University of South Bohemia
Faculty of Science

Ph.D. Thesis

The dynamics of the MRP1/2 complex and the function of intact MRB1 core for RNA editing in *Trypanosoma brucei*

Zhenqiu Huang

Supervisor: Mgr. Mir Mohamod Hassan Hashimi, Ph.D.

Co-supervisor: Prof. RNDr. Julius Lukeš, CSc.

Institute of Parasitology

Biology Centre of the Academy of Sciences, the Czech Republic

České Budějovice, 2015

Zhenqiu H. (2015): **The dynamics of the MRP1/2 complex and the function of intact MRB1 core for RNA editing in *Trypanosoma brucei.***Ph.D. thesis, in English – 125 pages, University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic.

ANOTATION

This thesis describes the dynamics of mitochondrial RNA-binding protein 1 and 2 (MRP1/2) complex in different cell lines of *Trypanosoma brucei* under an optimized immobilized condition. This study reveals the influence of RNA on the complex's dynamics. Furthermore, the function of RNA-binding complex 1 (MRB1) core has been studied via reverse genetic, biochemical and molecular techniques, with its role in RNA editing being proposed.

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, September 26, 2015   Zhenqiu Huang

Statement regarding contribution

1. Huang Z, Kaltenbrunner S, Šimková E, Staněk D, Lukeš J, Hashimi H. Dynamics of Mitochondrial RNA-Binding Protein Complex in *Trypanosoma brucei* and Its Petite Mutant under Optimized Immobilization Conditions. **Eukaryot Cell.** 2014; 13: 1232-1240.

Zhenqiu Huang optimized the immobilization method, generated YFP tagging cell lines, performed FRAP assay, Immunoprecipation, and Western blot, which is about 80% of the data accumulated. He also analyzed data and helped writing the manuscript.

2. Huang Z, Faktorová D, Křížová A, Kafková L, Read L, Lukeš J, Hashimi H. Assembly of the Core Mitochondrial RNA Binding Complex 1 is vital for Trypanosome RNA Editing. **RNA**, in press.

Zhenqiu Huang generated MRB3010 V5 tagged cell lines, verified the RNAi cell line, did growth curve analyses, Glycerol gradient fraction, Immunoprecipitation, Real-time PCR and Western blot, about 70% of the data accumulated. Moreover, he analysed the data and participated in writing the manuscript.

4. Verner Z, Basu S, Benz C, Dixit S, Dobáková E, Faktorová D, Hashimi H, Horáková E, **Huang Z**, Paris Z, et al. Malleable Mitochondrion of *Trypanosoma brucei*. **Int Rev Cell Mol Biol.** 2014; 315: 73-151.

Zhenqiu Huang co-wrote the "RNA editing" section. Julius Lukeš, the (co-)corresponding author of all mentioned papers, approves the contribution of Zhenqiu Huang in these papers as described above.

………..……………………

Prof. RNDr. Julius Lukeš CSc.

Acknowledgement

"There is no means of testing which decision is better, because there is no basis for comparison. We live everything as it comes, without warning, like an actor going on cold. And what can life be worth if the first rehearsal for life is life itself?" ― Milan Kundera, *The Unbearable Lightness of Being*

With science research as the theme, lots of fun could have been missed. However, It is my great pleasure to have a nice company during the leisure time. Guys, you know who I am talking, don't you?

Last but not the least, thanks to my parents, who are my persistent supporter and source of motivation.

The four years my PhD study will be a memory I keep with me forever. With the belief of science, as well as life, the story will never end, our story will never end. Let us keep moving forward while bearing in mind "Anyone whose goal is 'something higher' must expect someday to suffer vertigo. What is vertigo? Fear of falling? No, Vertigo is something other than fear of falling. It is the voice of the emptiness below us which tempts and lures us, it is the desire to fall, against which, terrified, we defend ourselves." ― Milan Kundera, *The Unbearable Lightness of Being*

Contents

Abbreviations

$\Delta\Psi m$	mitochondrial membrane potential
A	adenosine
A6	F_1/F_0-ATP synthase subunit 6
ak	akinetoplastic
complex I	NADH dehydrogenase
complex III	cytochrome bc_1 complex
complex IV	cytochrome c oxidase
complex V	F_1/F_0-ATP synthase
coxX	cytochrome c oxidase
cyB	cytochrome b
dk	dyskinetoplastic
dsRNA	double stranded RNA
FRAP	florescence recover after photobleaching
G	guanosine
GAP	guide RNA associated protein
NAPDH	glyceraldehyde-3-phosphate
gRNA	guide RNA
HAT	human African trypanosomiasis
kDNA	kinetoplast DNA
KPAP	poly(A) polymerase
KREL	kinetoplastid RNA editing ligase
KREN	kinetoplastid RNA editing nuclease
KREP	kinetoplastid RNA editing proteins
KRET	kinetoplastid RNA editing 3' terminal uridylyltransferase
KREX	kinetoplastid RNA editing exonuclease
LSU	large subunit of risosome
mt	mitochondrial
MRB1	mitochondrial RNA binding complex 1
MRP	mitochondrial RNA binding protein
MURF	mitochondrial unidentified reading frame
nt	nucleotide
ORF	open reading frame
PPR	pentatricopeptide repeat
RECC	RNA editing core complex
RNAi	RNA interference
RNAP	RNA polymerase
RPS12	ribosomal protein S12
SSU	small subunit of ribosome
T	thymidine
tet	tetracycline
tetR	tetracycline repressor
U	uridine
UTR	untranslated region
VSG	variable surface glycoprotein
YFP	yellow fluorescent protein

1. SUMMARY

Summary

Trypanosoma brucei, the causative agent of the human sleeping sickness and nagana in livestock, is a unicellular parasite and has served as a model organism due to its amenity to genetic manipulation as well as easy cultivation. A distinguishing feature of *T. brucei* is mitochondrial uridine insertion/deletion RNA editing, which is the main interest of this thesis.

In the first part of the thesis, an optimized immobilization method has been demonstrated for application of a live cell imaging technique called fluorescence recovery after photobleaching (FRAP). Via this technique, the dynamics of yellow fluorescent protein (YFP) and YFP C-terminally tagged MRP1 (MRP1-YFP) have been characterized in *T. brucei brucei* procyclic stage (PS), bloodstream stage (BS), and *T. brucei evansi*, which is similar to the BS stage but lacks mitochondrially-encoded RNA. The difference between the dynamics of MRP1-YFP in BS and *T. b. evansi* suggested the hindering influence of RNA on MRP1, the proxy of the MRP1/2 heterotetramer complex involved in RNA editing **(3. Published results, study # 1, Huang *et al.* 2014, *Euk. Cell.*)**.

In the second part of the thesis, it was discovered that the silencing of one core subunit of mitochondrial RNA binding complex 1 (MRB1), called MRB8620, compromised the integrity MRB1 core, causing the dampening of the RNA editing. This phenotype was subtle, however, and an effect on PS stage fitness was only observed when cells were grown in glucose-poor conditions that force energy metabolism via an active mitochondrion. Additionally, it also revealed the hindered dissociation between the guide-RNA-stabilizing GAP1/2 subcomplex and mRNA, as well as reduced association between GAP1/2 and editosome due to the disruption of MRB1 core upon the MRB8620 depletion **(3. Published results, study # 2, Huang *et al.* 2015, *RNA* in press.)**.

In the third part of the thesis, a comparison of RNA abundance when PS is grown in glucose-rich and -poor conditions suggests either the elevated transcription of never-edited mRNAs or slowdown in the degradation, as seen from their increased levels in glucose-poor growth conditions. A slight decrease in pre-edited while prevalent increase in almost all edited RNAs indicates that editing is accelerated under these conditions, which may contribute more to the levels of these transcripts than an increase in transcription. The interesting exception is the decrease in the level of 9S rRNA. This observation may prove to be an interesting model to study the mechanism of the complicated regulation on transcription, editing and RNA degradation in the mitochondrion of *T. brucei*.

2. OVERVIEW

2.1. *Trypanosoma* species and trypansomiasis

Trypanosoma, a genus from the class Kinetoplastida and family Trypanosomatidae, is transmitted principally by biting insects. According to the transmission route from the insect vector, *Trypanosoma* is split into 2 branches: *Stercoraria* and *Salivaria*. *Trypanosoma cruzi*, the etiological agent of Chagas disease, is characterized as *Stercoraria* because it inhabits the posterior part of the triatominae digestive tract and is transmitted to the host when the bug deposits its faeces on the host's skin surface. Penetration of protists from infected faeces is facilitated by scratching at the bite area. In contrast, the sleeping sickness causative agent *Trypanosoma brucei*, included in *Salivaria*, develops in the anterior part of the insect digestive tract and is transmitted to a mammalian host via its injection from an insect bite (1,2).

Diseases caused by trypanosomes are termed trypanosomiasis, including sleeping sickness and Chagas disease in humans, and several other diseases in animals. Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. Many of the affected populations live in remote rural areas with limited access to adequate health services, which complicates the surveillance and therefore diagnosis and treatment of cases. In addition, displacement of populations, war and poverty are important factors that facilitate transmission. According to the latest report from WHO, approximately 30,000 people in 36 countries of sub-Saharan Africa have Sleeping sickness. In Latin America, Chagas disease causes almost 21,000 deaths per year (3). Animal trypanosomiasis, known sometimes as Nagana, is most severe in cattle but also can cause losses in pigs, camels, goats, and sheep (4).

