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PROSPECTS

RNA Binding Motif (RBM) Proteins: A Novel Family of Apoptosis Modulators?

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Abstract RBM5 is a known modulator of apoptosis, an RNA binding protein, and a putative tumor suppressor. Originally identified as LUCA-15, and subsequently as H37, it was designated ''RBM'' (for RNA Binding Motif) due to the presence of two RRM (RNA Recognition Motif) domains within the protein coding sequence. Recently, a number of proteins have been attributed with this same RBM designation, based on the presence of one or more RRM consensus sequences. One such protein, RBM3, was also recently found to have apoptotic modulatory capabilities. The high sequence homology at the amino acid level between RBM5, RBM6, and particularly, RBM10 suggests that they, too, may play an important role in regulating apoptosis. It is the intent of this article to ammalgamate the data on the ten originally identified RBM proteins in order to question the existence of a novel family of RNA binding apoptosis regulators. J. Cell. Biochem. 94: 5–24, 2005. © 2004 Wiley-Liss, Inc.

Key words: RBM; apoptosis; LUCA-15; RBM5

Single-stranded RNA binding proteins are involved in every aspect of RNA metabolism, including splicing, transport, translation, and stability. The interaction of any particular RNA binding protein and its substrate is determined by specific sequences within the RNA binding protein, such as the RNA-recognition motif (RRM)/RNA-binding motif (RBM)/ribonucleoprotein (RNP) motif [Burd and Dreyfuss, 1994], the arginine-rich motif (ARM) [Burd and Dreyfuss, 1994], the cold shock domain (CSD) [Manival et al., 2001], the K homology (KH) domain [Gibson et al., 1993; Siomi et al., 1993; Adinolfi et al., 1999] and the arginine–glycine–glycine (RGG) box [Kiledjian and Dreyfuss, 1992]. Many such proteins have more than one RNA binding sequence, and this is often reflected in the ability of a single protein to bind more than one type of RNA molecule and have more than one RNA-associated function.

This review will focus on the "RBM" proteins, an apparent subgroup of the RRM/RBM/RNP containing proteins, as officially designated by the HUGO Gene Nomenclature Committee. The RBM designation has not been classified as an official "family" designation, but is understood to be an attempt to more accurately describe novel proteins having one or more RRM domains, but, generally, about which very little else is known. It should be noted here that there is a plethora of RRM-containing RNA binding proteins that are not designated RBM. In time the "RBM" designation may be removed, once more accurate descriptives are revealed about each protein.

Recently, the list of proteins with the "RBM" designation has almost doubled: because little more than a name has been assigned to the new RBM members, this review will maintain its focus on the initial ten. It is the objective of this review to examine these RBM "family" proteins from the viewpoint of RBM5, a known apoptosis regulator [Mourtada-Maarabouni and Williams, 2002b]. It is hoped that through studying these other, novel, RNA binding

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proteins some unifying characteristics might be revealed that would help to delineate a common function and provide insight into the molecular mechanisms by which RBM5 acts as both a modulator of apoptosis and an RNA binding protein.

A summary of the most salient points regarding the structure and function of each of the ten RBM proteins is followed by a section concerning the evolutionary aspects of the RBM "family," a section regarding the role of RNA binding proteins in apoptosis regulation, and, finally, a section involving a comparison of sequences as predictive of apoptotic regulatory ability.

STRUCTURE

Members of the RBM protein "family" contain the primary structural motif most commonly referred to as the RNA-recognition motif (RRM), but which is also referred to as an RNA-binding domain (RBD), a consensus sequence RNA-binding domain (CS-RBD), a ribonucleoprotein domain (RNP), and an RNP consensus sequence (RNP-CS). The RRM domain was first described in 1989 [Bandziulis et al., 1989; Query et al., 1989], and shown to bind RNA shortly thereafter [Keene and Query, 1991]. It spans 80–100 amino acids, and is fairly loosely conserved except for two sub-domains termed RNP-1 and RNP-2 (see Fig. 1). RNP-1 has the octapeptide consensus sequence (K/R)G(F/Y)(G/A)FVx(F/Y) [Birney et al., 1993; Dreyfuss et al., 1993], while RNP-2 has the less well conserved hexapeptide consensus sequence (L/I)(F/Y)(V/I)(G/K)(G/N)L [Burd and Dreyfuss, 1994; Sachetto-Martins et al., 2000] or IYIKGM, which is rich in aromatic and aliphatic amino acids [Bandziulis et al., 1989; Query et al., 1989; Dreyfuss et al., 1993]. RNP-2 is usually located 25–35 amino acids amino-terminal to RNP-1. The structure formed by the RRM contains four β-sheets and two α-helices [Nagai et al., 1990; Hoffman et al., 1991; Kenan et al., 1991].

FUNCTION

The importance of the biological function(s) of the RRM domain is without doubt since it is the most prevalent of the RNA binding motifs (RBM), is present in practically every organelle of the cell where RNA is found, and is conserved in animals, plants, yeast, viruses, and bacteria. The fact that each individual RRM structure appears to have unique binding characteristics suggests that RRM containing proteins have a multitude of functions. Indeed, RRM proteins bind to pre-messenger RNA and are members of the hnRNP particle [Dreyfuss et al., 1993; Krecic and Swanson, 1999], are involved in RNA splicing, and are part of the snRNP [Zieve and Sauterer, 1990], and are involved in RNA stability and translation [Keene and Query, 1991]. The majority of RRM proteins, however, appear to participate predominantly in pre-mRNA processing.

The sequences recognized by RRM domains vary widely [Kenan et al., 1991]. Because each individual RRM even in a single protein may have a unique binding specificity, an RRM protein may bind to more than one RNA molecule at the same time. For instance, hnRNP A1 protein can simultaneously bind two different RNA molecules: pre-mRNA through one of its RRMs and snRNA through the other [Lutz and Alwine, 1994]. Williams and colleagues, however, demonstrate that when equilibrium techniques are used in the analysis, the hnRNP A1 binds RNA non-discriminately [Abdul-Manan and Williams, 1996]. It has been suggested that outlying sequences determine the RRM binding

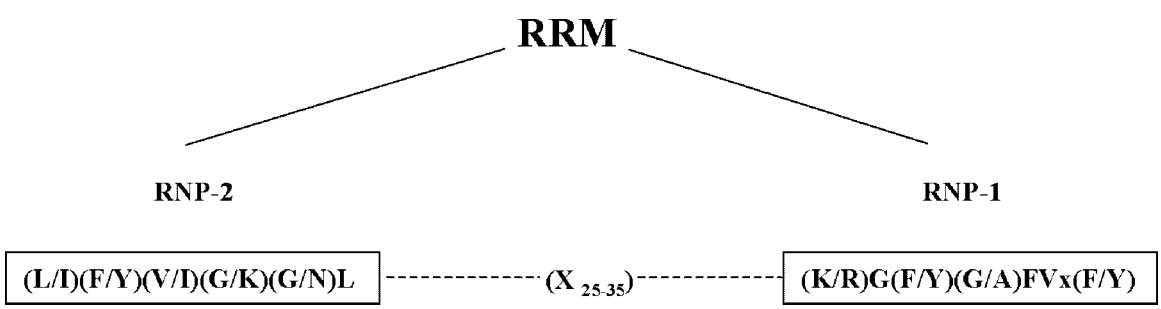

Fig. 1. RRM structure. The RNA recognition motif (RRM) further delineated to encompass the two subdomains RNP-2 and RNP-1. X represents any amino acid. For sources of sequence information, see text.

specificity, while the core consensus RRM sequences contribute to the binding energy [Query et al., 1989; Scherly et al., 1990; Bentley and Keene, 1991].

