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Does The Hepn Domain of Sacsin Interact with Rna? The Answer Is Relevant for the Etiology of the Rare Disease Arsacs

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a childhood-onset neurodegenerative disorder caused by point mutations in the SACS gene 13q11, encoding the Sacsin protein. Sacsin contains a HEPN domain, structurally similar to the HEPN domain of other known RNA-binding proteins. Interestingly, patients carrying a single missense mutation in the HEPN domain show symptoms similar to other ARSACS patients, suggesting that impaired RNA binding may contribute to ARSACS etiology. Here, we investigated the RNA-binding properties of the HEPN domain. RNAA and RNAB, were selected based on their predicted higher (A) and lower (B) affinity for the HEPN domain, respectively. Their interaction with HEPN was analyzed via intrinsic fluorescence, circular dichroism (CD), size-exclusion chromatography (SEC), and molecular modelling. Minimal protein secondary structure changes and altered fluorescence quantum yield were observed in the presence of RNA_A/B. The HEPN domain showed reduced stability in the presence of RNA_A while RNA_B had no effect, as indicated by the CD thermal unfolding curves. SEC and GST-pulldown assays supported HEPN-RNA_A interaction. The use of Alphafold3 and MD simulations suggested the presence of a RNA-binding site at the monomer-monomer interface. These findings suggest that the RNA-binding properties of HEPN may play a role in ARSACS pathogenesis.