African pathogenic trypanosomes mainly consists by three subgenera: *Nannomonas* (*Trypanosoma congolense*), *Duttonella* (*Trypanosoma vivax*), and *Trypanozoon* (*Trypanosoma brucei* group). There is rare epidemiological evidence for insect transmission in the field for *T. congolense* due to its low occurrence of parasitaemia in its main host cattle (5,6). *T. vivax* is mechanically transmitted by tabanids flies. Its geographical distribution remains in Africa and Latin American, limited by a narrow host range of mainly bovines and horses (7,8). Both *T. congolense* and *T. vivax* are described as blood parasites, based on the correlation of their presence in the blood and corresponding pathogenic effects. The *Trypanozoon* is distinct among these three subgenera because its transmission is attributed to sucking flies, serial biting insects such as tabanids and stomoxes, plus tsetse flies (9-11). The capacity of some *Trypanozoon* species to invade a host's tissues, such as nervous system, causes difficulties in disease diagnosis and results in pathogenic effects that lead to often underestimated health and economic threats (12).

In the subgenus of *Trypanozoon*, there are the sub-species *T. brucei brucei, T. brucei rhodesiense, T. brucei gambiense, T. brucei evansi* and *T. brucei equiperdum*.
(I)   The subspecies *T. b. brucei, T. b. rhodesiense* and *T. b. gambiense*, are

transmitted by tsetse flies. The first subspecies together with *T. congolense* and *T. vivax* cause Nagana in livestock, which has a great impact on cattle breeding in Africa (13). *T. b. rhodesiense* and *T. b. gambiense* are pathogens causing Human African Trypanosomiasis (HAT), commonly known as sleeping sickness, and the disease is most often fatal in the absence of treatment.

(II) *T. b. evansi* is described as derived from *T. brucei* through the loss of mitochondrial DNA, otherwise known as kinetoplast DNA (kDNA), which is indispensible for the inhabitation of the flagellate in tsetse flies. This condition has enabled the spread of this subspecies outside of the tsetse fly belt of Africa *T. b. evansi* is termed as akinetoplast during the complete loss of kDNA or dyskinetoplast because of maxicircles and minicircles deletion, loss and/or homogenization(14). kDNA will be discussed in section 2.3.1 of this introduction.

(III) *T. b. equiperdum* is the causative agent of dourine in Equidae, a sexually transmitted disease. As with *T. b. evansi*, *T. b. equiperdum* has also lost significant amounts of its kDNA and has spread outside of Africa.

As reported by the WHO, until 2014 *T. b. gambiense* is still endemic in 24 countries of west and central Africa and responsible for more than 98% of reported cases of sleeping sickness. *T. b. rhodesiense* is prevalent in 13 countries of eastern and southern Africa, causing about 3% of reported cases (**Fig. 1**). From 1999 to 2014, the number of reported new cases of the chronic form of HAT (*T. b. gambiense*) declined by 77%, from 27 862 to 3 679. During the same period, the number of newly reported cases of the acute form of HAT (*T.b. rhodesiense*) fell by 71% from 619 to 117. However, there is a possibility of many cases remaining undiagnosed or unreported, posing a threat to health and economics in these affected regions (15).

2.2. *T. brucei*: Life cycle, morphology and energy metabolism

T. brucei is transmitted to the mammalian host by the bite of a tsetse fly. In the insect vector, the midgut-multiplying parasites are termed the procyclic stage (PS). Around 2 weeks after infection, the PS move to the proventriculus, the terminal portion of the foregut and turn into the epimastigote stage, which later differentiates into the non-proliferative, salivary gland attached metacyclic stage (Fig. 2). In the mammalian host, *T. brucei* is termed the bloodstream stage (BS), including the proliferative long slender form and the non-proliferative, infective short stumpy form (16), which is competent for uptake by the tsetse fly to continue its lifecycle. The protist is extracellular during this stage, and develops variant surface glycoprotein (VSG), which forms a coat around the flagellate to help it evade the host's immune system (17). This VSG coat is continuously changed while the long slender forms are dividing, presenting a different antigen that the immune system has to develop a response, which is a mechanism called antigenic variation. In this introduction, the

terms PS and BS will be used to denote the form that propagates in the midgut of the fly and the proliferative long slender form in mammalian host, respectively. These two stages exist as *in vitro* cultures that are used to study *T. b. brucei* biology.
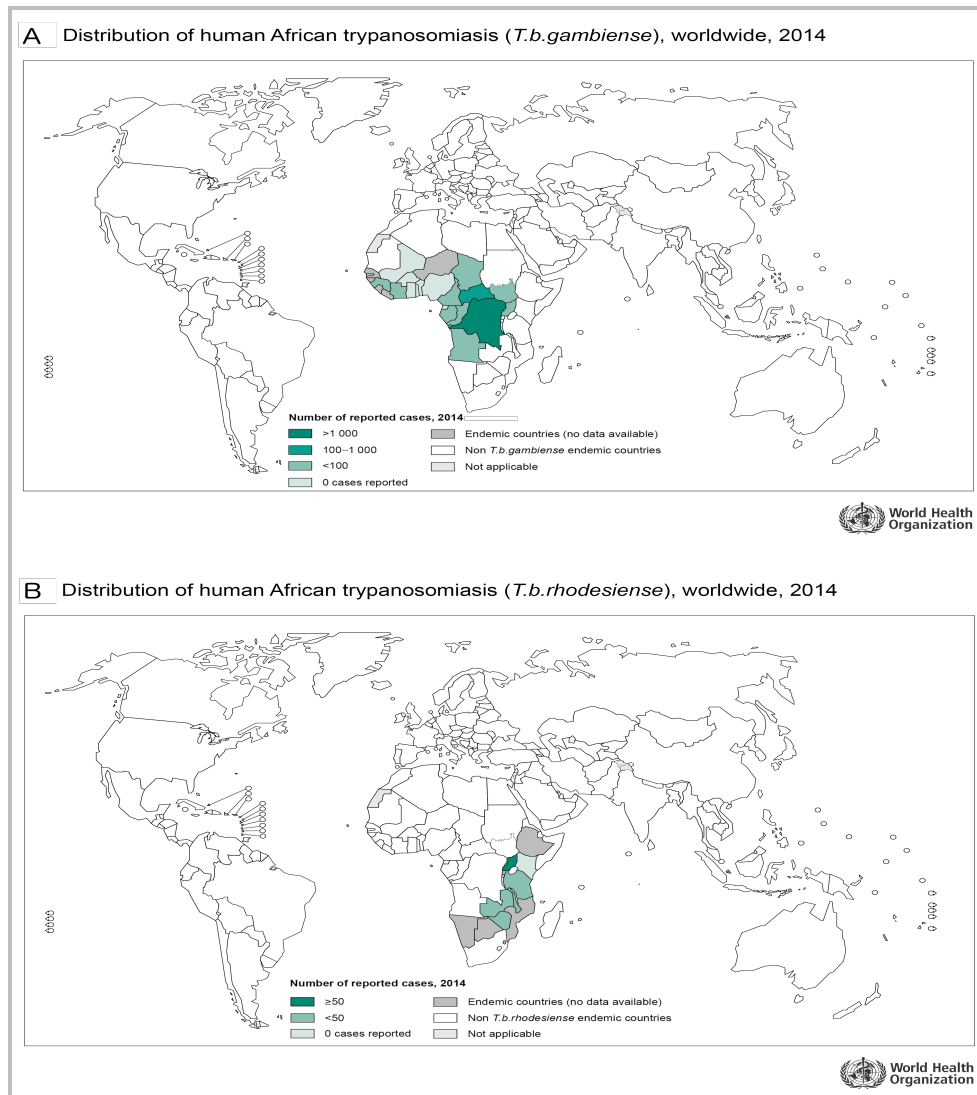
Figure 1: Distribution of human African trypanosomiasis (A-*T. b. gambiense* and B-*T. b. rhodesiense*), worldwide, 2014 (http://gamapserver.who.int /mapLibrary/app/search Results.aspx).

From alimentary canal of the insect vector to bloodstream of the mammalian host, there are drastic changes encountered by the flagellate from these environments, e.g. glucose concentration, pH, osmolarity and the absence of proteolytic enzymes (18). *T. brucei* therefore developed morphological and metabolic adaptions for the survival in these different environments, particularly in the mitochondrion.

In PS, the cristae-rich mitochondrion is equipped with the cytochrome-containing complexes of the electron transport chain (ETC). Krebs cycle activity was earlier thought to be fully present in PS *T. brucei* (19-21). However, more recent studies

revealed that Krebs cycle is not used for the complete oxidation of acetyl-CoA to carbon dioxide, although genes encoding the set of enzymes of this cycle are present in the genome and expressed in the PS (22-25). Living in the tsetse fly midgut where glucose is scarce or absent, PS flagellates have developed an energy metabolism based on amino acids in the mitochondrion. Proline is the main carbon source for the midgut PS. NADH from the catabolism of proline is supplied to ETC for maintaining the mitochondrial (mt) membrane potential $\Delta\psi_m$, which is in turn coupled with ATP synthesis by F_oF_1-ATP synthase (26). However, when PS flagellates are grown in standard glucose-rich conditions, glucose is preferentially selected for glycolysis, which takes place in specialized peroxisome-derived organelles called glycosomes. Under these conditions, PS mitochondrial metabolism is attenuated (27,28).

