Cytochrome c and its Interaction with the Histone Chaperone ANP32B: Tackling the Chaperone Disordered Regions

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Extra-mitochondrial cytochrome *c* (C*c*) has recently emerged as an inhibitor of histone chaperones, namely SET/TAF-1β in mammals and NRP1 in plants, upon DNA damage. Likewise, C*c* can target other histone chaperones, such as mammalian Acidic leucine-rich Nuclear Phosphoprotein 32 family member B (ANP32B). ANP32B modulates mRNA nucleocytoplasmic trafficking upon Thr244 phosphorylation, ANP32B comprises four Leucine-Rich Repeats (LRR) at its N-terminal end and an unstructured Low-Complexity Acidic Region (LCAR) at the C-terminal end.

Here, we used co-immunoprecipitation assays to confirm the cytoplasmic interaction between ANP32B and C*c* upon DNA damage. Then, we analyzed such interaction by Isothermal Titration Calorimetry (ITC) and Nuclear Magnetic Resonance (NMR) spectroscopy. Notably, wild-type (WT) full-length ANP32B and its phosphomimetic T244E mutant interacted with C*c*, whereas a deletion mutant lacking the LCAR region failed to bind the hemeprotein, confirming that the LCAR region is responsible for the ANP32B-C*c* interaction.

The LCAR contains the topogenic KRKR motif near T244. Hence, we tested two synthetic peptides containing LCAR residues from 231 to 251. T244 was replaced by glutamate in the second one. Both peptides were able to bind C*c*; albeit the T244E mutant exhibited a higher affinity.

Simulations by Replica Exchange Molecular Dynamics (REMD) using distance restraints derived from 2D ^{1}H-^{1}H TOCSY, NOESY and ROESY NMR spectra showed that both peptides adopted a compact structure, despite secondary structure predictions suggesting that the LCAR is fully unstructured. Brownian Dynamics calculations using C*c* and the peptide structures derived from REMD showed that both peptides explored the heme-surrounding cleft and part of the opposite-side of C*c,* in agreement with the chemical-shift perturbations maps inferred from NMR titration assays.

Further functional assays will be performed to shed light onto the functional relevance of the ANP32B-C*c* interaction.