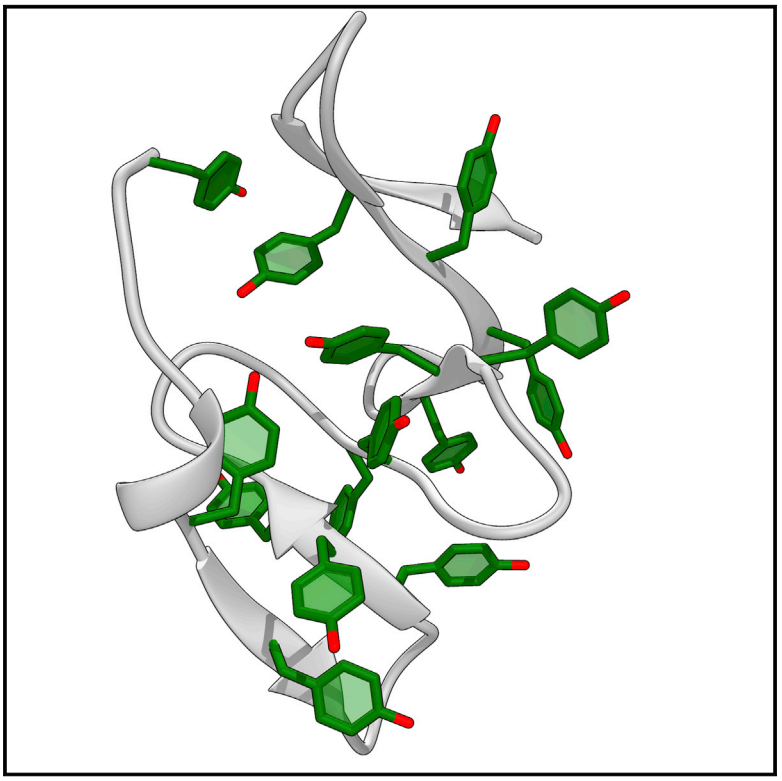
Short Article

Structure

Nuclear Magnetic Resonance Structure of a Novel Globular Domain in RBM10 Containing OCRE, the Octamer Repeat Sequence Motif

Graphical Abstract



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In Brief

The octamer repeat (OCRE) sequence in RMB10 regulates alternative splicing of *Fas* and other apoptosis-related proteins. Martin et al. show that OCRE is part of a globular domain with a tyrosine-rich, anti-parallel β sheet fold. This novel domain is highly conserved across the animal kingdom, indicating an important physiological role.

Highlights

- The RBM10 octamer repeat (OCRE) sequence motif is part of a stable globular domain
- 16 aromatic residues result in a unique architecture and surface-exposed tyrosines
- The OCRE globular domain is conserved across the animal kingdom in RBM10 and RBM5

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Nuclear Magnetic Resonance Structure of a Novel Globular Domain in RBM10 Containing OCRE, the Octamer Repeat Sequence Motif

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SUMMARY

The OCtamer REpeat (OCRE) has been annotated as a 42-residue sequence motif with 12 tyrosine residues in the spliceosome *trans*-regulatory elements RBM5 and RBM10 (RBM [RNA-binding motif]), which are known to regulate alternative splicing of *Fas* and *Bcl-x* pre-mRNA transcripts. Nuclear magnetic resonance structure determination showed that the RBM10 OCRE sequence motif is part of a 55-residue globular domain containing 16 aromatic amino acids, which consists of an anti-parallel arrangement of six β strands, with the first five strands containing complete or incomplete Tyr triplets. This OCRE globular domain is a distinctive component of RBM10 and is more widely conserved in RBM10s across the animal kingdom than the ubiquitous RNA recognition components. It is also found in the functionally related RBM5. Thus, it appears that the three-dimensional structure of the globular OCRE domain, rather than the 42-residue OCRE sequence motif alone, confers specificity on RBM10 intermolecular interactions in the spliceosome.

INTRODUCTION

The family of RNA-binding motifs (RBMs), which includes RBM5 and RBM10, is a ubiquitous group of splicing factors present throughout the spliceosome (Callebaut and Mornon, 2005; Sutherland et al., 2005). RBM5- and RBM10-mediated effects in regulating mature RNA isoforms (Matera and Wang, 2014; Wahl et al., 2009) have been documented in lung adenocarcinoma (David and Manley, 2010; Imielinski et al., 2012; Sutherland et al., 2010) and in diverse developmental processes (Johnston et al., 2010; Loiselle and Sutherland, 2014). Their functions in the early spliceosome (Behzadnia et al., 2007) are to regulate pre-mRNA splicing by promoting exon skipping and alternate 5′-splice site selection in *Fas* and *Bcl-x* pre-mRNA (Bonnal et al., 2008; Inoue et al., 2014; Wang et al., 2013). The functions of Fas and Bcl-x in regulating apoptosis can switch between pro- or anti-apoptotic behavior, either through alternative splicing of a transmembrane domain in *Fas* exon 6 (Cheng et al., 1994) or by selection of an alternate 5′-splice site in exon 2 of *Bcl-x* (Boise et al., 1993). Overexpression of RBM5 or RBM10 promotes exon 6 skipping in *Fas* or elongation of exon 2 in *Bcl-x* pre-mRNA, and both events yield anti-apoptotic isoforms of both proteins (Bonnal et al., 2008; Inoue et al., 2014). Physiological functions of RBM5 and RBM10 have been shown to be related to a tyrosine-rich polypeptide segment, the OCtamer REpeat (OCRE), since deletion of this OCRE sequence motif disrupts RBM5 function by affecting the isoform bias in *Fas* pre-mRNA (Bonnal et al., 2008). Here, we investigated the structural properties of the OCRE sequence motif and its conservation in the animal kingdom.

The domain organization of RBM5 and RBM10 consists of a zinc finger flanked by two RNA recognition motifs (RRM) (Kielkopf et al., 2004; Maris et al., 2005; Nguyen et al., 2011; Ray et al., 2013; Song et al., 2012). A linker region of variable length connects these common RBM components to an OCRE sequence motif (Callebaut and Mornon, 2005), which is followed by another zinc finger and a G patch (Figure 1A). The sequence identity for corresponding annotated globular domains of RBM5 and RBM10 is above 60%, and within the RBM family the annotated OCRE sequence motif with 12 tyrosines within 42 residues is unique to RBM5 and RBM10. OCRE sequence motifs with five imperfect tyrosine repeats have been observed in only two other human proteins, RBM6 and AGGF1 (Callebaut and Mornon, 2005). The mechanism of action by which RBM5 and RBM10 regulate pre-mRNA processing is the subject of intensive studies, but is still largely unknown (Bechara et al., 2013; Inoue et al., 2014; Loiselle and Sutherland, 2014; Ray et al., 2013; Wang et al., 2013). At this early state of work on the mechanistic basis of RBM functionality, structure determination of RBM building blocks and their intermolecular interactions greatly contribute to the advancement of the field. Here, we report spectroscopic studies in solution of the RBM10 OCRE sequence motif, showing that it is part of a globular OCRE domain. We present the nuclear magnetic resonance (NMR) structure of the OCRE globular domain, provide insights into the structural role of the high tyrosine content, and investigate the conservation of the three-dimensional structure of the OCRE globular domain in the animal kingdom.