In contrast, the long slender BS relies mainly on glycolysis for energy production. Thus, its mitochondrion is reduced, lacking significant cristae, cytochrome-containing respiratory complexes and key enzymes of the Krebs cycle (29). Interestingly, the hydrolytic activity of the F_oF_1-ATPase in BS sustains the indispensable $\Delta\psi_m$, even without the proton-pumping respiratory complexes III and IV. Thus, F_oF_1-ATPase of integrity possesses an essential, unique function in the mitochondrion (30). However, *T. b. equiperdum* and *T. b. evansi* and a laboratory strain of kDNA independent *T. b. brucei* (31) are able to maintain their $\Delta\psi_m$ without the kDNA encoded F_oF_1-ATPase subunit 6 (A6). Single point mutations in the nuclear-encoded subunit γ of the mitochondrial F_1F_0-ATPase were identified these flagellates (30). Some of these mutations enabled the compensation for the complete loss of kDNA in BF *T. brucei*, with F_0-independent generation of the essential $\Delta\psi m$ (32,33).
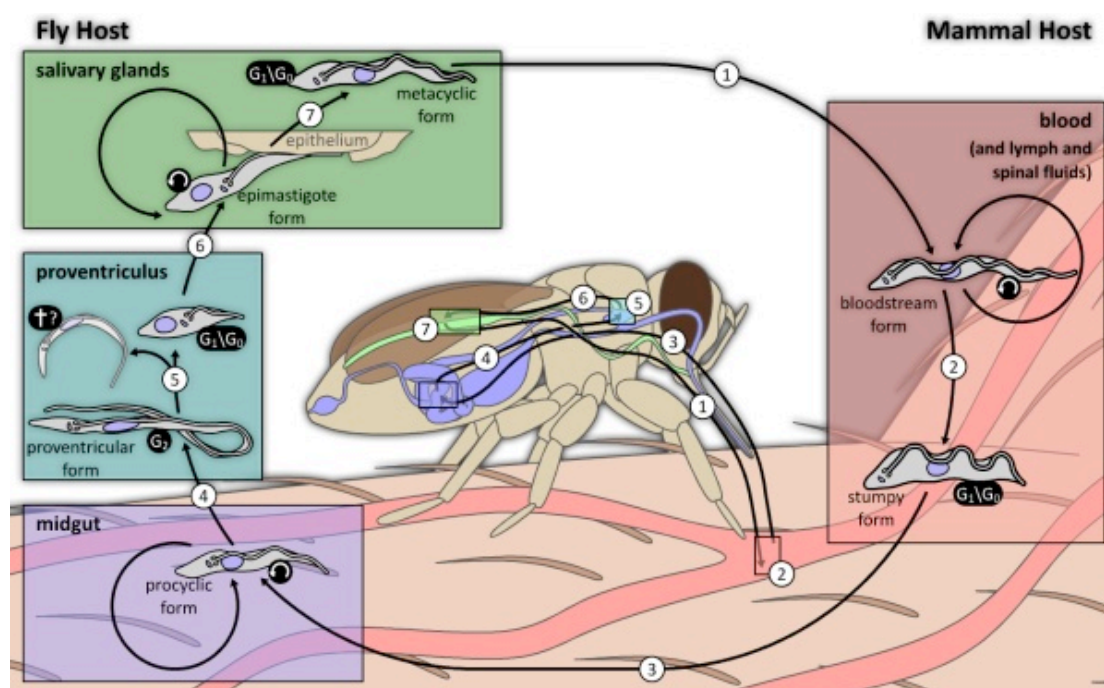
Figure 2: An outline of the lifecycle of *T. brucei* through the mammalian and Tsetse

fly hosts. The peripheral and internal number labeling indicate the life cycle step and corresponding locus: 1. Tsetse fly sting; 2. Density dependent differentiation; 3. Tsetse bloodmeal; 4. Transition to proventriculus; 5. The extension in length and decrease in width occurs during a single cell cycle in proventriculus; 6. Entering salivary glands; 7. Differentiation of the epimastigote to the metacyclic. From (http://www.richardwheeler/net/contentpages/image.php?gallery=Scientific_Illustratio n&img=Trypanosome_Life_Cycle&type=jpg).

2.3. The central dogma of the mitochondrion: the kinetoplast，its transcription, RNA editing and translation

2.3.1. Hallmark: the kinetoplast

A defining characteristic of the kinetoplastid flagellates is the kinetoplast, also known as kDNA, which represents the trypanosome's mitochondrial genome (Figure 3). It is a compact network composed of thousands of the mutually concatenated DNA minicircles and tens of maxicircles adjacent to the flagellar basal body. In *T. brucei*, the maxicircles encode two rRNAs, one small guide RNA (gRNA) and 18 protein-coding genes for subunits of respiratory chain, F_oF_1-ATPase and mt ribosome (34). Among the 18 protein-coding transcripts, twelve of them go through a process called RNA editing as a maturation step to make them translatable. Minicircles encode almost all the gRNAs required for RNA editing, which will be discussed later in this section.

1.	Nucleus	5.	Flagellar pocket
2.	Kinetoplast	6.	Axoneme and paraflagellar rod
3.	Mitochondrion	7.	Glycosome
4.	Basal body and probasal body		

Figure 3: Basic morphology of *T. brucei* taken from (35).

During the cell cycle, the kDNA network is faithfully replicated for distribution to both daughter cells. More than one hundred proteins are employed to accomplish this

sophisticated process. Minicircle replication is initiated by topoisomerase II, which directs the release of individual minicircles to the so-called kinetoflagellar zone between the kDNA and flagellar pocket (Figure 3). A universal minicircle sequence-binding protein (UMSBP) (36) and the so-called p38 protein recognize and bind the origin of replication on minicircles. The Pif1 helicase (37) and Pri2 primase (38) bind to free minicircles for DNA unpackaging and RNA primer synthesis, respectively, so that Pol1B polymerase can generate the leading and lagging strands (39). In this process, topoisomerase IA removes the intermediate structure formed during the replication of minicircles, termed as theta structure (40), with primers from Okazaki fragments resolved by the Pif5 helicase.

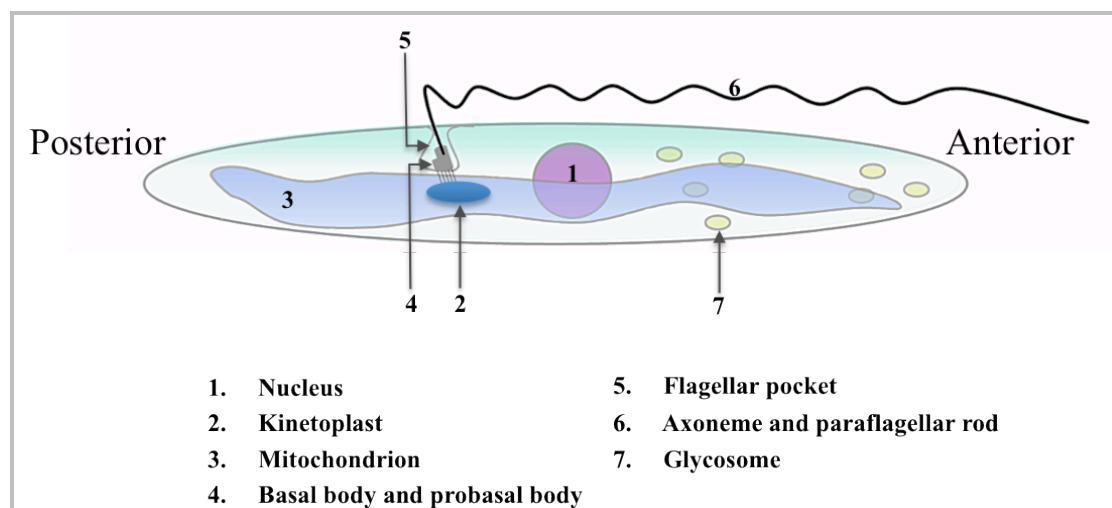The replication mechanism of maxicircles remains less understood. It has been reported that the Pif2 helicase is indispensible for the maxicircle replication (41). The Pri1 primase, polymerases Pol1C and Pol1D have also been reported to be involved in maxicircle duplication (38).

During the evolution, some sub-species without the intact kDNA have emerged from *T. b. brucei*, such as *T. b. equiperdum* and *T. b. evansi*. A laboratory strain of kDNA independent *T. b. brucei* has also been generated (31). Although these cells lack the A6 subunit of the membrane-embedded F_O-moiety of the ATPase, a condition that normally leads to disruption of the whole complex (42), they are still able to maintain $\Delta\psi_m$. This situation has also been observed in petite mutant yeast and ρ^0/ρ^- mammalian cells, which also lack (ρ^0) or have aberrant (ρ^-) mt DNA (43,44). It has been discovered that single point mutations in subunit γ of the mitochondrial F_1F_0-ATPase in these flagellates (32,33,45,46) may be analogous to those in petite mutant yeast (45), which facilitate ATP hydrolysis by only the soluble F_1 moiety of the ATPase. In this situation, ATP, which has three phosphate groups that contribute four negative charges, is converted to ADP, having one less negative charge, by the F_1-ATPase. This action results in a sufficiently low mitochondrial ATP concentration to allow the antipodal exchange of these two nucleotides by the ATP/ADP carrier protein (AAC) of ADP^{3-} for ATP^{4-}. This exchange process generates $\Delta\psi_m$ across the inner membrane by making the intermembrane space more positively charged as compared to matrix.

