University of South Bohemia

Faculty of Science

Department of Molecular Biology

Master thesis

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Functional analysis of Iron-Sulfur cluster assembly protein Isd11 in procyclic and bloodstream *Trypanosoma brucei*

Master thesis

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Anotation:

In this study we used RNA interference to knock down Isd11, protein that participates in iron-sulfur cluster biogenesis. The results show that Isd11 is essential in the procyclic form of *Trypanosoma brucei*. Moreover, other key and conserved components of the pathway - IscU and Nfs, are down-regulated in the Isd11 knock down cells, while levels of frataxin, the putative iron donor, remain unaltered. The depletion for Isd11 results in the drop of activities of iron-sulfur cluster-containing enzymes aconitase and fumarase, followed by the decrease of mitochondrial membrane potential. Fractionation shows that in both the procyclic and bloodstream forms Isd11 is located exclusively in their mitochondria.

I hereby declare that I did all work, summarized in this thesis, on my own or on the basis of consultations with my supervisors, and only using the cited literature.

České Budějovice, April 13, 2009

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Piya Changmai

Acknowlegements

I would like to thank Jula Lukeš for the great opportunity to work in this lab, his supervision and a lot of supports. Thank you Zdeněk Paris for supervising and teaching me in every step and on a daily basis. Thank you all members of Laboratory of Molecular Biology of Protists - Zdeňka Čičová, Lucie Hanzálková , Hassan Hashimi , Milan Jirků , Shao Jun Long , Lucie Novotná , Pavel Poliak . Lenka Richterová , Jiří Týč, Zuzana Vávrová , Zdeněk Verner , Yan Zi Wen, De Hua Lai and Petr Růžička for suggestion and such a nice time in the lab (and in pubs). I can not write all what you did to me. I would like to also thank the Czech government for scholarship and opportunity to study in the Czech Republic. I would like to thank other people the names of which I don't mention, yet your names will always be in my heart. I would like to mention the most important thing in my life, my family. You are my first love and this love will last until my body will be burned. I dedicate all of good things in my life to you. Even we are far away, actually in different sides of the world, but I always feel your love in every breath.

ขอขอบคุณทุกสิ่งทุกอย่าง ทุกๆคน ทุกเหตุการณ์ที่เกิดขึ้น ที่ทำให้ผมได้มีวันนี้

Ještě jednou *děkuji všem za všechno*

Attachments

Paper's preface

I am a co-author of the article: Isd11 but not Mtu1 is essential for tRNA thiolation in the excavate protist *Trypanosoma brucei* (Paris et al., 2009). My contribution to this paper was as follows:

I have measured the growth curve of the non-induced and RNAi-induced Isd11 knock downs both in the procyclic and bloodstream forms. I have determined the level of iron sulfur cluster proteins using Western analyses. I also measured mitochondrial membrane potential in the procyclic form. I prepared construct for RNAi knock down in the bloodstream form and electroporated it into *T. brucei*. I also prepared the publication-quality pictures for this article. I did not participate in experiments involving thiolation of

tRNAs and functional analysis of Mtu1, most of which are being performed by the collaborating laboratory of Prof. J.D.Alfonzo.

Below, please find signature of the corresponding author confirming my contribution.

Declaration

I declare that Piya Changmai substantially contributed to the results presented in the attached paper, which is at present in preparation for publication, pending some additional experiments describing tRNA thiolation.

Julius Lukeš

corresponding author

Contents

Abstract

Isd11 functions as a stabilizer and adaptor for Nfs, forming cysteine desulfurase complex which provides sulfur to the scaffold protein IscU in the ISC system of the Iron sulfur (Fe/S) proteins assembly. The ISC system is highly conserved in eukaryotes and was derived from prokaryotes. However, Isd11 is its sole component unique to eukaryotes. Isd11 is essential in *Saccharomyces cerevisiae* under both fermentable and non-fermentable conditions, and was also shown to be indispensable in mammalian cells. Using RNAi to knock down Isd11, here we show that the protein is essential in the procyclic form of *Trypanosoma brucei*. Its depletion resulted in reduced activity of the marker Fe/S enzymes aconitase and fumarase in both the cytosol and mitochondrion, followed by a decrease of mitochondrial inner membrane potential. Cell fractionation using digitonin showed that Isd11 is located in the mitochondrion of both procyclic and

bloodstream forms of *T. brucei*. Interestingly, Isd11 knock down in the bloodstream form of *T. brucei* does not affect cell growth (data not shown here).