RBM FAMILY PROTEINS

Each of the ten proteins designated as RBM by the HUGO Gene Nomenclature Committee contains from 1–4 copies of the RRM consensus sequence (Fig. 2). As depicted in Figure 3, some of these proteins have additional modules that mediate either RNA or protein binding, such as a serine/arginine-rich region (RBM5, RBM8), an arginine/glycine-rich domain (RBM3, RBM8), a SPOC domain (RBM15), a D111/G-patch (RBM5, RBM6, RBM10), a proline-rich region (RBM12), an alanine-rich region (RBM4), and/or a zinc finger (RBM4, RBM5, RBM6, RBM10). Table I lists the RBM "family" proteins and highlights some of their key features. A more detailed description of each RBM protein follows.

RBMY

There are approximately 30 *RBMY* genes, including pseudogenes, all located on the Y chromosome [Prosser et al., 1996]. It is surmised that *RBMY* originated from the autosomal *hnRNP G* gene and underwent translocation and amplification to create the diverse and extensive cluster on the Y chromosome [Delbridge et al., 1999; Mazeyrat et al., 1999]. The gene designations have changed frequently and are confusing. Suffice it to say, the majority of genes within the *RBMY* clusters on both the short and long arms of the Y chromosome appear to be pseudogenes, while the *RBMY1* subfamily appears to be the only one expressing functional product [Prosser et al., 1996; Chai et al., 1997; Delbridge et al., 1997].

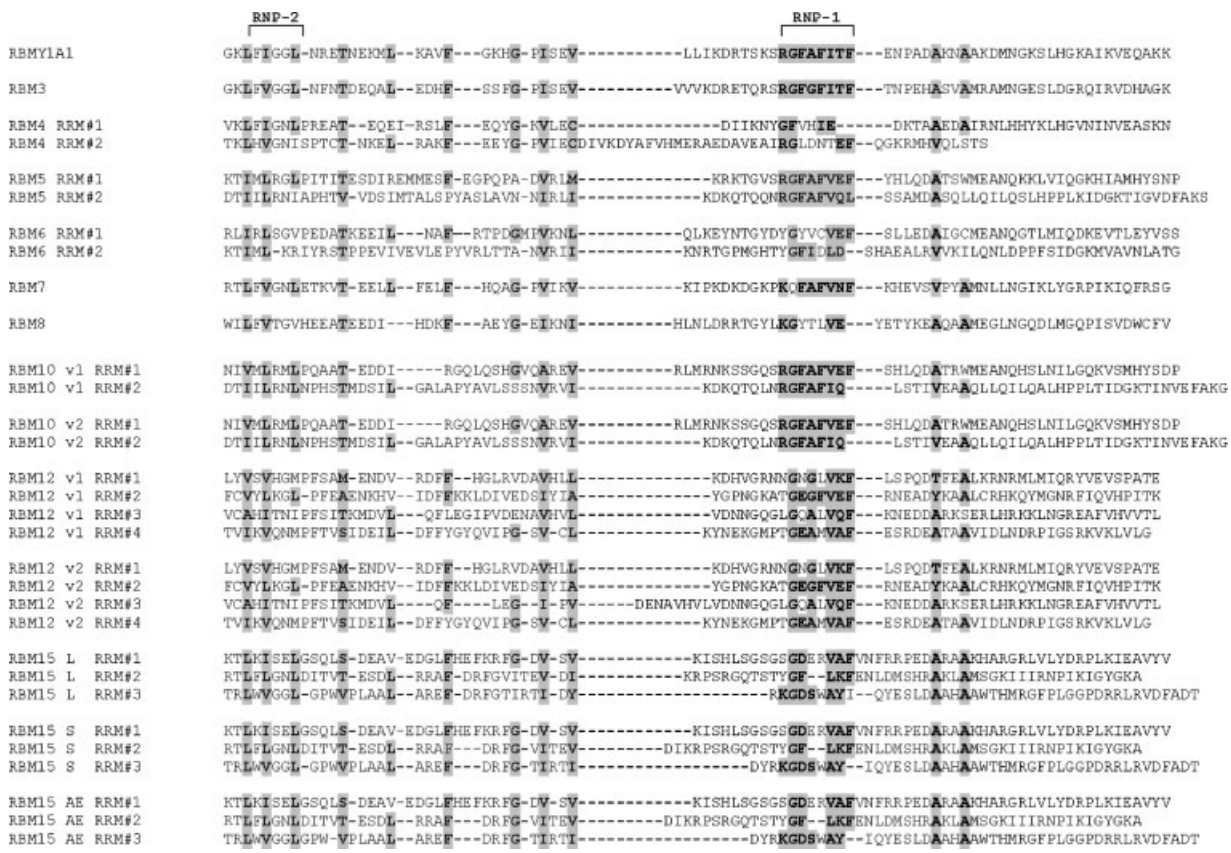

Fig. 2. Alignment of RBM "family" proteins. The accession numbers (www.ncbi.nlm.nih.gov) for the sequences are given in Table I. For proteins with more than one RRM, the RRM is indicated with a #, beginning from the amino terminal. The RRMs (delineated by PROSITE: http://kr.expasy.org/prosite/) were aligned manually to conform with known secondary structure requirements [Birney et al., 1993]. Alignment gaps are indicated by dashes. The positions of conserved amino acids are shaded. The core RNP-1 and RNP-2 motifs are delineated above the alignment.