2.3.2. Transcription in mitochondrion

The transcription mechanism of kDNA in *T. brucei* is still unknown. So far, only a mitochondrial RNA polymerase (mtRNAP) has been reported in the transcription of both maxicircles and minicircles (47,48). In the maxicircle, there is a conservative region consisting of rRNA and protein coding genes with the length of 17 kb (Fig. 4), while the remaining variable region is comprised of repeated sequences (49). The polycistronic transcription of the maxicircle initiates at a site around 1.2 kb upstream of the 12S rRNA gene (50). In the case of the heterogeneous minicircles, it has been

estimated number that is around 400 classes of these molecules are present in kDNA, each of which encoding 2-5 gRNAs in *T. brucei* (51,52). Endonucleases are recruited post-transcriptionally for the cleavage of primary polycistronic transcripts into monocistrons for both maxi- and minicircles as a step in RNA maturation.
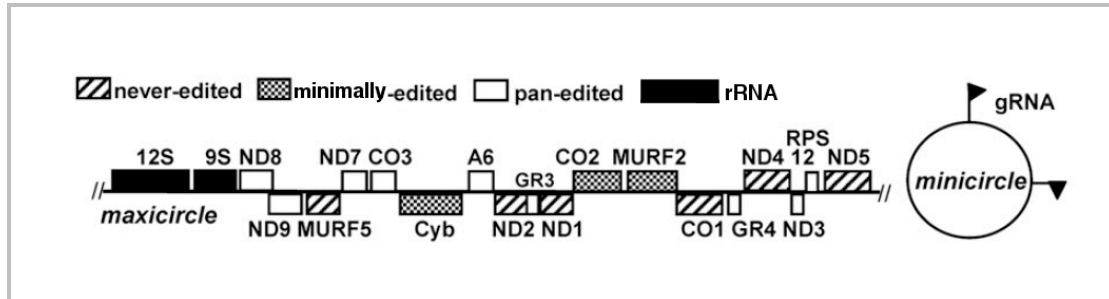
Figure 4: Genes arrangement on maxicircle (conserved region) and minicircle DNA from (53). A6, ATP synthase subunit 6; CO1, cytochrome *c* oxidase subunit 1; CO2, cytochrome *c* oxidase subunit 2; CO3, cytochrome *c* oxidase subunit 3; CYb, cytochrome *b*; GR, G-rich region; MURF2, maxicircle unidentified reading frame 2; MURF5, maxicircle unidentified reading frame 5; ND1, NADH dehydrogenase subunit 1; ND3, NADH dehydrogenase subunit 3; ND4, NADH dehydrogenase subunit 4; ND5, NADH dehydrogenase subunit 5; ND7, NADH dehydrogenase subunit 7; ND8, NADH dehydrogenase subunit 8; ND9, NADH dehydrogenase subunit 9; MURFl, maxicircle unidentified reading frame 1; RPS12, 40S ribosomal protein S12. Flag represents the start of gRNA coding gene.

2.3.3. RNA editing

RNA editing refers to a post-transcriptional processing step introducing changes into a transcript that changes its information content, with the exception of 5' capping, 3' polyadenylation and splicing (54). Prevalent in the eukaryotic domain, from rRNA, mRNA, microRNAs to tRNAs (55-59), RNA editing was first reported as four uridine (U) residue insertion into the mitochondrial transcript encoding subunit II of cytochrome oxidase (CO2) in *T. brucei* (60), followed with more extensive U-insertion/deletion discovered in other mitochondrial mRNA transcripts (60,61). The restriction of U-insertion/deletion editing exclusively to kinetoplastids indicates that this process originated in order Kinetoplastida (62,63).

2.3.3.1 Editing profile in *T. brucei*

The 20 maxicircle genes in *T. brucei* are categorized into never-edited (MURF5, MURF1, ND1, COI, ND4, ND5, 9S and 12S rRNAs); pan-edited (ND8, ND9, ND7, COIII, A6, CR3, CR4, ND3, RPS12); minimally-edited (CYb, COII, MURF2) (Fig. 4; abbreviations defined in the legend) (64). The grouping is mainly based on the general

editing coverage of maxicircle transcripts. In pan-edited transcripts, U-insertion/deletion occurs throughout the protein coding region. In contrast, this process happens within limited regions in minimally-edited transcripts. However, the RNA editing pattern appears to be developmentally regulated at different stages of the life cycle, e.g. COII and CYb transcripts are preferentially edited in PS and not in BS, which lacks the respiratory complexes that incorporate these two subunits (65). The differential editing profile between these two life stages suggests that this process may serve to regulate the expression of maxicircle genes.

2.3.3.2 Editing: substrates and machinery

U-insertion/deletion editing in *T. brucei* has been demonstrated in a single site with synthetic mRNA, gRNA and a crude mitochondrial extract providing the editing activity. (66,67). While mRNA is the substrate in the editing reaction, gRNA serves to direct the precise U-insertion/deletion at the site. The protein editing machinery is the catalytic center for the multistep U-insertion/deletion process. (68-71).

2.3.3.3 mRNA

Transcription on maxicircle provides polycistronic RNAs as substrate for both endolytic cleavage and editing to produce mature mRNAs. The maintenance of mRNA through its maturation is attributed to the protective function of the short poly(A) tail, synthesized by mitochondrial (kinetoplast) poly(A) polymerase (KPAP)1 (72). This protein is responsible for the polyadenylation of pre-edited, edited and never-edited mRNA. However, both the pattern and stabilizing effect of polyadenylation vary among these transcripts. Pre-edited mRNAs tend to have short (20-50 nt) poly(A) tails, while edited and never-edited molecules typically possess either short or long (200-300 nt) poly(A/U) tails (73-76). In pre-edited mRNAs, the loss of short poly(A)-tails causes decay results *in vitro* (77,78), whereas, *in vivo* it does not affect the steady-state levels, indicating the neutral role of short poly(A)-tails in stabilizing pre-edited mRNAs. Once editing initiates, short poly(A)-tails in these pre-edited mRNAs will be switched from either destabilizing or neutral mode into the stabilizing mode for edited transcripts (79). When editing of the transcript approaches the end, long tails are attached to the fully or nearly-fully edited mRNA via KPAP1 and a terminal uridylyl transferase named RET1 (for RNA editing Tutase 1) (72,80-82). Kinetoplast polyadenylation/ uridylation factors (KPAFs) 1 and 2 enhance the processivity of KPAP1 and RET1 (79). The role of long tail in these mRNA does not significantly affect the stability, instead it is believed to facilitate mRNA binding to the ribosome, thus forming a defining *cis*-elements of translation-competent mRNAs in *T. brucei* (79).

2.3.3.4   gRNA

The maturation process of gRNA post-transcription remains unclear. It has only been reported that RET1 participates in post-transcriptionally adding a poly-U tail to the gRNAs, while a mitochondrial exoribonuclease TbRND affects gRNA metabolism by trimming this structure (79,83). To conduct editing, a gRNA needs to hybridize to its cognate mRNA. This binding is attributed to both Watson-Crick base and G:U pairing via the 5' end 8-12 nucleotides anchor region and 3' end poly-U tail flanking the information domain, which may contain a stem-loop (Fig. 5) (68,84). The start site of editing is the first mismatch of the RNA duplex by a purine-rich sequence of the mRNA and 3' end poly-U tail in gRNA (Fig. 5). It is from this site the information domain of the gRNA starts to specify U-insertions and deletions in a small region of mRNA (85). Eventually, the newly edited region of mRNA is complementary to the gRNA via Watson-Crick and G:U base pairing. The gRNA of CO II editing is a gRNA like element 3′ untranslated region (UTR) of the mRNA, downstream of COII open reading frame and directs editing by hybridizing its cognate mRNA part *in cis*, hereby forming a hairpin loop (86).
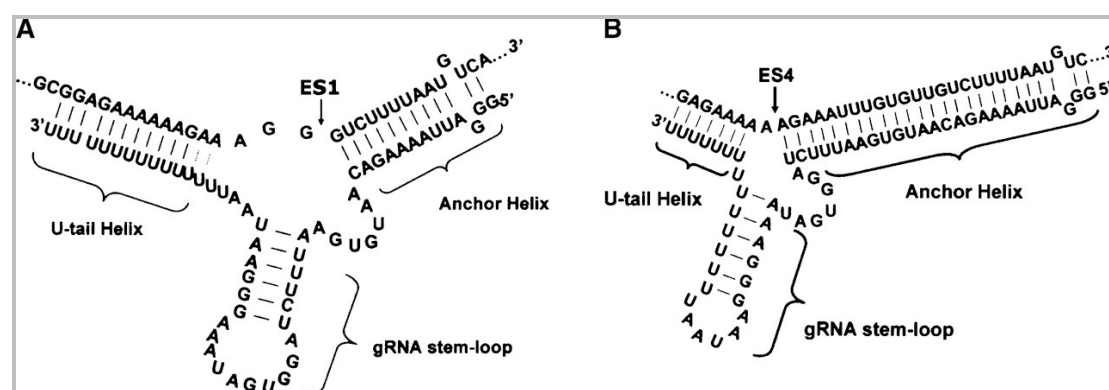
Figure 5: Duplex of gRNA and mRNA at the editing site (Interactions of mRNAs and gRNAs involved in trypanosome mitochondrial RNA editing: Structure probing of a gRNA bound to its cognate mRNA), taken from (87)

An interesting observation is that some gRNAs maintain 5'-triphosphates, which is unusual as these moieties not usually present in mRNA and rRNAs (68). The biological function of this 5′-triphosphate remains unknown.

2.3.3.5   Editing machinery

The gRNA:mRNA duplex serves as a substrate for the multistep enzymatic editing reactions that are conducted via RNA editing core complex (RECC, also referred to as the ~20S editosome), constituted with components responsible for each enzymatic step and structural proteins (Table 1) (53). The editing process can be generalized as three steps: cleavage of mRNA, U-deletion or insertion and mRNA ligation (Fig. 6).

