	Isopropylmalate isomerase (leucine biosynthesis)
Absent	Ecm17	[4Fe–4S]	Sulfite reductase, required for biosynthesis of methionine
Absent	Glt1	[4Fe–4S]	Glutamate synthase
AOX1	Absent	2× [2Fe–2S]	Aldehyde oxidase (catabolism of xenobiotics)
DPYD	Absent	4× [4Fe–4S]	Dihydropyrimidine dehydrogenase (pyrimidine catabolism)
GPAT	Absent	[4Fe–4S]	Phosphoribosyl pyrophosphate amidotransferase (purine biosynthesis)
XDH	Absent	2× [2Fe–2S]	Xanthine dehydrogenase/oxidase
Ribosome function and tRNA modification			
ABCE1	Rli1	2× [4Fe–4S]	Ribosome biogenesis, translation initiation and termination
ELP3	Elp3	2× [4Fe–4S]	Elongator protein 3 (tRNA wobble base modification), SAM
TYW1	Tyw1	2× [4Fe–4S]	tRNA wybutosine biosynthesis, SAM
CDKAL1	Absent	[4Fe–4S]	tRNA modification
CDKRAP1	Absent	[4Fe–4S]	tRNA modification
Other processes			
GRX3 (PICOT)	Grx3–Grx4	[2Fe–2S]	Intracellular iron homeostasis
IRP1	Absent	[4Fe–4S]	Intracellular iron regulation
RSAD1 (Viperin)	Absent	[4Fe–4S]	Antiviral activity, SAM
mitoNEET (CISD1)	Absent	[2Fe–2S]	Insulin sensitivity, unknown (located in mitochondrial outer membrane facing cytosol)
MINER1 (NAF1, CISD2)	Absent	[2Fe–2S]	Ca^{2+} metabolism, unknown
MINER2	Absent	[2Fe–2S]	Ca^{2+} metabolism, unknown

Table 3: Cytosolic and nuclear Fe/S proteins in eukaryotes [Source: (Netz et al., 2013)]

3. REVIEW ARTICLES

Review article # 1

Fe/S protein biogenesis in trypanosomes – a review

Julius Lukeš and **Somsuvro Basu**

Trypanosoma brucei, the causative agent of the African sleeping sickness of humans and lifestock, and other kinetoplastid flagellates belong to the eukarytotic supergroup Excavata. This early-branching model protist is known for a broad range of unique features. As it is amenable to most techniques of forward and reverse genetics, *T. brucei* was subject to several studies of its iron-sulfur (Fe/S) protein biogenesis and thus represents the best studied excavate eukaryote. Here we review what is known about the Fe/S protein biogenesis of *T. brucei*, and focus especially on the comparative and evolutionary interesting aspects. We also explore the connections between the well-known and quite conserved ISC and CIA machineries and the tRNA thiolation pathway. Moreover, the Fe/S cluster protein biogenesis is dissected in the procyclic stage of *T. brucei* which has an active mitochondrion, as well as in its pathogenic bloodstream stage with a metabolically repressed organelle.

Invited review article, re-submitted to BBA Molecular Cell Research, for the special issue on 'Fe/S proteins'

Review article # 2

The malleable mitochondrion of *Trypanosoma brucei*

Zdeněk Verner, **Somsuvro Basu**, Corinna Benz, Sameer Dixit, Eva Dobáková, Drahomíra Faktorová, Hassan Hashimi, Eva Horáková, Zhenqiu Huang, Zdeněk Paris, Priscila Peña-Diaz, Lucie Ridlon, Jiří Týč, David Wildridge, Alena Zíková and Julius Lukeš

The importance of mitochondria for a typical aerobic eukaryotic cell is undeniable, as the list of necessary mitochondrial processes is steadily growing. Here, we summarize the current knowledge of mitochondrial biology of an early-branching parasitic protist, *Trypanosoma brucei*, a causative agent of serious human and cattle diseases. We present a comprehensive survey of its mitochondrial pathways including kinetoplast DNA replication and maintenance, gene expression, protein and metabolite import, major metabolic pathways, Fe-S cluster synthesis, ion homeostasis and organellar dynamics, and other processes. As we describe in this review, the *T. brucei* single mitochondrion is everything but simple and as such rivals mitochondria of multicellular organisms.