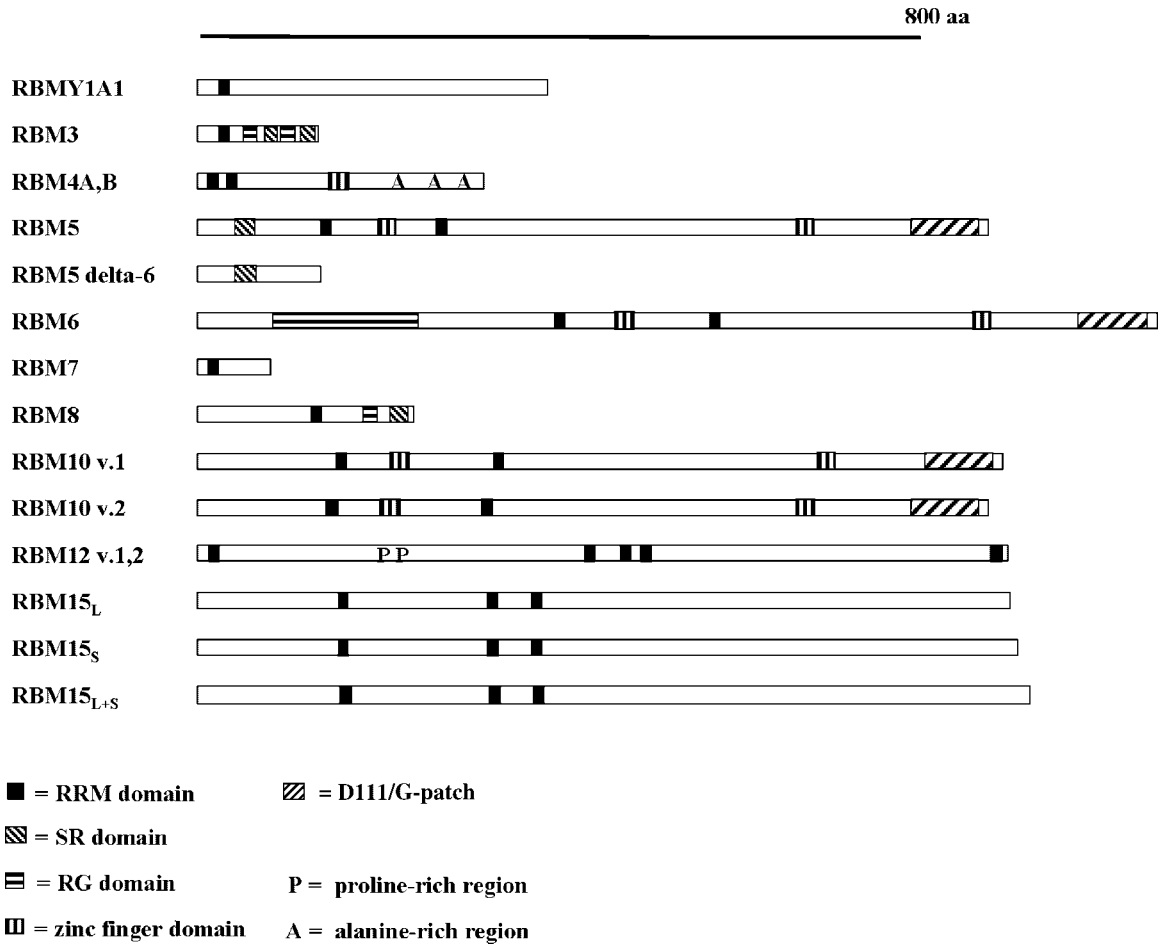

Fig. 3. Protein motif and size correlation's amongst the RBM proteins. Each protein is represented by a horizontal box that is drawn according to scale. Various motifs are delineated within each protein, with no consideration to scale, only placement within the protein.

RBMY1A1 was cloned in 1993 [Ma et al., 1993] from an adult human testis cDNA library. Many synonyms for this gene exist, including *YRRM1* [Ma et al., 1993], *RBMY1* [Chai et al., 1997], and *RBM2* (*YRRM2*). Two genes, *RBMY1A1* and *RBMY1A2* mapping to Yq11.23, encode the same protein of 496 amino acids with a predicted molecular mass of ~56 kDa [Chai et al., 1997]. Most, if not all, *RBMY1* genes have testis-specific expression [Ma et al., 1993]; however, *RBMY1A1* was also recently cloned from adult medulla [Strausberg et al., 2002].

All of the *RBMY1* genes have highly similar sequences, with only a few single base differences in both the exonic and intronic sequences [Chai et al., 1998]. The *RBMY1* genes encode RNA-binding proteins that contain a single N-terminal RRM. The C-terminal domain consists of four repeated segments with a high arginine (20%), serine (15%), tyrosine (14%), and glycine (9%) content, but no aliphatic leucine, isoleucine, methionine, or valine residues [Ma et al., 1993]. This serine–arginine–glycine–tyrosine tetrapeptide sequence, now referred to as the SRGY box, or a similar sequence, occurs twice in each of the four repeats [Ma et al., 1993]. An additional level of complexity is observed, since the products of each of the *RBMY1* genes are alternatively spliced [Chai et al., 1997, 1998].

The protein is expressed in foetal, prepubertal, and adult male germ cells [Elliott et al., 1997]. The Y chromosome location, structure, and testis-specific expression of *RBMY* imply a critical, male-specific function and suggest an important role in germ cell development and adult spermatogenesis [Delbridge et al., 1997; Elliott et al., 1997; Mazeyrat et al., 1999]. The fact that the gene is so highly conserved between marsupials and eutherions suggests this function is critical [Delbridge

TABLE I. RBM Family Members

Gene symbol	Genes	Splice variants	RefSeq ID	Chromosomal locus	Amino acids	Molecular mass (kDa)	Function	Binding partners	Synonyms
RBMY	*RBMY1A1*		NM_005058	Yq11.23	496	56	Splice site selection (spermatogenesis and germ cell development)	RBMY1A1 SRp20 SRp30c 9G8 T-STAR Tra2B	RBM1 RBM2 YRRM1 YRRM2 RBMY
	RBMY1A2			Yq11.23	496	56			
	RBMY1A3P			Yq11.23					
	RBMY1C			Yq11.23					
	RBMY1G			Yq11.23					
	RBMY1H			Yq11.23			Pseudogene		
	RBMY2A			Yq11.23					
	RBMY2B			Yq11.23					
RBM3	*RBM3*		NM_006743	Xp11.23	157	17	(Apoptosis, cold shock)		IS1-RNPL
RBM4	*RBM4a*		NM_002896	11q13	366	41	Splice site selection	TRN-SR2	LARK
	RBM4b		NM_002896	11q13	366	41	Splice site selection		
RBM5	*RBM5*		NM_005778	3p21.3	815	100–120	(Apoptosis enhancement)		LUCA-15 H37
		Delta-6 +introns5,6 +intron6			150	17	(Apoptosis suppression)		
RBM6	*RBM6*	A	NM_005777	3p21.3	1123	129			3G2 (g16) NY-LU-12 DEF-3
		B C D			1177				
RBM7	*RBM7*		NM_016090	11q23.1-q23.2	266	30	Splicing (meiosis)	SAP145 SRp20	
RBM8	*RBM8A*		NM_005105	1q12-q21	174	20	Exon-exon junction complex Nonsense-mediated decay Nucleocytoplasmic shuttling	MAGOH Importin13 TAP Aly/REF RNPS1 Upf3 RanBP5 OVCA1	RBM8 BOV-1 ZNRP Y14 MDS014
	RBM8B		AF403013	14q22	158	18	Pseudogene		
RBM10	*RBM10*	Variant 1	NM_005676	Xp11.23	930	103			MGC 997 MGC 1132 DXS8237E KIAA0122
		Variant 2	NM_152856		852	95			
RBM12	*RBM12*	Variant 1	NM_152838	20q11.21	932	97	(Membrane trafficking)		SWAN HRIHFB2091
		Variant 2							
RBM15	*RBM15*	Long	AF368064	1p13	957		(Cell fate specification)		
		short	AF368062		969				
		short+exon	AF368063		977	107			OTT

et al., 1997]. RBMY may be involved in RNA processing or translational control during spermatogenesis, and is a putative azoospermia factor.