Due to the presence of different types of duplexes, three RECC isoforms are responsible for U-deletion, U-insertion and *cis*-editing, respectively. However, RECC isoforms carry out their enzymatic activities with a core complex (Fig. 6), except that mRNA cleavage in each duplex is performed by site specific RNA editing endonuclease: REN1 for a deletion site, REN2 for insertion site and REN3 for the COII *Cis*-editing site (70,88). The mutually exclusive association of site specific RNA editing endonuclease contained module REN1, REN2 and REN3 with component-identical core complex bearing U-deletion (RNA editing exonuclease 2, REX2), U-insertion (RNA editing terminal uridyltransferase 2, RET2) and ligation (RNA editing ligase 1 and 2, REL1 and REL2) activities implies the switching module between these isoforms is coupled with the specific editing site on board (Fig. 6) (70,71,88-90).

The substrate specific endonuclease launches editing by cleavage at the first mismatch nucleoside immediately upstream of the anchor duplex on the mRNA, perhaps either the bulged out uridine from the mRNA at the deletion site or a purine from the gRNA in the insertion and *cis*-editing sites. The endonuclease cleavage results in 5' and 3' cleavage products of the mRNA that is bridged by the binding of the gRNA poly(U) tail and anchor domain. For gRNA directed editing, RNA editing exonuclease REX1 (90), which is not part of the core RECC but is associated with REN1, removes single-stranded Us from the 5' product in deletion editing. The terminal uridylyl transferase RET2 adds Us to the 5' products for insertion editing (91). As for *cis*-editing, which also requires the common set of enzymatic activities needed U-insertion and RNA ligation, have this core module associated with the REN3 endonuclease, which recognizes these substrates. The enzymatic reactions above all result in a nicked mRNA ready for repair by RNA editing ligases REL1 and possibly REL2 to seal one site of editing (Fig. 6) (92-94).

Table 1: Subunits of RECC. Dual abbreviations of RECC components in the current literature (53,95,96).

Alternative names for proteins	Gene ID	Proposed function
MP81/KREPA1	Tb927.2.2470	Structural, U-insertion subdomain organizer
MP63/KREPA2	Tb927.10.8210	Structural, U-deletion subdomain organizer
MP42/KREPA3	Tb927.8.620	Structural
MP24/KREPA4	Tb927.10.5110	Structural, RNA binding
MP19/KREPA5	Tb927.8.680	Structural
MP18/KREPA6	Tb927.10.5120	Structural, RNA binding
REN1/KREN1	Tb927.1.1690	Insertion site specific endonuclease
REN2/KREN2	Tb927.10.5440	Deletion site specific endonuclease
REN3/KREN3	Tb927.10.5320	*Cis*-editing site specific endonuclease
MP46/KREPB4	Tb927.11.2990	Structural, heterodimer with endonuclease
MP44/KREPB5	Tb927.11.940	Structural, endonuclease
MP49/KREPB6	Tb927.3.3990	Structural, part of KREN3 module
MP47/KREPB7	Tb927.9.5630	Structural, part of KREN2 module
MP41/ KREPB8	Tb927.9.5690	Structural, part of KREN1 module
REX1/KREX1	Tb927.7.1070	U-specific exonuclease
REX2/KREX2	Tb927.10.3570	U-specific exonuclease
REL1/KREL1	Tb927.9.4360	RNA ligase
REL2/KREL2	Tb927.1.3030	RNA ligase
RET2/KRET2	Tb927.7.1550	TUTase

For each pan-edited transcript, a series of multiple gRNA-mediated editing without unedited sequence has been called as one editing domain. The polarity of editing within the domain has been reported as from the 3' to 5' due to that the anchoring region for upstream gRNA usually is based on the downstream editing (97).
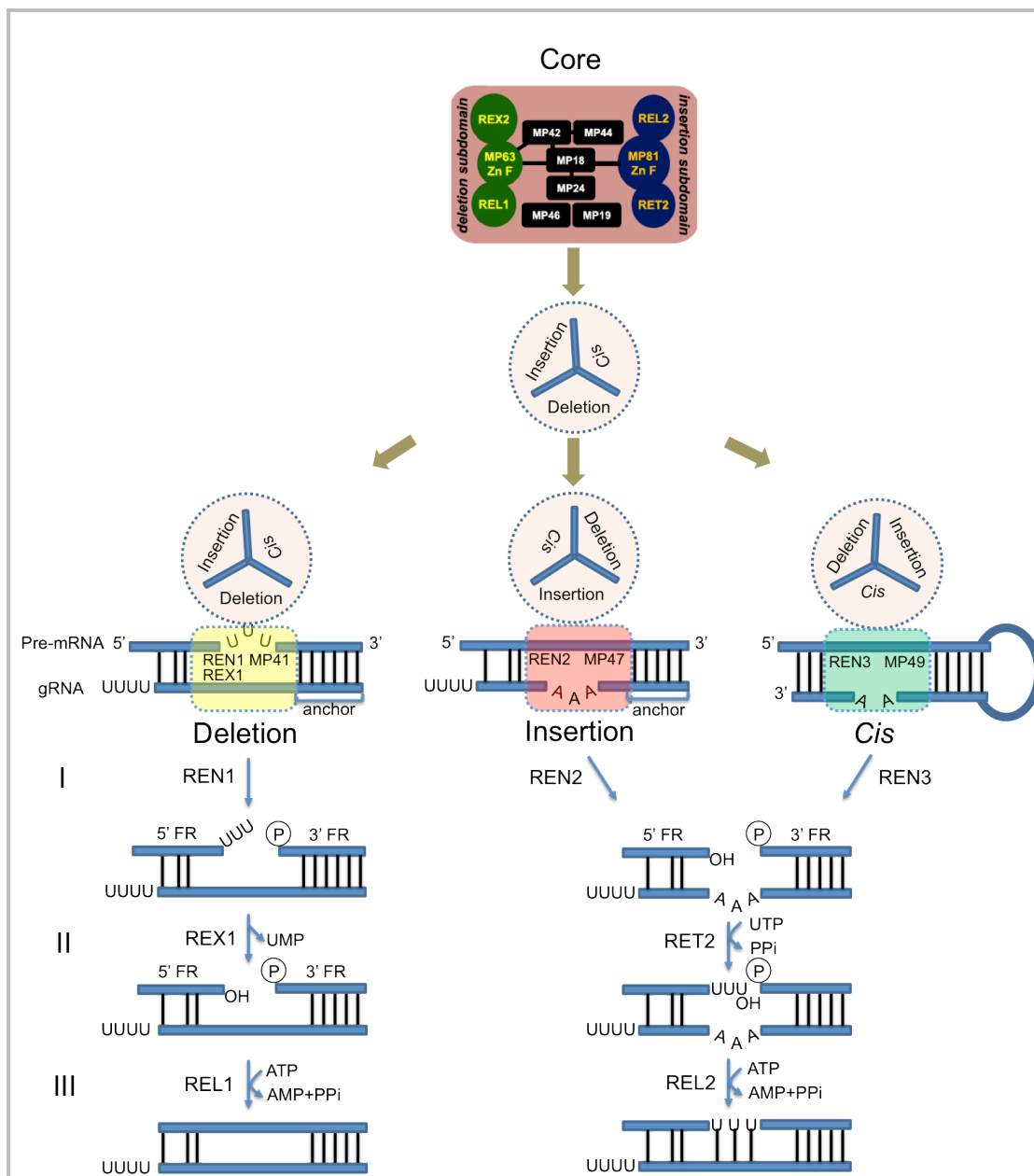
Figure 6: The construction and functioning process of three editosome isoforms. Associations of subunits in the core complex are presented by black bars or connections, modified from (53). The three general steps of RNA editing are indicated by roman numerals: I Cleavage of mRNA, II U-deletion or insertion and III mRNA ligation. MP: mitochondrial protein (structural and/or RNA binding components); REX: RNA editing exonuclease; REN: RNA editing ligase; REL: RNA editing ligase; RET: RNA editing TUTase (53).

While RECC provides the main catalytic activities needed for RNA editing, the mt RNA-binding complex 1 (MRB1) is another indispensible player in this process. MRB1 has been identified as an interacting protein group, with approximately 30 members in various configurations. These proteins are either directly interacting with RNA via binding motifs or as mediators by associating with RNA-bound proteins