Invited review article, submitted to International Review of Cell and Molecular Biology

Only the 'Fe-S cluster and protein biogenesis' section of the review, which is contributed by me, is included in the printed version of the thesis

4. PUBLISHED RESULTS

Study # 1

Divergence of Erv1-associated mitochondrial import and export pathways in trypanosomes and anaerobic protists

Somsuvro Basu, Joanne C. Leonard, Nishal Desai, Despoina A. I. Mavridou, Kong Ho Tang, Alan D. Goddard, Michael L. Ginger, Julius Lukeš and James W. A. Allen

In yeast (*Saccharomyces cerevisiae*) and animals, the sulfhydryl oxidase Erv1 functions with Mia40 in the import and oxidative folding of numerous cysteine-rich proteins in the mitochondrial intermembrane space (IMS). Erv1 is also required for Fe-S clus- ter assembly in the cytosol, which uses at least one mitochondrially derived precursor. Here, we characterize an essential Erv1 orthologue from the protist *Trypanosoma brucei* (TbERV1), which naturally lacks a Mia40 homolog. We report kinetic parame- ters for physiologically relevant oxidants cytochrome *c* and O_2, unexpectedly find O_2 and cytochrome *c* are reduced simultane- ously, and demonstrate that efficient reduction of O_2 by TbERV1 is not dependent upon a simple O_2 channel defined by con- served histidine and tyrosine residues. Massive mitochondrial swelling following Tb*ERV1* RNA interference (RNAi) provides evidence that trypanosome Erv1 functions in IMS protein import despite the natural absence of the key player in the yeast and animal import pathways, Mia40. This suggests significant evolutionary divergence from a recently established paradigm in mito- chondrial cell biology. Phylogenomic profiling of genes also points to a conserved role for TbERV1 in cytosolic Fe-S cluster as- sembly. Conversely, loss of genes implicated in precursor delivery for cytosolic Fe-S assembly in *Entamoeba*, *Trichomonas*, and *Giardia* suggests fundamental differences in intracellular trafficking pathways for activated iron or sulfur species in anaerobic versus aerobic eukaryotes.

Eukaryotic Cell, 12:343–55 (2013)

doi:10.1128/EC.00304-12

Study # 2

Cytosolic iron-sulphur protein assembly is functionally conserved and essential in procyclic and bloodstream *Trypanosoma brucei*

Somsuvro Basu, Daili J. Netz, Alexander C. Haindrich, Nils Herlerth, Thibaut J. Lagny, Antonio J. Pierik, Roland Lill and Julius Lukeš

Cytosolic and nuclear iron-sulphur (Fe/S) proteins include essential components involved in protein translation, DNA synthesis and DNA repair. In yeast and human cells, assembly of their Fe/S cofactor is accomplished by the CIA (cytosolic iron-sulphur protein assembly) machinery comprised of some 10 proteins. To investigate the extent of conservation of the CIA pathway, we examined its importance in the early-branching eukaryote *Trypanosoma brucei* that encodes all known CIA factors. Upon RNAi-mediated ablation of individual, early-acting CIA proteins, no major defects were observed in both procyclic and bloodstream stages. In contrast, parallel depletion of two CIA components was lethal, and severely dimin- ished cytosolic aconitase activity lending support for a direct role of the CIA proteins in cytosolic Fe/S protein biogenesis. In support of this conclusion, the *T. brucei* CIA proteins complemented the growth defects of their respective yeast CIA depletion mutants. Finally, the *T. brucei* CIA factor Tah18 was characterized as a flavo- protein, while its binding partner Dre2 functions as a Fe/S protein. Together, our results demonstrate the essential and conserved function of the CIA pathway in cytosolic Fe/S protein assembly in both develop- mental stages of this representative of supergroup Excavata.

In Press, Molecular Microbiology, 2014

doi:10.1111/mmi.12706

5. UNPUBLISHED RESULTS

This part is comprised of 13 pages unpublished data, which is present in the original thesis deposited at the Faculty of Science

6. CONCLUSIONS

My thesis revolved around the parasitic protist *T. brucei,* which includes subspecies that cause fatal human disease known as African sleeping sickness. *T. brucei* undergoes a series of transformations between the mammalian host (bloodstream stage, BS) and the tsetse fly insect vector (the procyclic stage, PS), which are characterized by distinct changes in morphology, metabolism, and biochemistry.