Protein–protein interaction assays demonstrate that RBMY1A1 interacts with itself [Elliott, 2000], the SR-related pre-mRNA splicing proteins SRp20, SRp30c, 9G8, and Tra2β [Elliott, 2000; Venables et al., 2000] and the pre-mRNA splicing protein T-STAR [Venables et al., 2000]. Protein–protein interactions with T-STAR and Tra2β are mediated through the SRGY boxes in the C-terminal region of RBMY1A1 [Venables et al., 1999, 2000]. Binding of RBMY1A1 to Tra2β, which is enhanced by SRGY-specific phosphorylation, has been shown to inhibit Tra2β-specific splicing [Venables et al., 2000].

No specific natural RNA target for RBMY1A1 has yet been identified.

RBM3

RBM3 was first identified by Derry et al. [1995] from a human foetal brain tissue cDNA library. The *RBM3* gene maps to Xp11.23 and encodes alternatively spliced RNA transcripts that are expressed in a wide variety of human tissues [Derry et al., 1995] and cell lines [Danno et al., 1997]. The longest open reading frame encodes a 157 amino acid protein with a predicted molecular mass of 17 kDa, containing one RRM domain and a glycine-rich region [Derry et al., 1995]. This protein binds to both RNA and DNA [Wright et al., 2001].

RBM3 is one of the first proteins synthesized in response to cold shock [Danno et al., 1997]. RBM3 expression is also upregulated (1) during late transcription of *Vaccina* virus proteins in vitro, suggesting a role for RBM3 in poxvirus replication [Wright et al., 2001], (2) in the TF-1 human erythroleukemic cell line, in the presence of granulocyte colony-stimulating factor (GM-CSF), suggesting a role for RBM3 in proliferative processes during hematopoiesis [Baghdoyan et al., 2000], and (3) in purified CD34+ cells [Baghdoyan et al., 2000]. RBM3 expression is downregulated in (1) differentiating TF-1 cells, suggesting that RBM3 may play a role in regulating proliferation versus differentiation [Baghdoyan et al., 2000], (2) the sertoli cells of mice with cryptorchid testis, a condition in which the testis do not descend and thus do not undergo cold stress [Danno et al., 2000], and (3) an in vitro human model of melanoma progression, suggesting a role for RBM3 in the development of cancer [Baldi et al., 2003]. Multiple isoforms of RBM3 have recently been shown to bind to the adenylate/uridylate-rich elements (AREs) in the 3′-UTR of the cyclooxygenase-2 (COX-2) mRNA, as a complex with the other RNA binding proteins TIAR, TIA-1, AUF1, HuR, CBF-A, hnRNP A3, and hnRNP A2/B1 [Cok et al., 2004]. Its role in COX-2 expression, however, remains undetermined.

The cold shock-induced overexpression of RBM3 is regulated by cap independent translation via an Internal Ribosome Entry Site (IRES) [Chappell et al., 2001]—a *cis*-acting RNA sequence able to mediate internal entry of the 40S ribosomal subunit on some eukaryotic and viral messenger RNAs, upstream of a translation initiation codon [Sachs et al., 1997]. RBM3 may function in a manner similar to the X-linked inhibitor of apoptosis (XIAP), which is also translated via an IRES in response to stress (such as low-dose irradiation or serum withdrawal) [Holcik et al., 1999]. Interestingly, like XIAP, a role for RBM3 in apoptosis regulation has been suggested: apoptosis triggered by induced expression of polyglutamine tracts results in downregulation of RBM3, and exogenous overexpression of RBM3 inhibits polyglutamine tract-induced apoptosis [Kita et al., 2002].

RBM4

Human RBM4 was cloned in 1997 by Jackson et al. from infant brain and foetal lung tissue libraries. There are two different *RBM4* genes, *RBM4a* and *RBM4b*, both located at 11q13, the entire *RBM4a* gene being located within the second intron of *RBM4b* [Lai et al., 2003]. The two *RBM4* genes have similar structures, with their coding sequences in exons 2 and 3; however, the untranslated regions (UTR) have no sequence homology.

RBM4a and *RBM4b* encode two highly similar proteins of 366 amino acids, with predicted molecular masses of ~41 kDa [Lai et al., 2003]. Both proteins contain two RRM-domains, located within their N-terminal regions, a C_2HC retroviral-type zinc finger and three alanine-rich regions within their C-terminal regions [Newby and Jackson, 1996; Jackson et al., 1997].

The human RBM4 is a putative mammalian homologue of the *Drosophila melanogaster*

RNA-binding protein Dlark [Newby and Jackson, 1993, 1996]. RBM4 and Dlark share ~50% identity within their N-terminal regions [Jackson et al., 1997; Lai et al., 2003]; however, both RBM4a and RBM4b proteins have three alanine-rich segments within their C-terminal regions, whereas Dlark has three proline-rich segments [Lai et al., 2003].

Dlark has been shown to play a role in embryonic development and is important for circadian regulation of adult eclosion [McNeil et al., 2001]. There is also evidence to suggest maternal inheritance of Dlark and protein function during oogenesis, and studies show that germ-line expression of Dlark is required for proper development [McNeil et al., 1999]. Mutational analyses indicate that the zinc finger of Dlark is important for the maternal function of the protein [McNeil et al., 1999], and that both RRM domains are important for normal development [McNeil et al., 2001]. Further evidence suggests, however, that neither the zinc finger nor either RRM is important for the circadian regulation of adult eclosion [McNeil et al., 2001].

It is anticipated that RBM4 has an important function in a variety of cell types since it is expressed in such a broad range of tissues and is highly evolutionarily conserved [Jackson et al., 1997]. Preliminary analyses have identified RBM4 as both a novel substrate of the nucleocytoplasmic serine/arginine(SR)-rich protein specific transport factor TRN-SR2 (transportin-SR2), and as a regulator of alternative splice-site selection [Lai et al., 2003]. RBM4 proteins are thus transported by the same nucleocytoplasmic factor that is responsible for the shuttling of SR proteins, a family of proteins that RBM4 has been shown to oppose in splice-site selection [Lai et al., 2003]. TRN-SR2 interacts directly with the C-terminal alanine-rich domain of RBM4, in a Ran-sensitive manner, to mediate its nuclear import [Lai et al., 2003], and both the RRM and alanine-rich domains have been shown to have a critical function in the modulation of splice-site selection [Lai et al., 2003].

