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Short communication

The selenoproteome is dispensable in bloodstream forms of *Trypanosoma brucei*

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ABSTRACT

Here we show that absence of Sep-tRNA:Sec-tRNA synthase (SepSecS) a key enzyme required for the synthesis of the three trypanosomal selenoproteins does not affect growth of bloodstream forms of *Trypanosoma brucei*. Both life cycle stages of *T. brucei* are highly sensitive to auranofin, a compound known to target selenoproteins. However, the same sensitivity is observed in the SepSecS double knockout cell lines indicating that the trypanocidal action of auranofin is not connected to selenoproteins. Finally, we show that absence of selenoproteins does not increase sensitivity to H_2O_2-induced oxidative stress. Thus in cell culture normal growth of procyclic and bloodstream *T. brucei* does not depend on selenoproteins.

1. Introduction, results and discussion

Selenium is an essential dietary trace element. It is present in proteins as selenocysteine (Sec), a cotranslationally inserted amino acid encoded by UGA. Sec is formed by the tRNA-dependent conversion of serine (reviewed in [1–4]). In the first step $tRNA^{Sec}$ is mis-acylated by seryl-tRNA synthetase. The subsequent conversion to Sec proceeds by two different pathways. In bacteria $Ser\text{-}tRNA^{Sec}$ is directly transformed to $Sec\text{-}tRNA^{Sec}$ by selenocysteine synthase [5]. Archaea and eukaryotes require an additional step, the formation of the intermediate phosphoserine (Sep) by $phosphoseryl\text{-}tRNA^{Sec}$ kinase (PSTK). The resultant $Sep\text{-}tRNA^{Sec}$ is then converted into the $Sec\text{-}tRNA^{Sec}$ by Sep-tRNA:Sec-tRNA synthase (SepSecS) [6,7]. Recently, the Sec-insertion pathway in trypanosomatids has attracted a lot of interest [8–10] and *Trypanosoma brucei* was used as a model to elucidate the in vivo pathway of $Sec\text{-}tRNA^{Sec}$ formation in eukaryotes [11]. Bioinformatic analysis of the *T. brucei* genome predicts three selenoproteins, termed SelK, SelT and SelTryp. Trypanosomal SelK and SelT are distantly related to mammalian selenoproteins whereas SelTryp appears to be unique to trypanosomatids [10]. Moreover, in vivo labeling with radioactive selenium detects three bands whose molecular weights are consistent with the three predicted trypanosomal selenoproteins. Using double allelic knockout (KO) cell lines for PSTK and SepSecS we recently showed that the two proteins are individually required for $Sec\text{-}tRNA^{Sec}$ formation in procyclic *T. brucei* [11]. These results are fully consistent with reconstitution studies done with the mammalian Sec-insertion system [7] and suggest that eukaryotes have a single pathway of $Sec\text{-}tRNA^{Sec}$ synthesis that requires $Sep\text{-}tRNA^{Sec}$ as intermediate.

However, in trypanosomes in contrast to other systems neither PSTK nor SepSecS were essential for normal growth [11]. These studies were done in procyclic cells, it therefore remained possible that selenoproteins have an essential role in the disease relevant bloodstream form *T. brucei*. In order to test this we produced a double KO cell line of bloodstream *T. brucei* (NYSM single marker cell line [12]) that lacks SepSecS, the central enzyme for eukaryotic $Sec\text{-}tRNA^{Sec}$ formation.

The two alleles of the SepSecS gene in bloodstream *T. brucei* NYSM cells were replaced by homologous recombination using either the puromycine (for the 1. allele KO) or the blasticidine (for the 2. allele KO) resistance genes. The DNA fragments used for transfection consisted of the resistance genes flanked by the same sequences that flank the SepSecS gene (Fig. 1A). The Southern analysis verifies that both alleles of the SepSecS gene have been replaced by the resistance markers (Fig. 1A). Since $Sec\text{-}tRNA^{Sec}$ cannot form in these cells $Sep\text{-}tRNA^{Sec}$ should accumulate. Analysis by acid urea polyacrylamide-electrophoresis [13] which can separate the two forms of the $tRNA^{Sec}$ shows that this is indeed the case (Fig. 1A, right panel). However, when grown in standard bloodstream medium HMI-9 [14] the *T. brucei* NYSM strain and the SepSecS KO cells showed essentially the same mean generation time mirroring the results obtained in procyclic cells [11].