Initially, I performed a characterization of the mitochondrial sulphydryl oxidase Erv1 in *T. brucei* using biochemical, cellular, and comparative genomic approaches (Basu et al., 2013). In other organisms, Erv1 functions with Mia40 in the import and oxidative folding of cysteine-rich proteins in the mitochondrial intermembrane space. This is a fundamental process in the eukaryotic cells however, trypanosomes and their nearest evolutionary relatives lack the Mia40 protein. Since, at least in the yeast, Erv1 is a multi-functional protein with an essential, yet indefinite role in the cytosolic Fe/S cluster assembly, the absence of Mia40 raised the question of what Erv1 does in trypanosomes and how protein import into the mitochondrial intermembrane space proceeds. We provide convincing evidence that trypanosome Erv1 indeed functions in mitochondrial protein import in the absence of its Mia40 partner (Basu et al., 2013). This is a significant divergence from the so-far characterized and conserved MIA pathway and is therefore of interest to cell and evolutionary biologists, as well as molecular parasitologists. The biochemical analysis of recombinant and mutagenized trypanosome Erv1 also provides insight into preferred downstream oxidant for the reduced protein, which is compatible with the physiology of the parasite in its mammalian host and insect vector (Basu et al., 2013).

Furthermore, in using comparative genomics to ask whether trypanosome Erv1 also influences the cytosolic Fe/S protein assembly we found an unexpected divergence in anaerobic protists. Our observations reveal that the basis of the as yet uncharacterized precursor exported from the mitochondria to the cytosol must have changed during independent adaptations to the anaerobic environment (Basu et al., 2013).

As an obvious subsequent step, I picked up the CIA machinery of *T. brucei.* Since nothing was known about it so far, I faced the arduous task of characterizing the whole machinery. Based on what was known about the yeast and human CIA pathways, I identified 9 components involved in this machinery. In frame of the CIA

project, I prepared 30 RNAi *T. brucei* cell lines with one or two genes ablated, and characterized them using a wide range of methods, including Western analysis, growth curves and enzymatic activity measurements. Complementation of several *Saccharomyces cerevisiae* CIA mutants with the *T. brucei* orthologs defined the extent of functional conservation of the pathway across eukaryotic super-groups. Characterization of candidates acting in the early and intermediate parts of the *T. brucei* CIA machinery has been published recently (Basu et al., 2014).

My thesis was not restricted to *T. brucei* only, as I am also involved in a project focusing on the CIA pathway of another human parasite, *Giardia intestinalis.* So far, I was able to show that two *G. intestinalis* Tah18-like proteins partially rescue the Tah18-Dre2 double knockdown in *T. brucei* (Pyrih *et al.*, in progress).

Finally, I participated in functional analysis of the mitochondrial chaperone Hsp70 (mtHsp70), a member of the 70-kDa heat shock protein (Hsp70) family, its protective co-chaperone Hep1 and the nucleotide exchange factor Mge1. I depleted Mge1 which proved to be essential in both stages of *T. brucei* (Týč *et al.*, in progress).

In a nutshell, the PhD study provided me with an opportunity to acquire knowledge about *T. brucei* and an extensive repertoire of molecular & cellular biology and biochemical methods.

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Zhang, Y., Lyver, E.R., Nakamaru-Ogiso, E., Yoon, H., Amutha, B., Lee, D.W., Bi, E., Ohnishi, T., Daldal, F., Pain, D., Dancis, A., 2008. Dre2, a conserved eukaryotic Fe/S cluster protein, functions in cytosolic Fe/S protein biogenesis. Mol. Cell. Biol. 28, 5569–82. doi:10.1128/MCB.00642-08

SOMSUVRO BASU, M.Sc.