RBM5

Alternative Splicing

RBM5 was first cloned as LUCA-15 in 1996, from islet cells [Wei et al., 1996], then subsequently as LUCA15 [Edamatsu et al., 2000], RBM5 [Timmer et al., 1999b], and H37 [Oh et al., 1999]. The gene maps to the putative human lung cancer tumor suppressor region 3p21.3, and encodes a number of alternative RNA splice variants, identified by reverse transcription-polymerase chain reaction (refer to Fig. 4). Full-length RBM5 RNA has ~2,500 bp and encodes a ubiquitously expressed protein with a molecular mass of between 100 and 120 kDa [Sutherland et al., 2000]. One RNA variant, RBM5Δ6, encodes a protein of ~17 kDa due to a frameshift caused by the deletion of exon 6 [Mourtada-Maarabouni et al., 2003]. Two other RNA splice variants retain intronic sequences: one retains introns 5 and 6 (RBM5 + 5 + 6), and—due to a pre-mature stop codon in intron 5—putatively encodes a protein of 17 kDa, while another variant retains only intron 6 (RBM5 + 6), and—due to a premature stop codon in intron 6—putatively encodes a protein of 21.5 kDa. Both intron-retaining transcripts are believed to be represented by an ~7 kb RNA transcript detected by Northern blot analysis [Drabkin et al., 1999; Timmer et al., 1999b; Sutherland et al., 2000]. Both intron-retaining variants are classic candidates for nonsense-mediated decay, a mechanism whereby RNA molecules containing premature stop codons followed by exon–exon junctions are degraded during/prior to translation [Maquat and Carmichael, 2001]. This may explain why a 21.5 kDa protein (putatively encoded by RBM5 + 6) has never been detected, and why a 17 kDa protein has rarely been detected (the 17 kDa protein reported [Sutherland et al., 2000] may have represented RBM5Δ6).

RBM5Δ6 cDNA was recently cloned [Mourtada-Maarabouni et al., 2003]. Full-length intron-retaining cDNA has not been cloned; however, a potentially partial cDNA, representing the 5′-end of the full-length RBM5 + 5 + 6 RNA, has been cloned by two groups (Clone 26 [Sutherland et al., 2000] and full-length Clone 86 [Edamatsu et al., 2000]). This potentially partial cDNA terminates within intron 6 in both clones, and contains an open reading frame that terminates within the proximal region of intron 6. Curiously, this clone, although retaining both introns 5 and 6, encodes a 21 kDa protein following in vitro transription/translation in a rabbit reticulocyte lysate, suggesting that intron 5 is spliced from the cDNA (Rintala-Maki and Sutherland, unpublished observations). In line with this

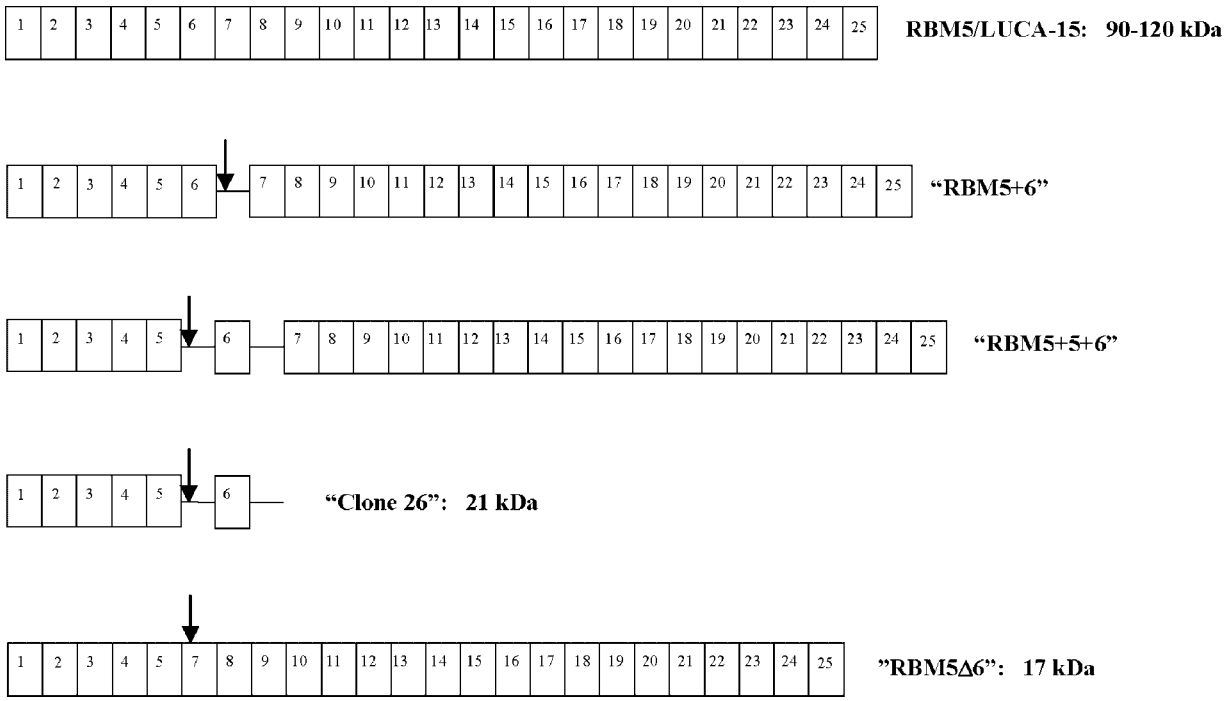

Fig. 4. RBM5 variant structures. Each box represents one exon, and horizontal lines represent introns. Downward arrows represent the positions of STOP codons in the protein coding sequence. Exons and introns are not drawn to scale.

finding is the fact that overexpression of Clone 26 cDNA in both Jurkat and TF-1 cell lines frequently results in production of an intron 5-less RT-PCR product, suggesting that the overexpressed intron 5 and 6-retaining cDNA is further processed (Sutherland, unpublished observations).

Deletion of intron 5 means that exon 6 is retained in the open reading frame, while retention of intron 5 means that any putatively encoded protein would lack exon 6 sequence. Since exon 6 harbors the RNP-1 motif of the first RRM of RBM5, it strongly suggests that RNA binding is important to the function of RBM5. The presence of RBM5Δ6, in which exon 6 is deleted, as the only other known splice variant of RBM5, supports this hypothesis.

And finally, one other cDNA that maps to the RBM5 locus is Je2, a 326 bp sequence that is antisense to intron 6 sequence of RBM5 and, as a consequence, the 3′-untranslated region of Clone 26 [Sutherland et al., 2000].

RNA Expression

Full-length RBM5, RBM5 + 5 + 6, and RBM5 +6 RNA all appear to be widely expressed in both primary tissue and cell lines, with highest expression generally being observed, by Northern blot, in muscle (heart and skeletal) and pancreas [Drabkin et al., 1999; Lerman and Minna, 2000; Sutherland et al., 2000; Oh et al., 2002]. RBM5Δ6 RNA is expressed in a tissue-specific manner, being highest in spleen [Sutherland et al., 2000] and transformed cells [Mourtada-Maarabouni et al., 2003]. A recent study determined that RBM5 RNA was downregulated in 82% of primary non-small-cell lung carcinoma specimens examined compared to normal adjacent tissue, and in many lung cancer cell lines [Oh et al., 2002], and in RAS-transformed Rat-1 cells [Edamatsu et al., 2000]. There is a 27-fold reduction of RBM5 RNA in vestibular schwannomas, as detected by gene chip array analysis [Welling et al., 2002]. Interestingly, it has also been reported that full-length RBM5 mRNA is upregulated following induced overexpression of the oncogene Her-2 in both MCF-7 breast cancer cells and CaOv-3 ovarian cancer cells (4.5 and ~3-fold, respectively), and demonstrates an 88% expression correlation with Her-2 in human primary breast cancer specimens [Oh et al., 1999]. It has conversely been reported that RBM5 mRNA is downregulated in breast cancer specimens, but no reference was made to the Her-2 expression levels in these specimens [Edamatsu et al.,

2000]. And finally, differential expression of RBM5 between the adult and foetal thymus indicates that RBM5 may be developmentally regulated [Drabkin et al., 1999].

