HuR Revealed as a Novel Target for Cytochrome c under DNA Damage

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Human antigen R (HuR) is a ubiquitously expressed RNA Binding Protein (RBP), whose coding region comprises three independent RNA Recognition Motifs (RRMs) and a hinge region between RRM 2 and 3; where the nucleo-cytoplasmic shuttling sequence is located. This RBP regulates the splicing, stability and translation of a diverse range of mRNAs, including that of Cytochrome *c* (C*c*). C*c*, in turn, is a multi-functional heme protein which plays a moonlighting role in cells, participating in their energetics, DNA damage response and apoptosis. We recently detected the interaction between HuR and C*c* in cells submitted to DNA damage by co-immunoprecipitation assays. Moreover, the signaling networks of HuR and C*c* converge by targeting ANP32B and SET/TAF-1β proteins which act as PP2A inhibitors. These findings suggest a novel cell death pathway regulated by the C*c*-HuR axis.

To explore the structural features of the C*c*-HuR complex, we performed Nuclear Magnetic Resonance (NMR) titrations using the following HuR constructs: RRM12, RRM23 and RRM3. We first tried HuR RRM12, but did not observe any interaction with C*c*. Next, we examined HuR RRM23 and RRM3 constructs, which were purified using the anionic detergent N-Laurylsarcosine (sarkosyl) in order to overcome the poor solubility of the third domain of HuR. NMR titrations using ^{15}N-labeled C*c* show no chemical-shift perturbations upon adding either ^{14}N-HuR RRM23 or ^{14}N-HuR RRM3. However, the 1D-NMR Met80-εCH_3 resonance of reduced C*c* broadens upon addition of the ^{14}N-HuR RRM23 construct. Thus, we speculate that sarkosyl encapsulates HuR inside detergent micelles.

Accordingly, we dispensed with sarkosyl and fused HuR RRM23 and RRM3 constructs with a GST tag to enhance HuR solubility. Indeed, HuR RRM3 has successfully been purified, allowing us to perform further structural analyses.