EDUCATION

2003-2006 B.Sc. in Zoology (Life Science), Visva Bharati University, India
2006-2008 M.Sc. in Zoology, Visva Bharati University, India
2010-present Ph.D. in Molecular and Cell Biology and Genetics, Faculty of Science, University of South Bohemia; Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice (Budweis), Czech Republic

AWARDS & ACHIEVEMENTS

- '**BEST POSTER AWARD**' in the "**7th International conference on iron-sulfur cluster biogenesis and regulation**", University of South Carolina, USA; 20-24 May, 2013
- Ph.D. (Doctoral) student grant for the period of January-June'2012 from the Institute of Parasitology, Biology Centre, Czech academy of science, Ceske Budejovice.
- Travel bursary for participation: "**6th International conference on Biogenesis of iron-sulfur proteins and regulatory functions",** Girton College, University of Cambridge; 22-25 August 2011.
- Selected for **Indian Academy of Sciences (IASc) Summer Research Fellowship Programme 2008** for teachers and students (an all India fellowship program).
- Selected for the **CCMB Summer Training Programme 2007** (declined).
- Stood 2nd in M.Sc final exam
- Merit scholarship based on results in M.Sc. 1st year, Visva Bharati University

PROFFESSIONAL EXPERIENCE

2008 Visiting fellow**, Centre for Cellular and Molecular Biology (CCMB)** under the **Indian Academy of Sciences (IASc) Summer Research Fellowship Programme 2008**

2010-present Graduate Research associate/Ph.D. student, **Laboratory of Molecular Biology of Protists, Institute of Parasitology, Biology Centre, ASCR.**

PEER REVIEWER (JOURNALS)

Journal of Clinical Microbiology, PLoS One, Free Radical Biology and Medicine, Antimicrobial Agents and Chemotherapy

RESEARCH PROJECTS

"Study of attenuation in *Leishmania donovani*" supervised by Dr. Tushar Vaidya, Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India through the IASc Summer research fellowship programme 2008.

Ph.D. projects: 1# "Cytosolic iron-sulfur cluster assembly in *Trypanosoma brucei*"
Supervisor: Prof. RNDr. Julius Lukeš CSc. In collaboration with: Prof. Dr. Roland Lill, University of Marburg, Germany and Prof. Dr. Antonio J. Pierik, TU Kaiserslautern, Germany. (**In Press**, *Molecular Microbiology,* 2014)

2# "Erv1-associated mitochondrial import and export pathways in trypanosomes"
Supervisor: Prof. RNDr. Julius Lukeš CSc.; In collaboration with: Dr. Michael Ginger, Lancaster University, UK & Dr. James W. Allen, UK. **(Published in *Eukaryotic Cell*, 2013)**

BENCH SKILLS

Handling Cell Cultures: Cultivation of both bloodstream and procyclic form life cycle stages, *Trypanosoma brucei,* Counting Cells using the Z2 Coulter® Particle Count and Size Analyzer (Beckman Coulter) or Burker Chamber, Preparation and Cultivation of Competent Cells (E. coli), Expression and purification of proteins in *E. coli.*, Preparation of Transgenic Organisms (*E. coli,*

Trypanosoma brucei). Generation of RNAi and overexpression strains in both life stages of *Trypanosoma brucei.*

Molecular biology: PCR and reverse transcriptase-PCR, quantitative real-time PCR, PCR cloning, DNA Restriction Analysis, DNA/RNA Isolation and Purification, Spectrophotometric Measurement of DNA/RNA/Protein Concentration using BioPhotometer (Eppendorf), DNA/RNA Electrophoresis, Phosphorylation of RNA/DNA, Reverse Transcription and cDNA Synthesis, End-repair, Low-melting-agarose gels, Electroelution of large fragments of DNA, Northern and Southern Blotting, Radioactive Labelling of Probes, Autoradiography.

Proteins: Protein analyses and purification (SDS-PAGE, immunoprecipitation, Ni-NTA agarose/glutathione sepharose/amylose resin affinity purification, Western blotting), Coomassie Protein Gel Staining, Silver staining of SDS-PAGE gels. Identifying protein:protein interactions: Tandem affinity purification (PTP-tagged protein purification). Immunolocalization, Digitonin Fractionation, Measurement of enzymatic activities (especially iron-sulfur containing proteins), Isolation of Mitochondria, Percoll Gradient.

Microscopy: Fluorescence Microscopy, Transmission electron microscope (JEOL JEM 1010).