DNA microarray technology has been used to examine the effect of stable expression of RBM5 or Je2 in CEM-C7 cells grown in the presence and absence of anti-Fas antibody. In the Je2 transfectants exposed to anti-Fas antibody, a number of differentially expressed genes were observed: most notably, transcription factor Stat5b was downregulated 17-fold, while the B cell translocation gene, *BTG2*, was upregulated 12-fold [Mourtada-Maarabouni et al., 2001]. Caspase-10, a protease involved in mediating death receptor-initiated apoptosis, was also downregulated, fivefold, while the Pim-1 oncogene was upregulated, sevenfold. In the RBM5 CEM-C7 transfectants exposed to anti-Fas antibody, the same genes demonstrated altered expression, but in the reverse direction [Mourtada-Maarabouni and Williams, 2002b].

Protein Expression

Protein expression data for RBM5 are less comprehensive. The predicted molecular mass of full-length RBM5 is ~90 kDa [Timmer et al., 1999b], and in vitro transcription/translation of the "H37" RBM5 cDNA (Accession No. AF103802), incorporating ^{35}S-methionine, confirms this [Oh et al., 1999]; however, some RBM5 antibodies detect a polypeptide of ~120 kDa [Rintala-Maki and Sutherland, 2004; Rintala-Maki et al., in press], suggesting that RBM5 is post-translationally modified. In Western blot analyses, rarely has an RBM5 immunoreactive protein of 17 kDa been detected, and, notably, most of the RBM5Δ6 protein overexpression analyses were carried out with the aid of a protein tag [Sutherland et al., 2000; Mourtada-Maarabouni et al., 2002a, 2003].

RBM5 protein was found to be downregulated in 73% of primary non-small-cell lung carcinoma specimens examined, compared to normal adjacent tissue [Oh et al., 2002]. Since 82% of the RBM5 RNA was downregulated in the same study, it suggests that ~10% of the cancers experienced deregulation between transcription and translation. RBM5 was recently identified as an autologous serum antigen in patients with renal cancer [Scanlan et al., 1999], suggesting that RBM5 may play a role in renal carcinoma.

Expression of Je2 RNA (antisense to intron 6) in CEM-C7 cells resulted in the downregulation of RBM5 protein and the upregulation of a lower molecular weight, possibly 17 kDa, band [Mourtada-Maarabouni et al., 2002a]. The downregulation of LUCA-15 expression suggests that Je2 functions by interfering with RBM5 transcription or translation. Upregulation of the smaller molecular weight protein, likely represented by RBM5Δ6, is not so easily explained. The fact that neither RBM5 nor RBM5Δ6 has Je2 homologous sequence suggests that the mechanism by which Je2 effects their expression is independent of a typical "antisense effect." This mechanism remains to be further clarified.

Motifs

Full-length RBM5 protein has an arginine-rich N′-terminal region (suggesting a nuclear speckle localization) [Li and Bingham, 1991], two bipartite nuclear localization signals (NLS), two zinc fingers (RANBP and C_2H_2), two RRM domains (with RNP-1 but not very well defined RNP-2 sequences), and a G-patch/D111 domain [Aravind and Koonin, 1999], suggesting that RBM5 localizes to the nucleus, and is involved in RNA binding. Exon 15 contains sequence that encodes a putative myristoylation site, transmembrane domain, and Asn-glycosylation site, suggesting that RBM5 may orient to a membrane. RBM5Δ6, lacking exon 6, thereby not only loses the core RNP-1 sequence of the first RRM, but, due to a frameshift resulting from the loss of exon 6 and subsequent truncation of the protein, all downstream functional motifs as well. All that is retained of any putative functional sequence in RBM5Δ6 is the arginine/glycine-rich amino terminal region. Thus, RNA binding is presumed to be a function of some, but not all, RBM5 variants.

RNA Binding

The consensus functional motifs within RBM5 suggest a role as an RNA splicing factor: RRM domains are necessary—but not sufficient—for localization to nuclear speckles, thought to be places of storage for splicing factors [Dye and Patton, 2001], and; zinc fingers are common motifs in RNA splicing factors [Dye and Patton, 2001]. In addition, the G-patch/D111 motif, also present in the 45 kDa splicing factor SPF45 [Aravind and Koonin, 1999], is

thought to be involved in protein–protein interactions of various RNA- and DNA-binding proteins [Courey and Tjian, 1988; Zhang et al., 1993]. Functional studies have demonstrated that RBM5 is indeed able to bind RNA. Epitope-tagged full-length, N′- and C′-terminal recombinant proteins, produced in either an *E. coli* expression system or in HEK293 cells, were shown to preferentially bind poly(G) homopolymer tracts in vitro [Drabkin et al., 1999; Edamatsu et al., 2000]. Of note, the C′-terminal region of RBM5, lacking RRM domains but containing both a zinc finger and the D111/G-patch, was also able to bind poly(G) tracts, but with less affinity than the RRM containing N'-terminal region. There is no known or predicted RNA substrate(s) for any of the RBM5 isoforms.

Proliferation

The effect of RBM5 expression on cell proliferation is poorly defined, and sometimes contradictory. For example, overexpression of RBM5 suppresses cell growth in human fibrosarcoma HT1080 cells [Edamatsu et al., 2000] and CEM-C7 T cells [Mourtada-Maarabouni et al., 2003], and stable RBM5 CEM-C7 transfectants are arrested in the G1 phase of the cell cycle [Mourtada-Maarabouni et al., 2003]; however, in Jurkat T cells, cell proliferation is unaffected by RBM5 overexpression [Rintala-Maki and Sutherland, 2004]. Overexpression of RBM5Δ6 increases CEM-C7 cell proliferation, as measured by propidium iodide staining [Mourtada-Maarabouni et al., 2003], and stable Clone 26 Jurkat transfectants have a reduced growth rate, resulting from either a cell proliferation regulatory effect, or a pro-apoptotic function [Sutherland et al., 2000].

Endogenous RBM5 and full-length Clone 86 transcripts are downregulated in Rat-1 rat embryonic fibroblast cells that express a mutant, constitutively activated Ras [Edamatsu et al., 2000]. Ras is involved in expression of early response genes and the regulation of cell proliferation, and is frequently mutated in tumors. This link between the constitutive expression of Ras and the downregulation of RBM5 suggests that RBM5 may act as a tumor suppressor through a dual mechanism that involves both augmentation of apoptotic signals and inhibition of cell proliferation.

