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New diagnostic and therapeutic tools for tuberculosis using anti-ESAT-6/CFP-10 aptamers

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INTRODUCTION

Where infectious diseases are concerned, tuberculosis (TB) is one of the biggest killers despite the worldwide use of a live attenuated vaccine and several antibiotics. An estimated eight million new cases are reported annually with two million deaths. The latter are compounded by the emergence of drug resistance TB and coinfections with HIV. In 2004, it was estimated that more than 4% of the world's infected people living with active TB are in South Africa. During this period, South Africa accounted for about 2% of the world's new cases; and approximately 3% of the total number of people who died of TB were in South Africa.

Current diagnostics and therapy have major drawbacks particularly in high prevalence, developing countries. We are currently exploring new approaches to diagnose and mitigate the virulence of TB by using aptamers. Aptamers are nucleic acid ligands selected in vitro to bind specifically to target molecules[1]. Early Secreted Antigen Target (ESAT)-6 and Culture Filtrate Protein (CFP)-10 are secreted by *M. tuberculosis* and form a tight 1:1 heterodimer. They are encoded by the region of difference (RD) 1, which is absent in the live attenuated TB vaccine Bacille de Calmette et Guérin (BCG). Moreover, mutations of the corresponding genes in *M. tuberculosis* result in diminished virulence. On the other hand, both proteins are potent activators of the host cell-mediated immunity[2].

OBJECTIVES

1. Expression of His-tagged versions of ESAT-6 and CFP-10 in E. coli
2. Purification and validation of recombinant ESAT-6 and CFP-10
3. Formation and characterisation of the ESAT-6/CFP-10 heterodimer in vitro
4. Isolation of RNA aptamers against ESAT-6, CFP-10 and the heterodimer

EXPERIMENTAL METHOD

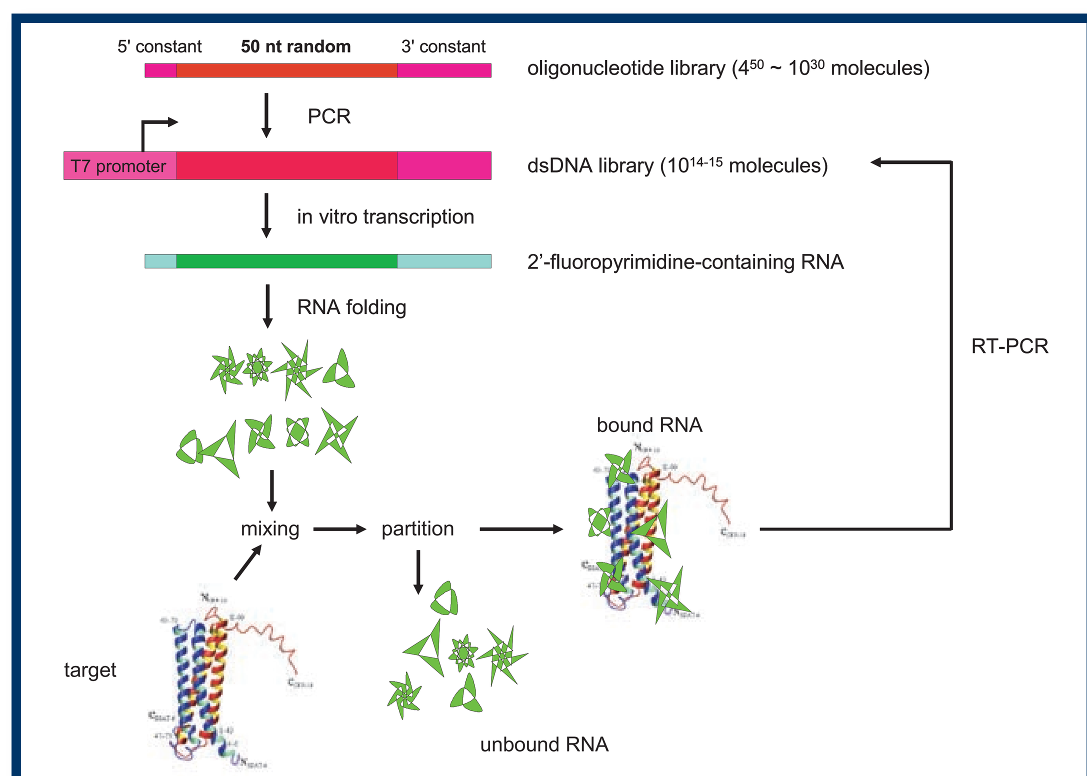


Figure 1. Isolation of 2'-fluoropyrimidine-containing RNA aptamers against ESAT-6, CFP-10 and the heterodimer by Systematic Evolution of Ligands by Exponential Enrichment (SELEX)3.

