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Cloning and functional complementation of *Schistosoma mansoni* cyclic nucleotide phosphodiesterases in Trypanosoma brucei

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1- Introduction

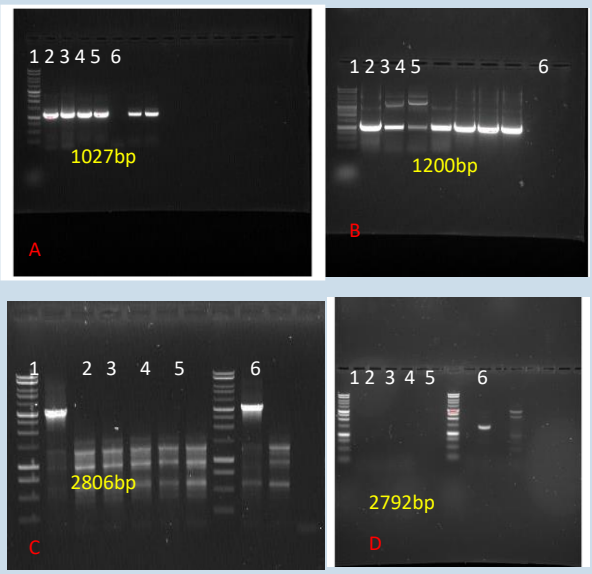
- Schistosomiasis is a neglected tropical disease caused by the parasitic flatworm genus Schistosoma
- The disease has the highest infection rates in the developing world. Currently, there is only one drug treatment available, an anthelmintic called Praziquantel.
- This reliance on one single medication has raised significant concerns over drug resistance in the worms and the continued effectiveness of the treatment. However, regulatory systems including cyclic nucleotide metabolism are emerging as primary candidates for drug discovery.

2. Objectives

- The cloning and expression of S. mansoni cyclic nucleotide phosphodiesterases (SmPDEs), specifically SmPDE7var, SmPDE1, SmPDE8, and SmPDE9C in a specialised Trypanosoma brucei cell line constructed as a model for heterologous expression of PDEs.
- a construct of TbrPDEB1 with the catalytic domain of SmPDEs 4A , SmPDE7var and SmPDE11 into *Trypanosoma brucei* conditional expression system.

3. Method

- amplify with stop codon full length SmPDEs in subcloning vector pRPa,i a tetracycline-inducible to integrate into a specific rRNA spacer region of T. brucei strain 2T1, Knocking out both alleles of TbPDEB1/B2 then makes the cell dependent on the tet-induced expression of the Schistosoma gene .
- express the catalytic domain of SmPDEs 4A, 7var and 11 in the complementation strain of T. brucei, through domain swap with TbrPDEB2 as the control in the pRPa,i-6myc plasmid.



PCR analysis of the SmPDEs(SmPDE7var- SmPDE1-SmPDE8 and SmPDE9C) complementation in *T. b. brucei*.

1= 1 Kb lader
2=dKOSmPDE7var
3= dKOSmPDE1
4= dKOSmPDE8
5= dKOSmPDE9C
6= WT
A- is Blasticidin resistance cassette was present in all the strains
B- is purocycin resistance cassette was present in all the stranis
C- is TbrPDEB1 seems to be absent in all the SmPDEs expressing line after 2rouds of ko except WT .
D- is TbrPDEB2 seems to be absent in all the SmPDEs expressing line after 2rouds of ko except WT

4. Results

- Cloning, and functional complementation of S. mansoni PDEs (7var,1,8 and 9C) in T.brucei successful for heterologous expression of PDEs, created for this purpose by the deletion both alleles of the essential locus TbrPDEB1/B2 .
- The growth of the final transfection was dependent on tetracycline-induced expression of the Schistosoma PDE gene (functional complementation);
- The catalytic domain of SmPDEs 4A or SmPDE7var, successfully complemented the PDEB1/B2 null strain of T. brucei. SmPDE11 failed to complement the T. brucei cell line and was assessed in a Schizosaccharomyces pombe system. It was found to hydrolyse cGMP over cAMP.

5. Conclusion

- S. mansoni PDEs 1, 7var, 8 and 9C are functional cAMP phosphodiesterases and that SmPDE 11 is a cGMP phosphodiesterase. The resulting cell lines are now employed to screen several classes of PDE inhibitors to establish the pharmacological profile of each PDE separately, in a cellular system.

6. References

1. Munday JC *et al* . (2020). doi: PLoS Negl Trop Dis. 2020 14(7):e0008447.
2. Kim HS *et al* ,2013. doi: 10.1016/j.molbiopara.2013.08.001