Induced expression of RBM5 resulted in reduced growth of anchorage-dependent and -independent cells in an MCF-7 RBM5$^{-/-}$ breast cancer cell subline, and in A9 mouse fibrosarcoma cells, but not in HBL-100 immortalized human cells that lack a malignant genotype/phenotype [Oh et al., 2002]. Ectopic expression of RBM5 in A9 cells injected subcutaneously into nude mice resulted in significant suppression of tumor growth [Oh et al., 2002]. Taken together, these results suggest that RBM5 modulates tumor cell growth and that regulation of proliferation observed as a result of induced RBM5 expression involves malignancy-specific factors.

Apoptosis Modulation

The majority of functional studies relating to RBM5 concern its ability to modulate apoptosis [reviewed in Mourtada-Maarabouni and Williams, 2002b]. Overexpression of full-length RBM5 in transformed cell lines leads to an enhancement of apoptosis. For instance, RBM5 sensitizes Jurkat human T lymphoblastoid cells to apoptosis induced by several death receptor ligands, including Fas, TNF-α, and TRAIL [Rintala-Maki and Sutherland, 2004]. Furthermore, RBM5 sensitizes MCF-7 human breast adenocarcinoma cells to TNF-α-mediated apoptosis [Rintala-Maki et al., in press].

Overexpression of RBM5 in the antisense orientation significantly suppresses apoptosis in Jurkat cells mediated by FasL, TNF-α, and staurosporine but not etoposide [Sutherland et al., 2001, 2004]. Overexpression of the small antisense cDNA, Je2, dramatically suppresses Fas-mediated apoptosis in Jurkat cells [Sutherland et al., 2000], as well as FasL-, TNF-α, and dexamethasone but not etoposide-mediated apoptosis in CEM-C7 cells [Mourtada-Maarabouni et al., 2002a]. In addition, there is a correlation between Je2 expression and upregulation of the Bcl-x_L apoptosis inhibitor protein [Mourtada-Maarabouni et al., 2002a], and antisense RBM5 expression and upregulation of the Bcl-2 apoptosis inhibitor protein [Sutherland et al., 2001, 2004], suggesting that Bcl-2 family proteins are involved in the regulation of apoptosis by RBM5 and its variants. Je2 may function by inhibiting RBM5/splice variant transcription, interfering with translation of either intron-retaining RNA variant, sequestering factors away from RBM5/splice variants or independently of RBM5. In Je2 transfected CEM-C7 T cells, there is a reduction

in the ~100 kDa RBM5 protein [Mourtada-Maarabouni et al., 2002a]; however, in Je2 transfected Jurkat T cells, there is a reduction in a 42 kDa RBM5 immunoreactive polypeptide, but no effect on the 100 kDa RBM5 protein [Sutherland et al., 2000]. These contradictory results may reflect cell type-specific effects of Je2 on apoptosis modulation. Much remains to be elucidated concerning the role of RBM5 reverse-strand expression on apoptosis regulation.

Considering that all putative functional motifs that are present in RBM5 downstream of the first RRM are absent in RBM5Δ6, it might be thought that RBM5Δ6 would have no effect on apoptosis. It is interesting, therefore, that the absence of exon 6 and subsequent truncation of RBM5 (thereby eliminating all downstream functional motifs) results in an active suppression of apoptosis in CEM-C7 cells [Mourtada-Maarabouni et al., 2003]. It remains to be determined whether exon 6 sequence is responsible for "overriding" an active, anti-apoptotic sequence contained within the amino-terminal region of RBM5, leading full-length RBM5 to facilitate apoptosis. This anti-apoptotic effect may be cell type-specific, since overexpression of RBM5Δ6 does not suppress Fas-mediated apoptosis in Jurkat cells [Mourtada-Maarabouni et al., 2003].

Jurkat cells transfected with Clone 26 are also more sensitive to Fas-mediated apoptosis [Sutherland et al., 2000], but it is unclear at this time which truncated variant is responsible for this enhancement, since intron 5 is apparently sometimes spliced out of Clone 26 when it is overexpressed in some transformed cell lines (as mentioned above). These results support a key role for exon 6 in modulating apoptosis because Clone 26 encodes a 21 kDa protein that retains exon 6, and Clone 26 overexpression, like that of full-length RBM5, sensitizes cells to apoptosis, whereas RBM5Δ6 suppresses apoptosis.

Homologies

RBM5 has highest homology to the other RBM family members RBM10 (~50%) and RBM6 (30%) (see below). Interestingly, outside of the RBM family, RBM5 has most homology to nucleolin, another RRM-containing RNA binding protein; however, this homology only extends to the two RRMs [Ginisty et al., 1999, 2001].

RBM6

Alternative Splice Variants

The gene encoding RBM6 is located at 3p21.3, and is immediately adjacent and telomeric to the gene encoding RBM5. RBM6 transcript was first cloned as g16, a partial cDNA of what is now known to be an alternative splice variant of RBM6 [Timmer et al., 1999b]. Alternative RBM6 transcripts, first cloned as NY-LU-12 A, B, C, and D [Gure et al., 1998], will herein be referred to as RBM6 transcripts A, B, C, and D. The predominant transcript, A (which includes exon 5) encodes a protein of 1123 amino acids with a predicted molecular mass of 129 kDa. A less abundant transcript, B, encodes a protein of 1177 amino acids, containing one additional exon and an altered N-terminal sequence [Gure et al., 1998]. Transcripts C and D are unlikely to encode protein, due to a very long UTR in the 5′-ends [Gure et al., 1998]. Another alternatively spliced RNA, RBM6 transcript A minus exon 5 (henceforth termed RBM6Δ5), is the complete sequence that is represented by the partial clone, g16: deletion of exon 5 results in a frame-shift and premature termination of the polypeptide [Gure et al., 1998; Timmer et al., 1999b]. Unless otherwise stated, RBM6 refers to RBM6 transcript A.

RNA Expression

RBM6 appears to be differentially expressed in adult tissues, including those of hematopoietic origin [Drabkin et al., 1999]. In hematopoietic tissues, RBM6 expression is highest in the thymus, lymph nodes, and peripheral blood leukocytes, and downregulated upon granulocytic differentiation, suggesting that RBM6 may be important for both T cell and granulocyte development and/or function [Drabkin et al., 1999; Hotfilder et al., 1999]. In non-hematopoietic tissue, RBM6 has highest expression in the heart, pancreas, and skeletal muscle (similar to RBM5) [Drabkin et al., 1999]. RBM6Δ5 is expressed in normal lung tissue, cancerous lung tissue, and lung cancer cell lines [Gure et al., 1998; Timmer et al., 1999b]; however, its expression is much higher in normal lung tissue than in lung cancer cell lines [Timmer et al., 1999b], suggesting that removal of exon 5, which contains an RNP-1 RBM, may be important for tumor suppression. Correlating with these observations is the identification of the RBM6 transcript A product

(NY-LU-12), which contains exon 5 encoded sequence, as an autologous serum antigen in patients with lung cancer [Gure et al., 1998]. In toto, these observations suggest that expression of exon 5 plays a role in lung carcinogenesis.