RESULTS

Expression and purification of CFP-10 and ESAT-6

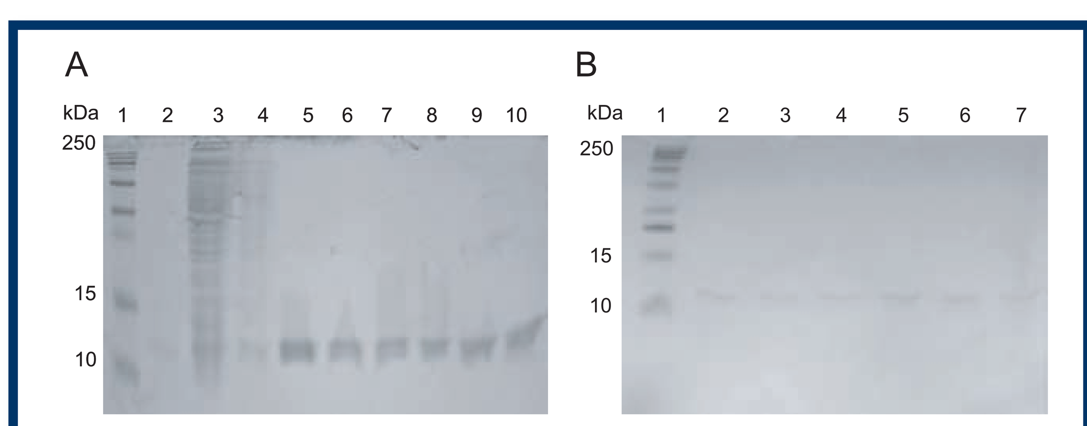
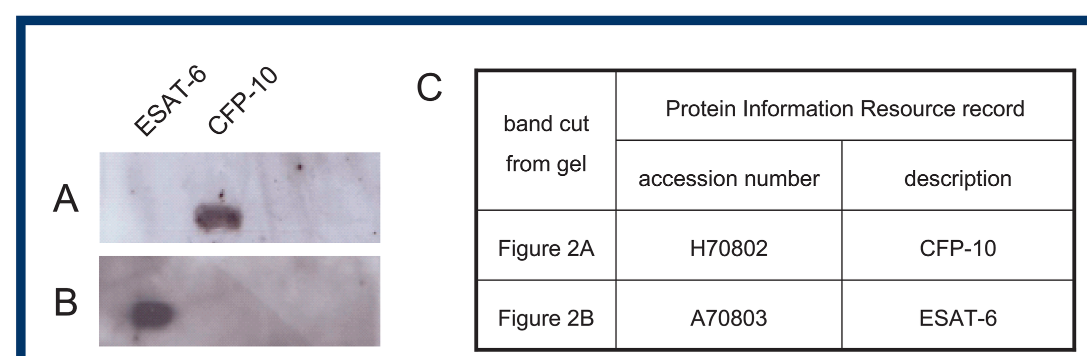


Figure 2. A. Purification and refolding of CFP-10 using immobilised metal affinity chromatography. CFP-10 was purified under denaturing conditions (6 M urea) and refolded while bound to the resin. SDS-PAGE (poly acrylimade gel electrophoresis) lane 1: Molecular weight marker (Precision Plus Protein™ Standards kaleidoscope, Biorad); lane 2: CFP-10 remained bound to the resin after refolding; lane 3: flow through; lane 4: 6 M urea wash; lane 5-10: purified CFP-10 fractions. B. Purification and refolding of ESAT-6. ESAT-6 was purified under the same conditions with two additional imidazole washes. Lane 1: Molecular weight marker; lane 2-7: purified ESAT-6 fractions.

Validation of the recombinant proteins



band cut from gel	Protein Information Resource record	
	accession number	description
Figure 2A	H70802	CFP-10
Figure 2B	A70803	ESAT-6

Figure 3. Western blot analysis of the purified recombinant proteins. A. anti-CFP-10 antibody. B. anti-ESAT-6 antibody. C. MALDI-MS (Matrix-assisted laser desorption/ionisation-mass spectrometry) analysis of the purified recombinant proteins.

