The structural and functional characterisation of the FOXO1 Forkhead domain - FOXP3 Promoter DNA complex.

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Introduction: The human body is protected by its immune system which responds to invading pathogens[1]. However, this response is sometimes excessive, leading to the body attacking self- antigens. These excessive immune responses cause autoimmune diseases. Regulatory T cells (Tregs) play an indispensable role in preventing autoimmune diseases, such as Multiple Sclerosis, Rheumatoid Arthritis, and Inflammatory Bowel disease. The most important transcription factor (TF) in Treg identity and function is the Forkhead Box P3 (FOXP3)[2]. The FOXP3 TF is in turn regulated by several other transcription factors - including FOXO1 - that bind to the promoter and enhancer elements within and up-stream of the *FOXP3* locus[1]. The aim of our work was to characterise the structure and function of FOXO1 FHD - *FOXP3* promoter DNA binding.

Methodology: The FOXO1 FHD (Forkhead domain) was cloned and expressed in *E. coli* T7 cells, followed by affinity purification and size exclusion chromatography. Thereafter, the following FOXO1 FHD structural and functional studies were done with *FOXP3* promoter DNA: Electrophoretic mobility shift assays (EMSAs), Circular Dichroism, Intrinsic Tryptophan Fluorescence, andFluorescence Anisotropy.

Results: Our data shows that: FOXO1 FHD exists as a monomer of 13.1 kilodaltons; FOXO1 is primarily alpha-helical in secondary structure; FOXO1 FHD has a stable tertiary structure; and FOXO1 FHD binds with strong affinity to a 20 base pair sequence from the promoter of the *FOXP3* gene. The dissociation constant (K_D) of the FOXO1 FHD and FOXP3 promoter DNA complex is 464 nanomolar.

Discussion and conclusion: We conclude that FOXO1 FHD binds specifically and strongly to *FOXP3* promoter DNA. It is likely that the spatial and temporal expression of FOXO1 and FOXP3 is essential in the regulation of Treg cells, and by extension, the prevention of autoimmunity. Ongoing Isothermal Titration Calorimetry (ITC) studies will determine the exact affinity constant, stoichiometry, and mechanism of binding.

References:
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