The normal growth of selenoprotein-lacking procyclic and bloodstream *T. brucei* cells was unexpected, since it had been reported that both life cycle stages of *T. brucei* are sensitive to nanomolar concentrations of auranofin, a compound suggested to inactivate selenoproteins [10]. Based on these results it was proposed that selenoproteins, especially the trypanosomatid-specific

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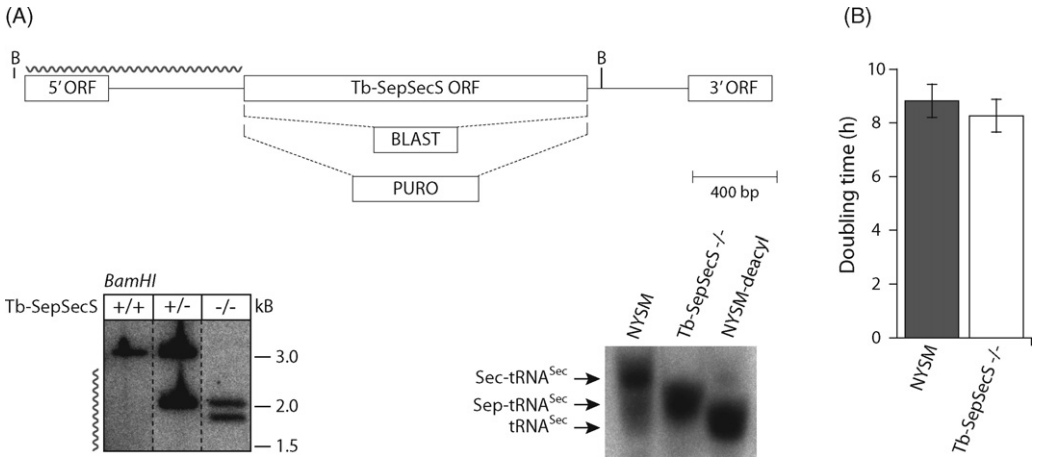

Fig. 1. Lack of SepSecS does not affect growth of bloodstream *T. brucei*. (A) Generation of a bloodstream SepSecS double KO cell line. Schematic to scale drawing of the wild-type Tb-SepSecS encoding locus and the situation after homologous recombination leading to replacement of the two loci by blasticidine (BLAST) and puromycin (PURO) resistance genes, respectively. BamHI (B) restriction sites are indicated. Left panel: Southern blot of BamHI-digested genomic DNA of the indicated cell lines using a probe corresponding to the 5′-flank of the Tb-SepSecS ORF gene. Right panel: Total RNA isolated from wild-type cells (NYSM) and from the Tb-SepSecS double KO (−/−) cells was separated on a long acidic urea gel and analyzed for the presence of the different forms of $tRNA^{Sec}$ by Northern analysis. The right most lane contains chemically deacylated total RNA (NYSM-deacyl). The positions of the selenocysteinyl-$tRNA^{Sec}$ (Sec-$tRNA^{Sec}$), the phosphoseryl-$tRNA^{Sec}$ (Sep-$tRNA^{Sec}$) and deacylated $tRNA^{Sec}$ ($tRNA^{Sec}$) are indicated. (B) Comparisons of doubling times of the parent bloodstream strain *T. brucei* NYSM and the corresponding Tb-SepSecS double KO cell line (Tb-SepSec −/−) grown in HMI-9 [14]. Standard errors ($n = 9$) are indicated.