during processing. Although a few proteins from MRB1 have been identified with protein–protein interaction, the assembly of MRB1 remains unknown. Recent work has further divided MRB1 into subcomplexes (72,98-101). The MRB1 core, a six protein subcomplex, has been identified in all MRB1 purifications (102). It contains the gRNAs stabilizer GAP1/2 heterotetramer (48,101), MRB3010, MRB11870 and MRB5390, which are essential in RNA editing (53,103-105), and MRB8620, whose role in RNA editing to was examined in this thesis. Although the MRB1 core seems to act as one subcomplex, the characterized subunits display distinct effects on gRNA stability. Unlike GAP1/2 heterotetramer, the RNAi-silencing of MRB3010 or MRB11870 consistently gives rise to an increase in gRNAs instead of causing gRNA degradation (Table 2) (103,104), which implies the accumulation of gRNA upon these editing compromised conditions. The coordination of how the MRB1 core subunits work together therefore will be the key to decipher its function in the complex editing process.
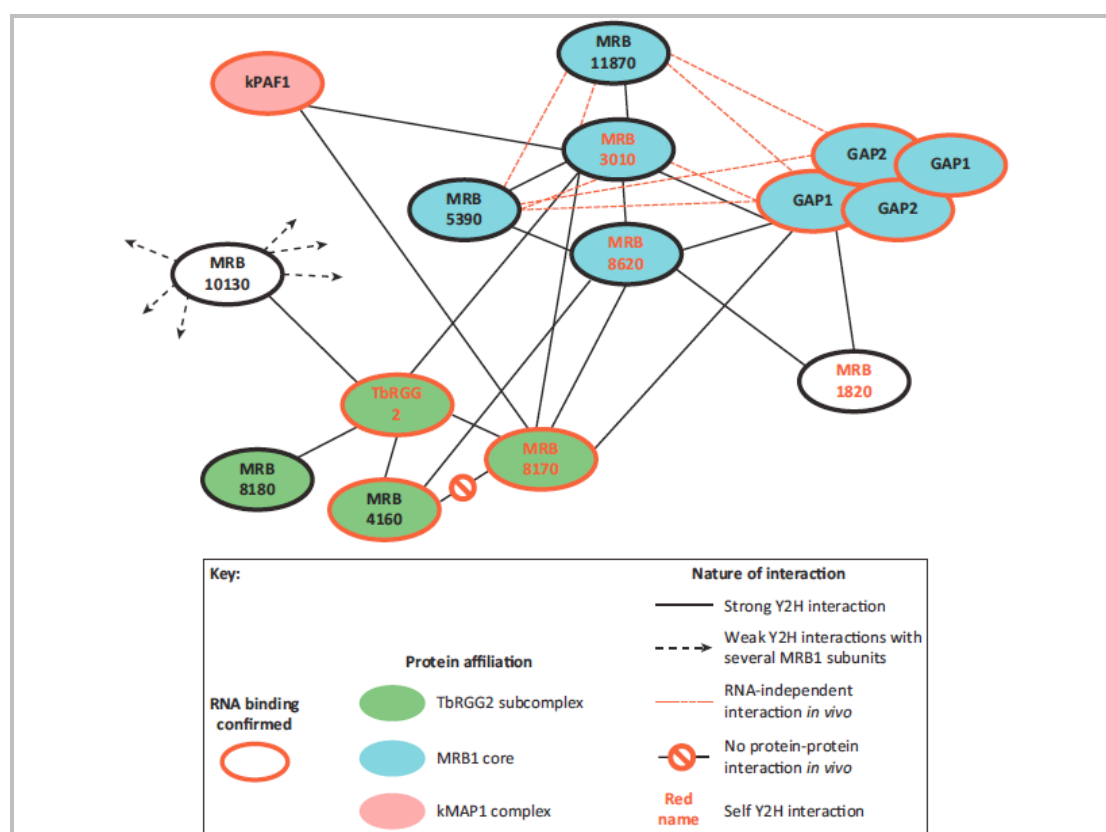
Figure 7: Architecture of MRB1. A summary of the pairwise yeast two-hybrid (Y2H) and immunoprecipitation (IP) *in vivo* mapped MRB1 subunit interactions (101,102,106,107). A key to the figure is shown at the bottom. The black lines represent 'strong' Y2H interactions in at least one direction. The promiscuous 'weak' Y2H interactions of the ARM/HEAT domain containing MRB10130 with various MRB1 subunits are also indicated by broken black lines projecting out. Taken from (107).

Subunits of the MRB1 core e.g. GAP1, MRB3010, MRB8620 have been shown to interact with the TbRGG2 subcomplex, constituted of four proteins and named after one RNA-binding protein, TbRGG2 (102). With both annealing and double-stranded RNA unwinding activities in its N-terminal and C-terminal regions, respectively (108), TbRGG2 has been hypothesized to have several roles: binding RNA, interacting with MRB8180 as well as two novel RNA-binding proteins, MRB8170 and MRB4160, in a mutually exclusive manner (Fig. 7) (102,106). All these proteins in the subcomplex appear to be key players particularly in pan-editing (106,109). Along with the biochemical properties of TbRGG2, it seems to indicate that this subcomplex ensures the multi-round editing process with a still unknown mechanism (Figure 8) (102,107). The RNA-editing helicase 2 (REH2) associates with MRB1 core and TbRGG2 subcomplexes, and is found to interact with RECC via RNA (99). Its double-stranded RNA unwinding activity appears involved in dislodging gRNA from an edited mRNA. Another RNA helicase REH1, with only an identified RECC association, has been proposed to facilitate editing in the gRNA release step (110).

Another intensely studied complex involved in RNA editing is the mitochondrial RNA-binding protein 1 and 2 (MRP1/2) complex. This abundant complex is a heterotetramer consisting of two each of the MRP1 (TriTrypDB accession no. Tb927.11.1710) and MRP2 (accession no. Tb927.11.13280) subunits (111,112). Though with low sequence identity, these two proteins all possess a "Whirly" transcription factor fold tertiary structure found in the plant ssDNA binding transcription factor p24 (113). It also has been shown *in vitro* that the electropositive face in this tetramer allows the complex's nonspecific interaction with the negatively charged phosphate groups of the RNA backbone, particularly with the high affinity for gRNA. Its capacity to promote the annealing of complementary RNAs *in vitro* has been proposed as the function of MRP1/2 complex in RNA editing (111,114,115). However, the downregulation of specific transcripts (both pre-edited and edited) upon dual MRP1/2 RNAi repression points to some still undefined function in mitochondrial RNA processing (116).

Table 2: Summary of MRB1 RNAi-silencing phenotype modified from (107).

Name[a]	TriTrpDB Ascension number	RNAi Growth		RNA abundance effect due to RNAi		RNA binding?			Complex	Notes	References
		PF[d]	BF[d]	Pan-edited RNA	Minimally-edited RNA	gRNA	Proven	Predicted domain[b]			
GAP1 (GRBC2)	Tb927.2.3800	Y	Y	Y	Y (not coll)	Y	Y		MRB1 core	-gRNA binding -Paralog GAP2	(48, 101, 102)
GAP2 (GRBC1)	Tb927.7.2570	Y	Y	Y	Y (not coll)	Y	Y		MRB1 core	-gRNA binding -Paralog GAP1	(48, 101, 102)
MRB3010	Tb927.5.3010	Y	Y	Y	Y	N	N		MRB1 core	-Role in early step kRNA editing	(102, 103)
MRB5390	Tb11.02.5390	Y	ND	Y (subset)	Y (subset)	N	ND		MRB1 core		(102, 105)
MRBB11870	Tb927.10.11870	Y	Y	Y	Y	N	N		MRB1 core	-Role in early step kRNA editing	(102, 104)
MRB8620	Tb927.11.16860	ND	ND	ND	ND	ND	ND		MRB1 core		
TbRGG2	Tb927.10.10830	Y	Y	Y	N	N	Y	RGG, RRM	TbRGG2	-3'-5' progression RNA editing	(102, 108)
MRB8170	Tb927.8.8170	N	ND	Y	N	N	Y		TbRGG2	-paralog MRB4160	(106)
MRB4160	Tb927.4.4160	N	ND	N	N	N	Y		TbRGG2	-paralog MRB8170	(106)
MRB8170/MRB4160[c]	--	Y	Y	Y	Y	N	--		--	-RNAi affects pan-edited more than minimally	(106)
MRB1680	Tb927.6.1680	Y	ND	Y	N	ND	ND	C2H2 Zn Finger			(107)
REH2	Tb927.4.1500	Y	ND	Y	Y (not coll)	Y	Y	dsRBP, DExD-box		-gRNA binding - RNA unwinding activity	(99)

[a]Aliases given in parentheses. [b]Common abbreviation of motifs.
[c]Describes simultaneous RNAi-mediated silencing of the paralogs MRB8170 and MRB4160.
[d]Abbreviations: BF, bloodstream form; gRNA, guide RNA; N, no; ND, not determined; PF, procyclic form; Y, yes.

2.3.4. Translation

The mt ribosome is a protein rich ribonucleoprotein complex constituted by the 30S small subunits (SSU) and 50S large subunits (LSU) ribosomal subunits, each with maxicircle-encoded 9S RNA and 12S RNA, respectively (117-119). In addition to these two ribonucleoprotein (RNP) particles, there are abundant stable 45S particles (SSU*) containing only 9S rRNA required for the translation, and are proposed as homodimers of the SSU or SSU associated with additional proteins. Variable minor amounts of 65S and 70S RNP particles are also existent in the mitochondrion, which may represent homodimers of the LSU and SSU*, respectively (120). Most of the proteins identified from mt ribosomes demonstrate no homology outside kinetoplastida, implying very low conservation and/or a divergent function in kinetoplastid mitochondria (121,122).

For mt translation, there are a number of ribosome-associated pentatricopeptide repeat-containing (PPR) proteins playing important roles. Six such proteins called TbPPR2-7 have shown the essentiality for rRNA biogenesis or stability, which is evidenced by the loss of either 9S or 12S rRNAs in their respective RNAi knockdowns (123). KPAF1 (also known as TbPPR1), and potentially KPAF2, also a PPR protein, have been reported as inducers for the synthesis of the long A/U tails, mainly attributed to KPAP1 and RET1 as previously described (124). With the long tail working as the *cis*-element on mitochondrial RNAs, the ORF-containing mRNA will be recruited to ribosomes for translation. The mt tRNAs that are also a component of the translation apparatus are all imported into the organelle, since none of them are encoded in the kDNA (125).

The association of RNA editing substrates (pre-edited mRNA and gRNA) and editing core and gRNA binding complexes with the LSU subunit begins to point that the mt ribosomes are involved in higher-order interactions with mRNA editing and polyadenylation machineries so that the fully edited mRNA templates are selected for translation (124,126).

