Deleting the *nat* gene and inhibiting the NAT enzyme prevents survival of the mycobacteria in macrophages and induces cell wall alterations, rendering the mycobacterium sensitive to antibiotics to which it is normally resistant. However, TBNAT has been very difficult to generate as a soluble protein. To date, NAT from *M. marinum* (MMNAT) is considered the best available model for the TBNAT enzyme. Inhibitors have been developed by high throughput screening using homologous NAT enzymes and shown to be active against TBNAT [1]. In this work we present the first structural study on the MMNAT-inhibitor complexes. Understanding the ligand protein interaction will give better insight on the structure activity relationship of the identified inhibitors and will help to design potent inhibitors.

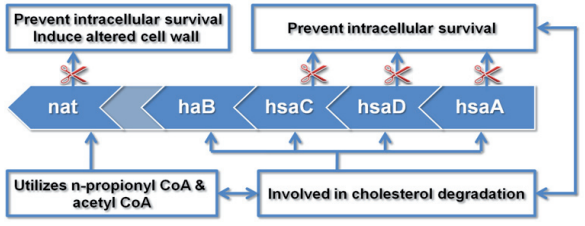

Figure 1: The nat operon in *M. tuberculosis*.

[1] I. Westwood, S. Bhakat, A. Russell, E. Fullam, M. Anderton, A. Kawamura, A. Mulvaney, R. Vickers, V. Bhowruth, G. Besra, A. Lalvani, S. Davies, E. Sim, *Protein Cell* **2010**, *1*, 82-95.

Keywords: latent TB, arylamine N-acetyltransferase, enzyme inhibition

MS16.P11

Acta Cryst. (2011) A**67,** C290

ADP-Mg^{2+} bound to the ATP-grasp domain of Human ATP-citrate lyase

Marie E. Fraser, Tianjun Sun, Koto Hayakawa *Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4 Canada*. E-mail: frasm@ucalgary.ca

ATP-citrate lyase is the cytoplasmic enzyme in humans linking energy metabolism from carbohydrates to the production of fatty acids. Acetyl-CoA, produced in mitochondria from the breakdown of carbohydrates, cannot be exported to the cytoplasm to serve as the precursor for fatty acids. Instead, citrate synthase transforms acetyl-CoA to citrate in the mitochondria, and citrate is exported to the cytoplasm where ATP-citrate lyase regenerates acetyl-CoA. Since fatty acids are required for the synthesis of both triacylglycerol and membrane lipids, ATP-citrate lyase has been considered as a possible drug target for the treatment of obesity and cancer.

Previously, a truncated form of the full-length human ATP-citrate lyase was crystallized using *in situ* proteolysis, and the structure was solved using X-ray crystallography [1]. Based on the structure, the plasmid was modified to include only the sequence encoding the 817 amino-terminal residues of the enzyme linked to a carboxy-terminal His_8-tag.

The reaction catalyzed by ATP-citrate lyase in the presence of magnesium ions is

citrate + CoA + ATP → acetyl-CoA + oxaloacetate + ADP + P_i. The amino-terminal portion of the enzyme had been shown to bind citrate or its competitive inhibitor, tartrate, and was predicted to bind ATP [1]. To investigate the nucleotide-binding site, the protein was crystallized in the presence of tartrate, ATP and magnesium ions. The crystals diffracted to 2.3-Å resolution at the macromolecular crystallography beamline of the Canadian Light Source. They belonged to space group C2 with unit cell dimensions a = 167.5 Å, b = 61.7 Å, c = 107.9 Å, α = 90°, β = 125.5°, γ = 90°. The structure shows ADP-Mg^{2+} bound to the domain that possesses the ATP-grasp fold and demonstrates that this crystal form could be used to investigate the structures of the complexes with inhibitors of ATP-citrate lyase that bind at either the citrate- or ATP-binding site.

[1] T. Sun, K. Hayakawa, K.S. Bateman, M.E. Fraser, *Journal of Biological Chemistry* **2010,** *285*, 27418-28.

Keywords: enzyme, complex, nucleotide

MS16.P12

Acta Cryst. (2011) A**67,** C290

The structure of SFPQ/PSF, a multifunctional nuclear protein

Mihwa Lee,[a] Daniel M. Passon,[a] Archa H. Fox,[b] and Charles S. Bond,[a] [a]*School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, WA, 6009, Australia.* [b]*Western Australian Institute for Medical Research, Perth, WA, 6000 (Australia).* E-mail: mihwa.lee@uwa.edu.au

SFPQ/PSF (Polypyrimidine tract-binding protein-associated splicing factor) is a multifunctional nuclear protein that has been implicated in a variety of nuclear processes including RNA biogenesis as well as DNA repair [1]. Importantly, SFPQ is a known tumour suppressor, binding to promoters of oncogenes and repressing transcription. A recent report of interactions of SFPQ with non-coding RNAs shows that these interactions release SFPQ from a proto-oncogene and activate transcription, thus, driving transformation and tumorigenesis [2]. A tight correlation to expression level of SFPQ with the progression of prostate cancer has also been reported [3].

In addition to the functions described above, SFPQ also plays a key role in the structural integrity and functions of paraspeckles, subnuclear bodies implicated in regulation of gene expressions by nuclear retention of hyperedited mRNAs. SFPQ along with two other paraspeckle proteins, NONO/p54nrb and paraspeckle protein component 1 (PSPC1) make up the mammalian DBHS (Drosophila Behaviour/Human Splicing) family. The conserved DBHS domains of these proteins, comprised of tendom RNA recognition motifs (RRMs) and a C-terminal coiled-coil domain, share more than 50% sequence identity. Reported interactions between DBHS proteins suggest that they exist as either homo-or heterodimers *in vivo*.

We have determined the first structure of human SFPQ homodimer and refined to 2.05 Å resolution. The structure shows the consistent arrangement of the DBHS domain with our previous structure of PSPC1/NONO heterodimer. Interestingly, the N-terminus of SFPQ reveals an additional secondary structure that was absent in the structure of PSPC1/NONO heterodimer and may be related to DNA binding activity of SFPQ. The structure of human SFPQ in comparison with PSPC1/NONO heterodimer will be discussed. In addition, DNA binding activity of SFPQ as well as possible RNA binding modes will be described in relation to the functions of SFPQ in tumorigenesis.

[1] Y. Shav-Tal, D. Zipori, *FEBS Lett* **2002**, *531*, 109-114 [2] L. Li, T. Feng, Y. Lian, G. Zhang, A. Garen, X. Song, *Proc Natl Acad Sci U S A* **2009**, *106*, 12956-12961 [3] R. Berretta, P. Moscato, *PLoS One* **2010**, *5*, e12262.

Keywords: SFPQ/PSF, DBHS domain, paraspeckle