Identification of New RNA Targets for KSRP by the Scaffold Independent Analysis of the Specificities of its KH Domains

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K-Homology Splicing Regulator Protein (KSRP) is a multi-functional RNA binding protein involved in different post-transcriptional regulatory processes. Originally identified as an important factor in the alternative splicing of N1 neuronal RNA[1], it has also been shown to play a key role in mRNA localisation[2], and the in-depth studied degradation of a subset of labile mRNAs containing adenosine-uridine-rich elements (AREs) at their 3' untranslated regions (UTRs) via the recruitment of the exosome and other de-adenylation factors[3]. More recently, it has also been demonstrated the regulatory role of KSRP in promoting the maturation of specific miRNAs related to several forms of cancer[4]. RNA binding is achieved by the four KH domains in the midle region of the protein.

The wide range of molecular mechanisms KSRP is involved in reflects its capability to recognise a broad range of sequences within its RNA targets. Although KSRP has been shown to directly interact with the AREs[5], there is little information on the binding specificity of the isolated KH domains of the protein to non-AU-rich sequences.

We have used the Scaffold-Independent Analysis (SIA) approach[6] to dissect the RNA binding potential of the four KH domains of KSRP. Our experiments reveal that the four domains have different sequence specificities and binding affinities[7]. The method identifies the highest affinity binding domain, KH3, as the most specific for RNA sequences containing G-rich stretches. Interestingly, all four KSRP KH domains share a negative selectivity for multiple adjacent cytosines, limiting the target choice and contributing to the specificity of target recognition. The method has been proved successful for the identification of new KSRP targets different from the AREs, and has unvealed a leading role for KH3 in the molecular recognition of the terminal loop of a subset of miRNA precursors (pre-miRNAs) important for correct embryonic development, linking KSRP to miRNA biogenesis[4]. This study provides a better understanding of the role of low sequence specificity domains in RNA recognition by multi-domain RNA binding proteins.

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