2.4. Prospect

With all the oddities mentioned above and the potential of being a pharmacological target, the mitochondrion of *T. brucei* has been a subject of intense research since the discovery of the kinetoplast.

T. brucei has served as a model organism for the order Kinetoplastida. In contrast to the other trypanosomatids, the presence of RNA interference machinery in *T. brucei* has allowed it to be used for reverse genetics. The ease of electroporation to introduce

transgenic constructs also makes *T. brucei* amenable for laboratory studies (127).

Nowadays live-cell imaging has been a powerful tool for real-time tracking of biological processes of individual cells. Microscopy-based techniques such as fluorescence recovery after photobleaching (FRAP), with the measurement time-lapse fluorescent intensity (Figure 8), are frequently utilized in studies about protein dynamics such as diffusion, assembly and interaction with partners (128).
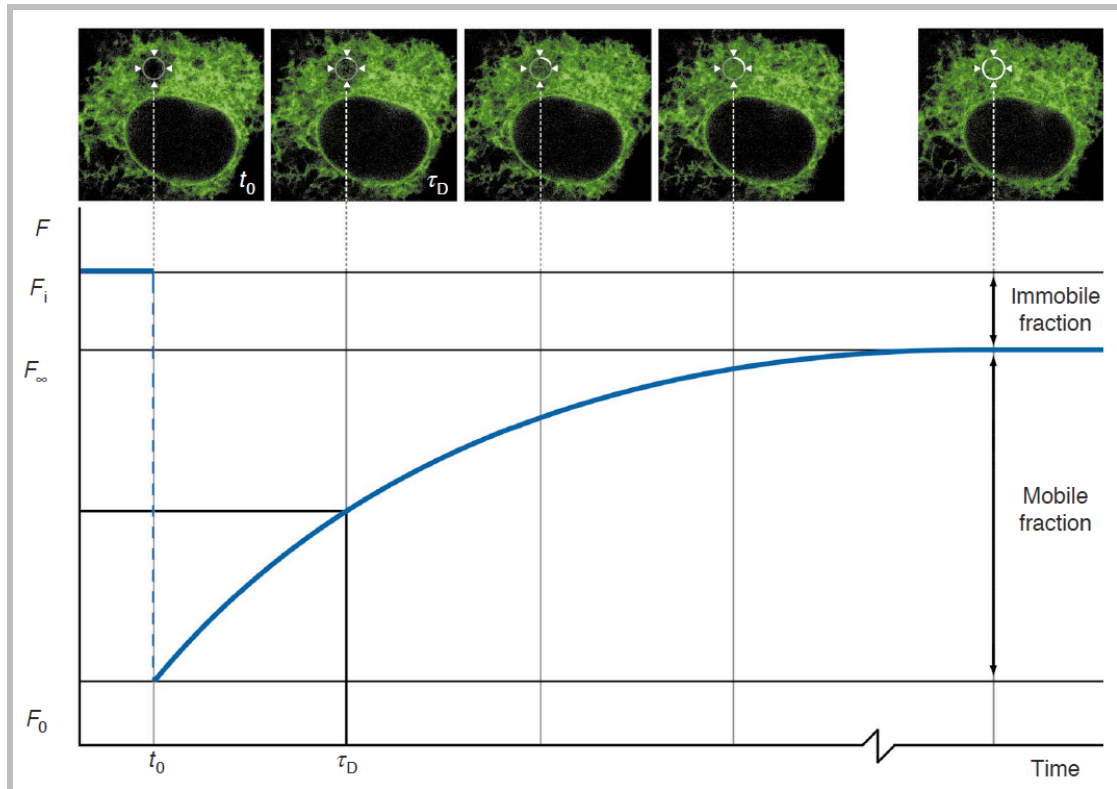

Figure 8: An example of a fluorescence recovery after photobleaching (FRAP) measurement taken from (129). When a region in the fluorescent area is bleached at time t_0 the fluorescence decreases from the initial fluorescence F_i to F_0. The fluorescence recovers over time by diffusion until it has full recovery (F_∞). The diffusion time τ_D indicates the time at which half of the fluorescence has recovered. The mobile fraction can be calculated by comparing the fluorescence in the bleached region after full recovery (F_∞) with that before bleaching (F_i) and immediately after bleaching (F_0).

The complex and highly ordered RNA processing in the mitochondrion of *T. brucei* is based on the coordination between protein, RNA and other molecules. Live cell imaging undoubtedly would be appropriate to implement to better understand these processes (130,131). However, the vigorous motility of *T. brucei* compromises the application of these powerful techniques. Methods of immobilization of cells were utilized in different studies, though the impact of these immobilization on cell viability remains to be addressed. For example, a study in which kinetoplastid protists were immobilized in a CyGEL matrix did systematically assay cell viability, claiming

its suitability for the PS and Leishmania major but not for the BS (132). This immobilization method was later used to study the trafficking of surface proteins in L. major by FRAP (133).

RNA editing is never an isolated series of events in the mitochondrion. Nowadays with the development of deep sequence technique, more systematic information can be extracted from the RNA processing studies, this application on mapping will certainly accelerate the unfolding of fine-procedures and regulation on editing.

3. PUBLISHED RESULTS

3.1.   Study # 1

Dynamics of Mitochondrial RNA-Binding Protein Complex in Trypanosoma brucei and Its Petite Mutant under Optimized Immobilization Conditions

Zhenqiu Huang, Sabine Kaltenbrunner, Eva Šimková,

David Staněk, Julius Lukeš, Hassan Hashimi

The dynamics of mitochondrial RNA-binding protein complex in *Trypanosoma brucei* and its petite mutant under optimized immobilization conditions

Zhenqiu Huang [1,2], Sabine Kaltenbrunner [2], Eva Šimková [3], David Staněk [3], Julius Lukeš [1,2,] & Hassan Hashimi [1,2,*]*

[1] *Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic*

[2] *Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic*

[3] *Institute for Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic*

[*] **Corresponding authors**

Tel: +420 38 777 5416; E-mail: jula@paru.cas.cz; hassan@paru.cas.cz

Running title: Dynamics of MRP in the mitochondrion of live trypanosomes

Keywords: Trypanosome, mitochondrion, organelle dynamics, FRAP, RNA editing, live cell imaging

1

ABSTRACT

There is a variety of complex metabolic processes ongoing simultaneously in the single, large mitochondrion of *Trypanosoma brucei*. Understanding the organellar environment and dynamics of its proteins requires quantitative measurement *in vivo*. In this study, we have validated a method for immobilizing both procyclic (PS) and bloodstream (BS) stages of *Trypanosoma brucei brucei* with a high level of cell viability over hours and verified its suitability for undertaking fluorescence recovery after photobleaching (FRAP), using mitochondrial-targeted yellow fluorescent protein (YFP). Next, we used this method for comparative analysis of the translational diffusion of mitochondrial RNA binding protein 1 (MRP1) in the BS and in *T. brucei evansi*, which is like petite mutant yeast because they lack organelle-encoded nucleic acids. FRAP measurement of YFP-tagged MRP1 revealed its different dynamics in both cell lines, illuminating from a new perspective how the absence or presence of RNA affects proteins involved in mitochondrial RNA metabolism. This work represents the first attempt to examine this process in live trypanosomes.

3.2.   Study # 2

Integrity of the core mitochondrial RNA binding complex 1 is vital

for trypanosome RNA editing

Zhenqiu Huang, Drahomíra Faktorová, Adéla Křížová, Lucie Kafková,

Laurie K. Read, Julius Lukeš and Hassan Hashimi

Integrity of the core mitochondrial RNA binding complex 1 is vital for trypanosome RNA editing

Zhenqiu Huang[1,2], Drahomíra Faktorová[1,2], Adéla Křížová[2], Lucie Kafková[3], Laurie K. Read[3], Julius Lukeš[1,2,4] and Hassan Hashimi[1,2,*]

[1]Biology Center, Institute of Parasitology, Czech Academy of Sciences and [2]Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic; [3]Department of Microbiology and Immunology, School of Medicine, State University of New York at Buffalo, Buffalo, New York, USA; [4] Canadian Institute for Advanced Research, Toronto, Canada

*Corresponding author

Abstract

The parasitic flagellate *Trypanosoma brucei* is the causative agent of the human and veterinarian diseases African sleeping sickness and nagana. A majority of its mitochondrial-encoded transcripts undergo RNA editing, an essential process of post-transcriptional uridine insertion and deletion to produce translatable mRNA. Several protein complexes are involved in this unique and complicated process. Besides the well-characterized RNA editing core complex, the mitochondrial RNA binding 1 (MRB1) complex is one of the key players. It comprises a core complex of about six proteins – guide RNA associated proteins (GAP) 1/2, which form a heterotetramer that binds and stabilizes gRNAs, plus MRB5390, MRB3010, and MRB11870, which play roles in initial stages of RNA editing, presumably guided by the first gRNA:mRNA duplex in the case of the latter two proteins. To better understand all functions of the MRB1 complex, we performed a functional analysis of the MRB8620 core subunit, the only one not characterized so far. Here we show that MRB8620 plays a role in RNA editing in both procyclic and bloodstream stages of *T. brucei*, which reside in the tse-tse fly vector and mammalian circulatory system, respectively. While RNAi silencing of MRB8620 does not affect procyclic *T. brucei* fitness when grown in glucose-containing media, it is somewhat compromised in cells grown in the absence of this carbon source. MRB8620 is crucial for integrity of the MRB1 core, such as its association with GAP1/2, which presumably acts to deliver gRNAs to this complex. In contrast, GAP1/2 are not required for the fabrication of the MRB1 core. Disruption of the MRB1 core assembly is followed by the accumulation of mRNAs associated with GAP1/2.