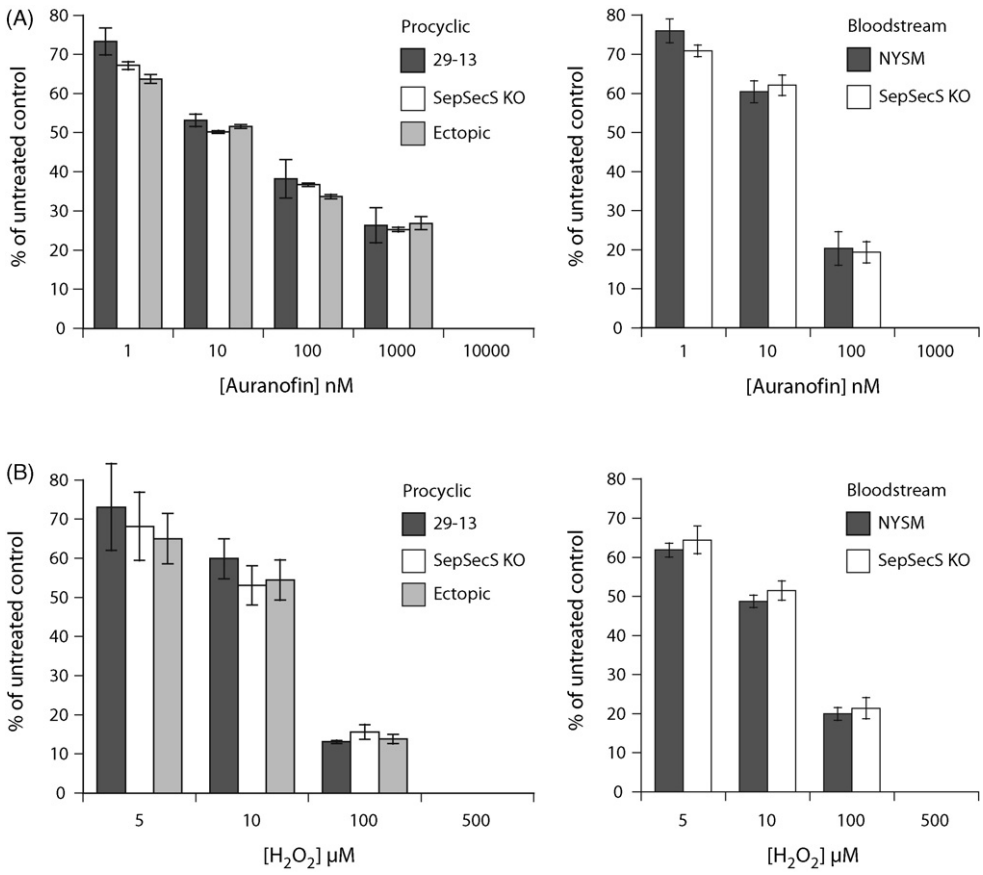

Fig. 2. Lack of SepSecS does not affect sensitivity to auranofin or H_2O_2, respectively. (A) Auranofin treatment: 2×10^6 cells each of procyclic *T. brucei* (29-13, the corresponding SepSecS KO strain, and the same strain complemented with an ectopic copy of the SepSec gene [11], top graph) as well as 10^5 cells each of bloodstream *T. brucei* (NYSM and the corresponding SepSecS KO strain, bottom graph) were incubated with the indicated concentration of auranofin. The graph shows the cell concentrations relative to untreated controls that are reached after 18 h of incubation at 28 °C for procyclics and at 37 °C for bloodstreams, respectively. The experiments were done in triplicate, the graph depicts the mean of the relative cell concentrations including standard errors. (B) H_2O_2 treatment: same as above but cells were treated with the indicated concentrations of H_2O_2.

SelTryp, might be novel targets for the development of an antiparasitic drug.

In order to address this discrepancy we compared the sensitivity of the parent cell line and the SepSecS double KO cells to auranofin. Fig. 2A shows that *T. brucei* is highly sensitive to nanomolar concentrations of auranofin in both life cycle stages and thus confirms the results reported before [10]. However, the same sensitivity was also observed for the procyclic and bloodstream SepSecS double KO cell lines. This shows that the trypanocidal action of auranofin cannot be due to inactivation of selenoproteins. Auranofin has been reported to interact with both selenol and thiol groups [15] suggesting that its effect on trypanosomes is due to binding to thiol groups.

In SelTryp selenocysteine is present in the C-terminal region in a CxxU motif [10] which is often found in selenoproteins that carry out redox functions. We therefore compared growth of the parent cell lines and the SepSecS double KO cells under oxidative stress conditions. To that end cells were incubated in standard growth medium supplemented with 5–1000 μM of H_2O_2 for 18 h. Fig. 2B shows that whereas growth of the procyclic and bloodstream parent cell lines was severely reduced with increasing concentrations of H_2O_2 the same was true for the corresponding KO cell lines. Thus, a redox function of SelTryp could not be verified.

In summary we show that normal growth of procyclic and bloodstream *T. brucei* in cell culture does not require any of the three selenoproteins. Moreover, the selenoproteins do not confer protection against added H_2O_2. This raises the question of why the selenocysteine insertion machinery and the three selenoproteins of *T. brucei* have not been lost during evolution? We believe there are at least two possible not mutually exclusive explanations. Lack of selenoproteins may have only a mild effect on growth which is not seen in in vitro cultures but which might be important in the in vivo situation when selenoprotein-lacking cells would compete with wild-type cells. Moreover, it is clear that the requirements for life in cell cultures are not identical to the requirements for life in the insect or the mammalian hosts. Thus, selenoproteins might be required for processes that are essential in the living hosts but that are bypassed in the in vitro cultures. However, this is speculation and it is clear that selenoproteins and the selenocysteine insertion machinery should at present not be considered as drug targets against *T. brucei*.

Acknowledgements

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