Structural Study of N-terminal domain of cardiac transcriptional factor Nkx2-5

Martinez CG, Kurtenbach E and Costa MW
IBCCF, UFRJ, Rio de Janeiro, Brazil.

Gene regulation occurs via activation or repression of specific genes mediated by interactions between the basal transcription machinery and specific transcriptional factors. Nkx2-5 is a transcriptional factor that belongs to the family of homeoproteins, possessing a highly conserved DNA binding region, and regulates important cardiac promoters, allowing the maintenance of cardiac homeostasis and development. It is expressed initially at heart precursor cells and persists until adulthood. We recently demonstrated that post-transcriptional modifications of Nkx2-5 in HEK293T cells mediated by SUMOylation leads to synergistic activation of cardiac regulatory genes. Bioinformatic and biochemical assays showed that Nkx2-5 is SUMOylated in at least two lysine residues at the N-terminal region and that this region is essential for the modulation of transcriptional activity mediated by Nkx2-5. SUMOylation usually changes protein-protein interactions leading to the recruitment of accessory proteins. In order to identify the structural changes promoted by the SUMOylation of Nkx2-5 we decided to resolve the 3D structure of the N-terminal domain of murine Nkx2-5 by solution NMR. For this purpose, the region of the N-terminal domain containing amino acids 1-140 from the mouse cDNA was amplified by PCR and digested with EcoRI and HindIII restriction enzymes. Subsequently, this fragment was subcloned into the expression vector pET28b. Clones containing the cDNA of interest were confirmed by PCR and DNA sequencing. Currently we are testing the ideal conditions for the protein expression, which was already set using the expression vector pThioHis. After purification using Ni-NTAagarose the identity of N-termNkx2-5 will be confirmed by mass spectrometry analysis. The structural comparison of Nkx2-5 and Nkx2-5-SUMOylated will reveal important protein interaction regions that further will be explored by dynamics NMR solution experiments. Supported by FAPERJ.