Computing skills and Bioinformatics: Highly acquainted with a variety of programmes [Power Point, Excel, Word, Vector NTi, CLC Workbench, DNA star, CDD (Conserved Domain Database), GeneDB, TritrypDB, Tigr, Sanger, NCBI blast, etc.].

MENTORING

2010 Training of Lindsay McDonald, ERASMUS exchange student from University of Glasgow.

2011-2013 Co-supervised bachelor thesis "**Iron-sulfur cluster assembly in *Trypanosoma brucei*"** of Alexander Haindrich (Johannes Kepler university of Linz, Austria & University of South Bohemia, Czech Republic joint bachelor degree program in Biological Chemistry). He received excellent grades for the thesis.

2013 Trained/demonstrated Ph.D. students in the frame of '**Advanced Methods in Molecular Biology (KMB/603) course**' at the Department of molecular biology, Faculty of Science, University of South Bohemia.

PUBLICATIONS

Basu S., Leonard J.C., Desai N., Mavridou D.A., Ho Tang K., Goddard A.D., Ginger M.L., Lukeš J., Allen J.W. (2013) **Divergence of Erv1-Associated Mitochondrial Import and Export Pathways in Trypanosomes and Anaerobic Protists**. *Eukaryotic Cell* **12**: 343-355.

Basu S., Netz D.J., Haindrich A.C, Herlerth N., Lagny T.J., Pierik A.J., Lill R., Lukeš J. (2014) **Cytosolic iron-sulphur protein assembly is functionally conserved and essential in procyclic and bloodstream *Trypanosoma brucei*** (**In Press**, *Molecular Microbiology*)

Lukeš J., **Basu S**. **Fe/S protein biogenesis in trypanosomes – a review** (Invited review article, **revision submitted**) *BBA Molecular Cell Research*

Verner Z., **Basu S.**, Benz C., Dixit S., Dobáková E., Faktorová D., Hashimi H., Horáková E., Huang Z., Paris Z., Pena P., Ridlon L., Týč J., Wildridge D., Zíková A., Lukeš J. **The malleable mitochondrion of *Trypanosoma brucei*** (Invited review article, **submitted**) *International Review of Cell and Molecular Biology*

INVITED LECTURE

Centre for Biotechnology, Visva Bharati University (A Central university and an institution of national importance), India, 2010

CONFERENCE PRESENTATIONS

2013 **43rd Jirovec's protozoological days: Oral presentation:** "**Cytosolic iron sulfur cluster assembly in *Trypanosoma brucei*",** RS Novy Dvur, Czech Republic, 6-10 May. *

7th **International conference on iron-sulfur cluster biogenesis and regulation**: Presented poster: "**Cytosolic iron sulfur cluster assembly in *Trypanosoma brucei*"**, University of South Carolina, Columbia, SC, USA; 20-24 May. The poster won the **"BEST POSTER AWARD"** *

2012 **GRC Iron-sulfur enzymes:** co-author in the poster: "**Cytosolic assembly of iron-sulphur cluster proteins in *Trypanosoma brucei***", Mount Holyoke College, MA, USA, 10-15 June.

44th Microsymposium on Parasite/Trypanosomatid metabolism, drug design and glycosomes: Oral presentation: "**Cytosolic assembly of iron-sulphur cluster proteins in *Trypanosoma brucei*", ** Vrij Universiteit Amsterdam, 25-26 October. *

2011 **Kinetoplastid molecular cell biology meeting IV:** Co-author on the poster "**Iron sulfur cluster assembly in *Trypanosoma brucei***", Woods Hole, Massachusetts, USA, April 8-12.

6th **International conference on Biogenesis of iron-sulfur proteins and regulatory functions:** Presented poster "Cytosolic assembly of iron-sulphur cluster proteins in *Trypanosoma brucei*", Girton College, University of Cambridge; 22-25 August. *

2010 7th **Mitochondrial Physiology conference:** Co-author of the poster "**The Fe/S cluster assembly protein Isd11 is essential for tRNA thiolation in *Trypanosoma brucei***", Obergurgl, Austria, 27th Sep – 1st Oct. *

* Personally presented at the conferences

PROFESSIONAL SOCIETIES

Member of *International Society of Protistologists*