Dimerisation of ESAT-6 and CFP-10

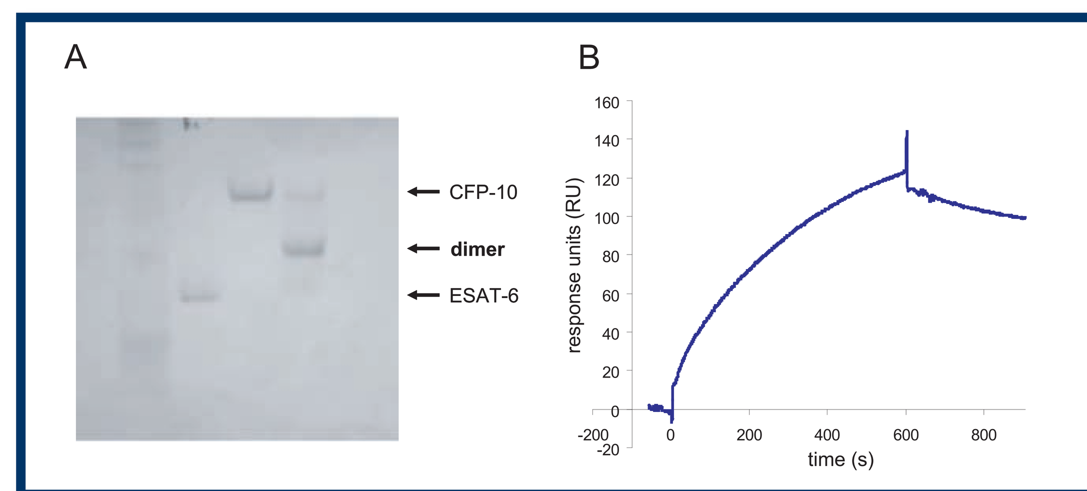


Figure 4. A. Native PAGE of the heterodimer. CFP-10 and ESAT-6 were dimerised using a previously described protocol[4].
B. Real-time binding of ESAT-6 to immobilised CFP-10 using surface plasmon resonance (SPR). Data was corrected by subtracting non-specific binding using a reference (empty) flow cell.

Isolation of RNA aptamers

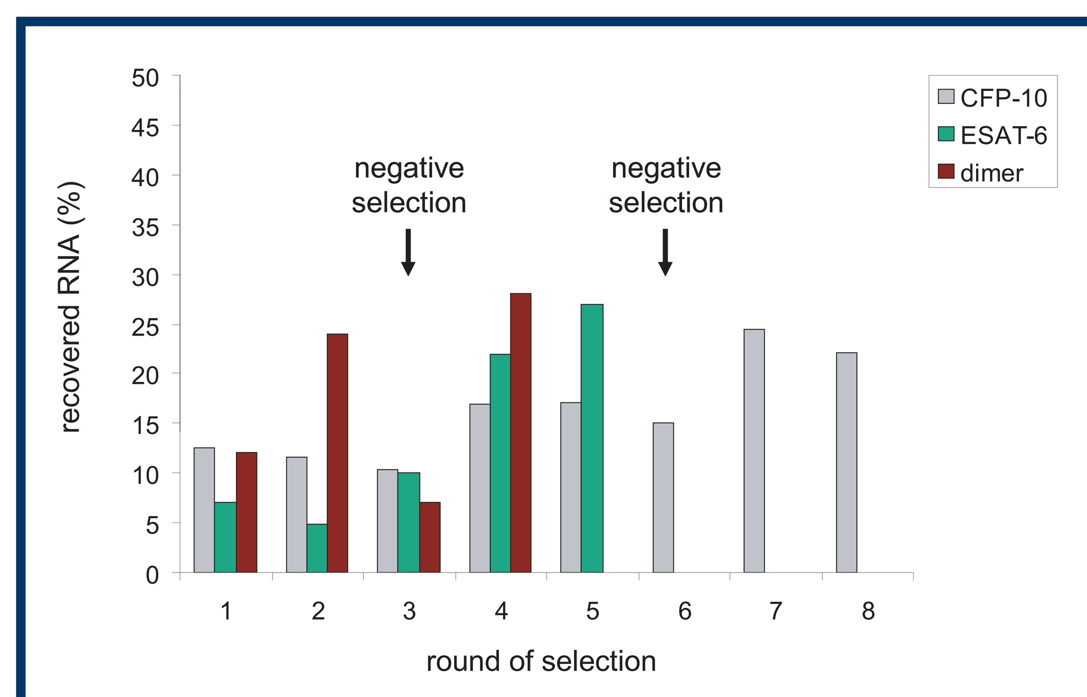


Figure 5. Ongoing selection of aptamers against CFP-10, ESAT-6 and the heterodimer. RNA-protein complexes were captured on a resin by immobilized metal affinity chromatography taking advantage of the protein His-tag. Negative selection against the resin was performed every third round.

DISCUSSION

We expressed and purified His-tagged versions of TB secreted proteins ESAT-6 and CFP-10. These monomers were used to form the heterodimer *in vitro*. We are currently selecting 2'-fluoropyrimidine-containing RNA aptamers against ESAT-6, CFP-10 and the heterodimer by systematic evolution of ligands by exponential enrichment (SELEX). The affinity of the generated aptamers to their target proteins will be assessed using the surface plasmon resonance-based Biacore technology. We will then test the ability of the aptamers to: 1) be used as TB diagnostic tools by detecting the presence of ESAT-6 and/or CFP-10 in blood or sputum samples; 2) inhibit the virulence of *M. tuberculosis in vitro* and in TB mouse models.

CSIR bioscientists are using aptamers to explore new strategies for diagnosis and mitigation of the virulence of TB. Current diagnostics and therapy interventions are unpredictable.

REFERENCES

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2. Brodin, P., Rosenkrands, I., Andersen, P., Cole, S. T. & Brosch, R., 2004. ESAT-6 proteins: protective antigens and virulence factors? Trends Microbiol 12, 500-8.
3. Tuerk, C. & Gold, L., 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249, 505-10.
4. Renshaw, P. S. et al., 2002. Conclusive evidence that the major T-cell antigens of the Mycobacterium tuberculosis complex ESAT-6 and CFP-10 form a tight, 1:1 complex and characterization of the structural properties of ESAT-6, CFP-10, and the ESAT-6*CFP-10 complex. Implications for pathogenesis and virulence. J Biol Chem 277, 21598-603.

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