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Faculty of Science

Department of Molecular Biology and Biochemistry

RNDr. thesis

Functions and cellular localization of cysteine desulfurase and selenocysteine lyase in *Trypanosoma brucei*

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Annotation:

Nfs-like proteins have cysteine desulfurase (CysD) activity, which removes sulfur (S) from cysteine, and provides S for iron–sulfur cluster assembly and the thiolation of tRNAs. These proteins also have selenocysteine lyase activity in vitro, and cleave selenocysteine into alanine and elemental selenium (Se). It was shown previously that the Nfs-like protein called Nfs from the parasitic protist Trypanosoma brucei is a genuine CysD. A second Nfs-like protein is encoded in the nuclear genome of T. brucei. We called this protein selenocysteine lyase (SCL) because phylogenetic analysis reveals that it is monophyletic with known eukaryotic selenocysteine lyases.
The Nfs protein is located in the mitochondrion, whereas the SCL protein seems to be present in the nucleus and cytoplasm. Unexpectedly, downregulation of either Nfs or SCL protein leads to a dramatic decrease in both CysD and selenocysteine lyase activities concurrently in the mitochondrion and the cytosolic fractions. Because loss of Nfs causes a growth phenotype but loss of SCL does not, we propose that Nfs can fully complement SCL, whereas SCL can only partially replace Nfs under our growth conditions.

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Declaration:

I hereby declare that I did all the work, presented in this thesis, by myself or in collaboration with the co-authors of the published article.

Further, I declare that in accordance with the Czech legal code § 47b law No. 111/1998 in its valid version, I consent to the publication of my RNDr. thesis (in an edition made by removing marked parts archived by the Faculty of Science) in an electronic way in the public access to the STAG database run by the University of South Bohemia in České Budějovice on its web pages.

České Budějovice, 30 April 2010 .....

Pavel Poliak

Co-authors agreements

We declare here that Pavel Poliak contributed the major part to the publication "Poliak P., Van Hoewyk D., Oborník M., Zíková A., Stuart K.D., Tachezy J., Pilon M. and Lukeš J. (2010). Functions and cellular localization of cysteine desulfurase and selenocysteine lyase in *Trypanosoma brucei*. *FEBS J.* 277, 383-393."

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the FEBS Journal

Functions and cellular localization of cysteine desulfurase and selenocysteine lyase in *Trypanosoma brucei*

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Keywords
Fe–S cluster; mitochondrion; RNAi; selenoprotein; *Trypanosoma*

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Nfs-like proteins have cysteine desulfurase (CysD) activity, which removes sulfur (S) from cysteine, and provides S for iron–sulfur cluster assembly and the thiolation of tRNAs. These proteins also have selenocysteine lyase activity *in vitro*, and cleave selenocysteine into alanine and elemental selenium (Se). It was shown previously that the Nfs-like protein called Nfs from the parasitic protist *Trypanosoma brucei* is a genuine CysD. A second Nfs-like protein is encoded in the nuclear genome of *T. brucei*. We called this protein selenocysteine lyase (SCL) because phylogenetic analysis reveals that it is monophyletic with known eukaryotic selenocysteine lyases. The Nfs protein is located in the mitochondrion, whereas the SCL protein seems to be present in the nucleus and cytoplasm. Unexpectedly, downregulation of either Nfs or SCL protein leads to a dramatic decrease in both CysD and selenocysteine lyase activities concurrently in the mitochondrion and the cytosolic fractions. Because loss of Nfs causes a growth phenotype but loss of SCL does not, we propose that Nfs can fully complement SCL, whereas SCL can only partially replace Nfs under our growth conditions.

Structured digital abstract

- MINT-7298305: *NFS* (uniprotkb:Q386Y7) and *PHB1* (uniprotkb:Q57UX1) *colocalize* (MI:0403) by *cosedimentation through density gradients* (MI:0029)
- MINT-7298357: *SCL* (uniprotkb:Q38DC4) and *Enolase* (uniprotkb:Q38BV6) *colocalize* (MI:0403) by *cosedimentation through density gradients* (MI:0029)