Protein recognition of RNA by sequence and shape

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RNA binding proteins employ a variety of recognition strategies in order to interact with a specific set of targets. We have used complementary in vitro and in vivo tools to explore these interactions, and we also create mutants to strategically perturb the binding in order to gain insight into the biological role of the protein-RNA complexes, or to mimic disease. One goal in the group is to understand how development in multicellular organisms relies on the regulation of splicing factor expression in order to create protein isoforms unique to a given tissue and at a specific time in development. In this context, we are currently investigating the atomic details of RNA recognition by the human splicing factor RBM20 essential for heart development, and the splicing factor MEC-8 required for neural function in C. elegans. For RBM20, we have used NMR spectroscopy and isothermal titration calorimetry to show that RNA recognition involves a coupled folding/binding mechanism with a C-terminal helix required for full affinity interaction with a UCUU motif. For MEC-8, we have discovered that despite low similarity in protein sequence, both the N-terminal and C-terminal RRM domains interact with the same GCAC motif sequence. The additional dimerization of the N-terminal domain [1] imparts further complexity to the interaction of MEC-8 with target RNA. Finally, we have also found and characterized a new double-stranded RNA (dsRNA) binding domain present on the human prolyl isomerase FKBP25 [2]. In this case, the conformation of the RNA rather than sequence features appear to govern RNA recognition. Similar to our other studies, characterization of the interaction between FKBP25 and dsRNA uses a combination of NMR spectroscopy, isothermal titration calorimetry, X-ray crystallography, and cellular assays.

[1] H. Soufari, C.D. Mackereth. (2017). Conserved binding of GCAC motifs by MEC-8, couch potato and the RBPMS protein family. **RNA** 23:308-316
[2] D. Dilworth, S.K. Upadhyay, P. Bonnafous, A.B. Edoo, S. Bourbigot, F. Pesek-Jardim, G. Gudavicius, J. Serpa, E. Petrotchenko, C. Borchers, C.J. Nelson, C.D. Mackereth. (2017). The basic tilted helix bundle domain of the prolyl isomerase FKBP25 is a novel double-stranded RNA binding module. **Nucleic Acids Res.** 45:11989-12004.