Institut Européen de Chimie et Biologie

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Soutenance de thèse

Etude Structurale et fonctionnelle du facteur d'épissage MEC-8.

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In multicellular organisms, proteomic diversity in each cell and tissue is provided initially by selective expression of gene subsets from the total genome, which are further subjected to alternative splicing, such that a different pattern of exons can be retained or excluded in the final protein-coding mRNA. We are investigating the molecular details of the tissue-specific splicing factor protein MEC-8 from the worm Caenorhabditis *elegans*. The MEC-8 mutant protein is responsible for a touch insensitive phenotype in Caenorhabditis *elegans*, relating to its role as an alternative splicing factor. More precisely, MEC-8 can bind to the mec-2 pre-mRNA, a component of mechanosensory receptor, to regulate the production of a certain isoform required for transducing the touch signal. Previous studies of the conserved RNA Recognition Motif (RRM) domain in orthologues from vertebrate (RBPMS) and insect (couch potato; CPO) have demonstrated a homodimerization motif in MEC-8 RRM1. However, MEC-8 also contains a second RRM domain in the C-terminus that is not found in the characterized RBPMS and CPO proteins. We have therefore expressed the independent RNA-binding domains of MEC-8 as well as the full-length protein and have used these constructs first in a variety of biophysical assays. We identified the optimal RNA binding sequence for both the RRM1 and RRM2, and quantified the penalty of sequence variations. The investigation has also been extended to the homologous domains from human RBPMS and Drosophila CPO, which show a high affinity to the same RNA sequence. We therefore find that despite differences in function and localization, the members of the RBPMS protein family all bind to the same RNA motif. Atomic details of binding have also been obtained by using a combination of NMR spectroscopy and X-ray crystallography. The ligand-bound complexes reveal a surprising similarity in the architecture of the bound ligand for the first and second RRM domains from MEC-8.

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