INVESTIGATION OF A PREDICTED N-TERMINAL ACETYLTRANSFERASE C (NATC) AS A POTENTIAL DRUG TARGET IN *TRYPANOSOMA BRUCEI* USING RNA INTERFERENCE

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INTRODUCTION

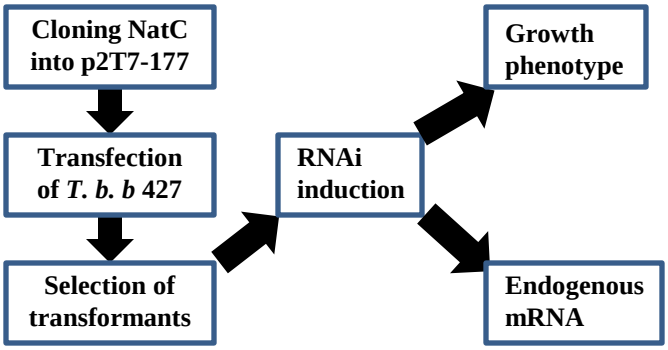
There is just a handful of available drugs against Human African Trypanosomiasis. Although these are becoming increasingly safer due to new combinations/formulations, the threat of drug resistance remains real. In this study, we have investigated NatC as a potential drug target.

OBJECTIVES

❖Determine the effect of silencing NatC catalytic subunit (NAA30) on growth of *T. b. b 427.*

❖Determine the effect of silencing NatC catalytic subunit (NAA30) on endogenous mRNA.

METHODOLOGY



RESULTS

A. Effect of NatC catalytic subunit (NAA30) RNAi on growth

❖A growth defect was observed 48hrs post RNAi induction.

❖There was background expression of dsRNA from the p2T7-177 vector as shown by the growth rate of uninduced transformants (Fig 1).

❖Occurrence of growth inhibition after RNAi varies depending on the stability and role of the target RNA/protein.

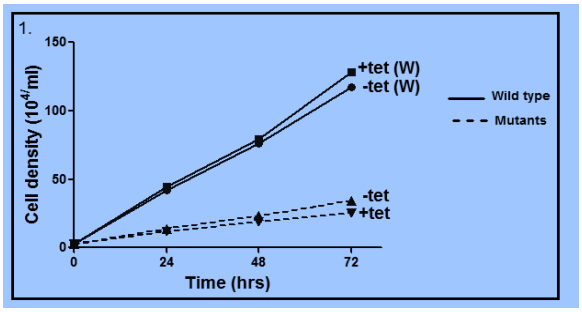


Fig 1: Growth curve of *T. b. b 427* (W) and mutants grown in the absence (-) and presence (+) of tetracycline over a period of 72hrs post RNAi induction. 2.5x10^4/ml was the starting cell density.

B. Effect of NatC catalytic subunit (NAA30) RNAi on endogenous mRNA

❖There was a reduction in the transcript of NatC catalytic subunit 48hrs post induction.

❖On day 0 (pre-induction), transcript of NAA30 was lower than that of the wild type (Fig 2). This can be explained by the 'background' expression of dsRNA leading to specific mRNA degradation.

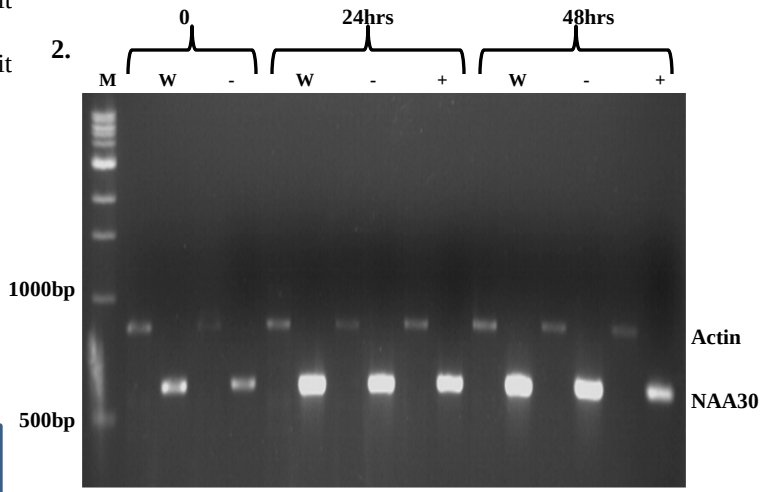


Fig 2: Agarose gel (2%) showing gene expression analysis of wild type (W), non-induced (-) and induced (+) cells. RNA was extracted from 1x10^5/ml pre-induction (time 0), 24hrs (2.73x10^{5}cells/ml); 48hrs (3.75x10^{5}cells/ml) and analyzed using Reverse Transcriptase PCR. Actin (700bp) was run as an internal control along side target NAA30 (573bp).

CONCLUSION

Knockdown of the NAA30 led to reduced growth rate of *Trypanosoma brucei 427*. This suggests that interfering with the normal function of NAA30 can affect proliferation of *T. brucei*. Therefore, NAA30 can possibly be used as a potential drug target.

RECOMMENDATIONS

❖Selective inhibitors of NatC catalytic subunit should be identified with the use of *insilico* analysis and tested *invitro* using *Trypanosoma brucei* species.

❖RNA interference of NatC catalytic should be done *invivo* using *T. b. b 427* infected mice to determine if there will be an effect on parasitaemia levels.

❖Knock out or use of the stem loop vector system should be used to confirm our findings.

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