Keywords: RNA editing; mitochondrion; trypanosome

5. CONCLUSIONS

In this thesis, I examined the dynamics of MRP1/2 complex with FRAP measurement on the *in situ* C-terminally tagged MRP1-YFP, its verified proxy in this research (35). The comparison of MRP1/2 dynamics in BS and AK *T. b. evansi* cells reflects the influence of mt-encoded RNAs, which are absent from the latter subspecies (46,137). More of the MRP1/2 complex in BS was in an immobile fraction than in AK *T. b. evansi* (35). The presence of mt-encoded RNA in the former clearly affects the dynamics of the MRP1/2 complex. The RNA mediated association of MRP1/2 with the RECC shown in the related trypanosomatid *Leishmania tarentolae*(114), suggests that RNA processing proteins in contact with RNA bound to MRP1/2 may hinder its dynamics. The incomplete recovery to pre-photobleached levels within the MRP1-YFP1 region of interest in AK *T. b. evansi* indicates an immobile fraction of the heterotetramer. The effects from interacting proteins, imported tRNA contacts, matrix sieving to tag or inner membrane hindering may contribute to this result (35).

The translational diffusion of the MRP1/2 complex in PS *T. b. brucei* also exhibits an immobile fraction of the complex, although smaller than that in the BS. The difference between the two stages might result from the presence of cristae, activity of energy metabolism as stated before or the developmentally regulated mitochondrial transcriptomes (35).

MRP1/2 complex, as part of the complex the RNA editing machinery, plays its dynamic role with the coordination of the other components. The RECC and its MRB1counterparts in RNA editing are of particular importance for understanding the dynamic process of this machinery as a whole.

Herein we studied MRB8620, a subunit of the MRB1 core subcomplex that functions in trypanosome RNA editing. The compromised integrity of MRB1 core by MRB8620 silencing gives rise to the impaired mt RNA editing in *T. brucei* and accumulated gRNAs, as was observed when MRB3010 and MRB11870 proteins were silenced by RNAi (53,104). The deletion of GAP1/2 heterotetramer, however, disrupted RNA editing due to the destabilization of gRNA instead of affecting the integrity of the MRB1 core. Therefore, GAP1/2 is proposed as functionally distinct entity in MRB1 core. And in this study, the disruptive MRB1 core appeared to hinder the dissociation of mRNA from the GAP1/2 subcomplex, at same time causing the reduced association between GAP1/2 and RECC. To sum up, the MRB1 core may serve to facilitate a conformational change in this super-complex machinery so that GAP1/2 is removed from the gRNA:mRNA duplex so that it does not sterically hinder RECC access to the editing site along the hybridized transcripts. Upon knockdown of MRB8620, the dynamics of this process is compromised.

However, the effect of MRB8620 knockdown on the doubling time of PS *T. brucei* in the glucose-rich SDM79 in contrast to that in glucose-poor SDM80 is subtle. This phenomenon is attributed to the reduced mitochondrial metabolism of flagellate in the glucose-rich SDM79, as glycolysis is the main source for cellular ATP (27). The

energy metabolism mode under this condition therefore masks the effects from impaired editing and translation of proteins.

As described above, the glucose based energy metabolism mode increases the resilience of the PS viability towards disrupted RNA editing. However, the effect of energy metabolism on RNA editing or mitochondrial RNA processing has been studied at different stage of life cycle and reported as developmental regulation by multiple mechanisms (136). Here, we tried to compare RNA abundance under the different PS growth conditions, glucose-rich and –poor environments. The observed regulation of different transcripts under these conditions suggested a potential effect from glucose based energy metabolism, also the regulation is fine-tuned upon each transcript.

6. REVIEW ARTICLE

Malleable Mitochondrion of *Trypanosoma brucei*

(2.2.3 section - RNA editing)

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, Julius Lukeš

(Abridged form of the 79 page article featuring the part that was co-written by me)

Malleable Mitochondrion of *Trypanosoma brucei* (2.2.3 section - RNA editing)

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, Julius Lukeš *International Review of Cell and Molecular Biology*, 315: 73–151 (2015) doi:10.1016/bs.ircmb.2014.11.00

Here, we summarize the current knowledge of mitochondrial biology of *Trypanosoma brucei*. 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. Only the 'RNA editing' of this review, which is contributed by me, is included in this thesis.

Zhenqiu Huang

Branisovska 31, České Budějovice ☎: +420 607786265   Email: zhenqiu@paru.cas.cz

----

RESEARCH EXPERIENCE

Sep 2011-
Present

Doctoral research (Parasitology): Institute of Parasitology Biology Centre of the Czech Academy of Sciences

- **Developed** an intuitive immobilization method for live cell imaging; **analysed** the dynamic property of mitochondrial protein.

- **Elucidated** that MRB1 core facilitated RNA editing by transforming the super complex conformation.

- **Investigating** the contacting manner of MRB1 proteins with RNA via **ICLIP & Sequence analysis**

Sep 2008-Jun 2011

Master research (Virology): State Key Laboratory for Biocontrol, Sun Yat-sen University

- Studied the **envelope formation** and **nuclear egress** of nucleocapsid on baculovirus.

- Investigated the **nucleocapsid assembly** of baculovirus

Sep 2008-Jun 2011

Bachelor research (Toxicology): Laboratory of environmental toxicology, Sun Yat-sen University

- Studying the time course distribution of compounds in the tissue and organs of mice
 (**Intravenous injection, organ harvesting, statistic analysis**)

SKILLS

Technical

- Cell culture, Real-time PCR, Viral infection/transfection and harvesting, Injecting mice, Dissecting mice and organ harvesting, Flow cytometry, Molecular cloning, Immunofluorescence staining, Confocal microscopy (Imaging, FRAP), Electron microscopy & Immunoelectron microscopy, Protein expression and purification, Immunoprecipitation, Glycerol/Sucrose gradient fraction.

Project management

- Project planning and delivering; Troubleshooting and protocol optimizing.
- Establishing collaboration; Team communication.
- Data analysis, presenting; Meeting organizing.
- Lab training and student supervision.

Computer

- Fluency in Windows & Microsoft office (Word, Excel, etc.).
- Image analysis software (Image J, Olympus Viewer, etc); Photoshop; GIMP.

EDUCATION

Sep 2011-
Present

Ph.D.: Molecular and Cell Biology and Genetics
Institute of Parasitology Biology Centre of the Czech Academy of Sciences
Advisor: Dr. Hassan Hashimi and C/o Prof. RNDr. Julius Lukeš CSc

Sep 2008-Jun 2011

M.Sc.: Microbiology Sun Yat-sen University, Guangzhou, China
Advisor: Prof. Kai Yang

Sep 2004-Jun 2008

B.Sc.: Biotechnology Sun Yat-sen University, Guangzhou, China
Advisor: Associate Prof. Meijin Yuan

SELECTED PUBLICATIONS

1. Huang Z, Kaltenbrunner S, Šimková E, Staněk D, Lukeš J, Hashimi H.
Dynamics of Mitochondrial RNA-Binding Protein Complex in *Trypanosoma brucei* and Its Petite
Mutant under Optimized Immobilization Conditions. **Eukaryot Cell.** 2014; 13: 1232-1240.

2. Huang Z, Faktorová D, Křížová A, Kafková L, Read L, Lukeš J, Hashimi H.
Assembly of the Core Mitochondrial RNA Binding Complex 1 is vital for   Trypanosome RNA
Editing. **RNA, in press**.

3. Yuan M, **Huang Z**, Wei D, Hu Z, Pang Y, Yang K.
Identification of *Autographa californica* Nucleopolyhedrovirus *ac93* as a Core Gene and Its
Requirement for Intranuclear Microvesicle Formation and Nuclear Egress of Nucleocapsids.
J Virol. 2011; 85(22): 11664-74.

4. Verner Z, Basu S, Benz C, Dixit S, Dobáková E, Faktorová D, Hashimi H, Horáková E, **Huang
Z**, Paris Z, et al. Malleable Mitochondrion of *Trypanosoma brucei*. **Int Rev Cell Mol Biol.** 2014;
315: 73-151.

CONFERENCE

Oct 2014	**The Complexed Life of mRNA.** Heidelberg, Germany **Poster**
	"MRB8620 is indispensible for RNA editing *Trypanosoma brucei*"
Sep 2014	**Annual RNA club meeting.** Prague, Czech **Presentation**
	"MRB8620 is indispensible for RNA editing *Trypanosoma brucei*"
Sep 2013	**Annual RNA club meeting.** Prague, Czech **Poster**
	"Dynamics study of MRP1 RNA binding protein in *Trypanosoma brucei*"
May 2012	**Mitochondria in Life, Death and Disease.** Fodele, Crete, Greece **Poster**
	"Characterization of mitoproteome in *Trypanosoma brucei*"

AWARDS

| Sep 2011-Present | ● | Research fellowship, Institute of Parasitology Biology Centre of the Czech Academy of Sciences (Excellent Ph.D. student) |
| 2004 - 2009 | ● | Outstanding student Scholarship, Sun Yat-sen University |

REFERENCE

Ph.D. Advisor	Julius Lukeš, Professor, Institute of Parasitology Biology Centre of the Czech Academy of Sciences
Ph.D. Advisor	Hassan Hashimi, Research scientist, Institute of Parasitology Biology Centre of the Czech Academy of Sciences
Frequent Collaborator	Laurie Read, Professor, Department of Microbiology and Immunology, University at Buffalo