Function

No function has yet been determined for any of the putative RBM6 proteins (encoded by RBM6 transcripts A or B, or RBM6Δ5). The fact that the putative product encoded by transcript A has 30% identity with RBM5 [Timmer et al., 1999b] suggests that the two proteins may have similar functions. The proteins encoded by RBM5 and RBM6 transcript A both contain two RRM domains (although the RRM domains of RBM6 are less conserved than those of RBM5 [Timmer et al., 1999b]: see Fig. 2), two zinc fingers, a nuclear localization signal, and a G-patch/D111 domain, while the proteins encoded by RBM5Δ6 and RBM6Δ5 lack all of the above. Unlike RBM5, each RBM6 variant has 20 repeats of a fairly conserved six amino acid sequence D(F/Y)R(G/D)(R/G)(D/E) separated by four to six amino acids in its N'-terminal [Drabkin et al., 1999]. It has been suggested that these repeat sequences may play a role in RNA binding (since some of the repeats contain the RNA binding sequence RGG [Burd and Dreyfuss, 1994; Gure et al., 1998; Drabkin et al., 1999]).

RNA Binding

Binding studies incorporating the RRMs of RBM6 in a recombinant in vitro system identified poly(G) RNA homopolymers as a binding substrate, as observed for RBM5 [Drabkin et al., 1999]. An interesting note in considering the contribution of RNA binding to RBM6—and indeed RBM5—function is that the deletions in both RBM6Δ5 and RBM5Δ6 span the RNP-1 sequence of the first RRM of each protein. A conundrum arises from the fact that expression patterns suggest a role for RBM6Δ5 in tumor suppression, while functional studies (involving apoptosis modulation) suggest a tumor suppressor role for the RBM5 exon 6-containing protein. Perhaps this is weak evidence for a lack of correlation between RNA binding and apoptotic function and/or tumor suppressor activity in these two proteins.

RBM7

RBM7 was recently cloned from a human testis cDNA library by Go et al. [2003]. The gene is located at 11q23.1-q23.2 and encodes a protein of 266 amino acids with a predicted molecular mass of 30–35 kDa. The RBM7 protein has one RRM-domain and a nuclear localization sequence [Guo et al., 2003]. RBM7 protein is ubiquitously expressed; however, expression is particularly abundant in certain brain cells and during spermatogenic meiosis. A role in cell type-specific RNA processing is therefore deduced, based on this differential expression, and the fact that RBM7 interacts specifically with the splicing factor SAP145 and the splicing regulator SRp20 [Guo et al., 2003].

RBM8

RBM8 was cloned by four separate groups in 2000 [Conklin et al., 2000; Kataoka et al., 2000; Salicioni et al., 2000; Zhao et al., 2000]. The gene maps to at least two chromosomal loci, 1q12-q21 (thought to be the originating locus) and 14q12-21 (a retroposon) [Zhao et al., 2000; Okubo et al., 2002]. The *RBM8A* gene at 1q12-q21 encodes a protein of 20 kDa. The *RBM8B* gene at 14q22 lacks a promoter or introns and is believed to be a pseudogene [Okubo et al., 2002; Lau et al., 2003].

The RBM8 protein has one RRM-domain and a C-terminal serine–arginine rich region and glycine–arginine rich region [Salicioni et al., 2000]. RBM8 associates with the other components of the exon–exon junction complex (EJC) Aly/REF, RNPS1, and Magoh [Zhao et al., 2000; Kataoka et al., 2001; Lau et al., 2003], the mRNA export factor TAP [Kataoka et al., 2000, 2001], the nonsense-mediated decay protein Upf3 [Gehring et al., 2003], and the nuclear import factors importin 13 [Mingot et al., 2001] and RanBP5 [Kataoka et al., 2000]. As a component of the EJC, RBM8 binds to mRNA 20–24 nucleotides upstream of a spliced exon–exon junction [Kataoka et al., 2000; Le Hir et al., 2000]. Furthermore, RBM8 plays a role in spliced mRNA nuclear export [Kataoka et al., 2000], and the process of nonsense-mediated decay of mRNAs with premature stop codons [Maquat and Carmichael, 2001].

RBM8 forms a specific complex with the protein Magoh [Zhao et al., 2000; Mingot et al., 2001; Lau et al., 2003]. Following mRNA splicing, the RBM8-Magoh complex associates

with Aly/REF, RNPS1, DEK, and SRm160 on the spliced mRNA [Le Hir et al., 2001]. The EJC-"tagged" mRNA is then actively transported through the nuclear pore complex by a process that involves an interaction between the nuclear export factor TAP and Magoh [Kataoka et al., 2000, 2001; Le Hir et al., 2001; Kim et al., 2001b]. RBM8 (complexed with Magoh) remains associated with the cytoplasmic spliced mRNA, serving as a splicing memory [Kataoka et al., 2000; Le Hir et al., 2001; Kim et al., 2001b]. If the spliced cytoplasmic mRNA contains a premature stop codon (defined by distance upstream of an EJC), it will be targeted for nonsense-mediated decay (NMD) by specific proteins, such as Upf2 and Upf3. Upf3 is recruited to the mRNA through interaction with RBM8 [Kim and Dreyfus, 2001a; Gehring et al., 2003]. If the mRNA is not targeted for NMD, the RBM8-Magoh complex is released from the mRNA during translation [Dostie and Dreyfuss, 2002], and translocated back into the nucleus. This cytonucleoplasmic transfer is mediated by importin 13, largely through its interaction with RBM8 [Mingot et al., 2001].

RBM10

RBM10 was first cloned by Nagase et al. in 1995 from bone marrow. The *RBM10* gene is located on the X chromosome, at p11.23, and although one allele is silenced in each somatic cell by the process of X chromosome inactivation, the remaining active allele is widely expressed in human cell lines and human tissues [Coleman et al., 1996; Thiselton et al., 2002]. RBM10 is alternatively spliced to produce RBM10 RNA variant 1 and variant 2. Both variants putatively encode proteins containing zinc finger motifs, a G-patch and two RRMs. RBM10 protein variants 1 and 2 share 85 and 96% of their respective amino acid sequence identities with the rat protein S1-1, a hypothetical RNA binding protein with poly(G) and poly(U) binding capabilities [Inoue et al., 1996].

RBM10 protein variants 1 and 2 share 49 and 53% of their respective identities with another RBM protein, RBM5. This identity jumps to 60 and 64%, respectively, when exons 4, 9, and 15 are eliminated from the comparison, demonstrating a high degree of amino acid sequence conservation between the two proteins (as graphically displayed in Fig. 5). The exon 4 sequence is completely different in all three proteins, while sequence in exons 9 and 15 is identical between the RBM10 variants but only 14% identical to RBM5. An additional stretch of 37 amino acids, which is unique to exon 9 of RBM10 variants 1 and 2 and lacking from exon 9 of RBM5, has no homology to other known protein sequences (www.ncbi.nlm.nih.gov/BLAST). The lack of sequence conservation in these specific exons between RBM5 and the RBM10 variants suggests that these sequences are either unimportant to a putative conserved function, or that the proteins have distinct functions, for which the sequences within exons 4, 9, and 15 are responsible.

Functional data on RBM10 are non-existent; however, the rat S1-1 homologue has been shown, in vitro, to preferentially interact with G and U polyribonucleotides [Inoue et al., 1996; Timmer et al., 1999b].

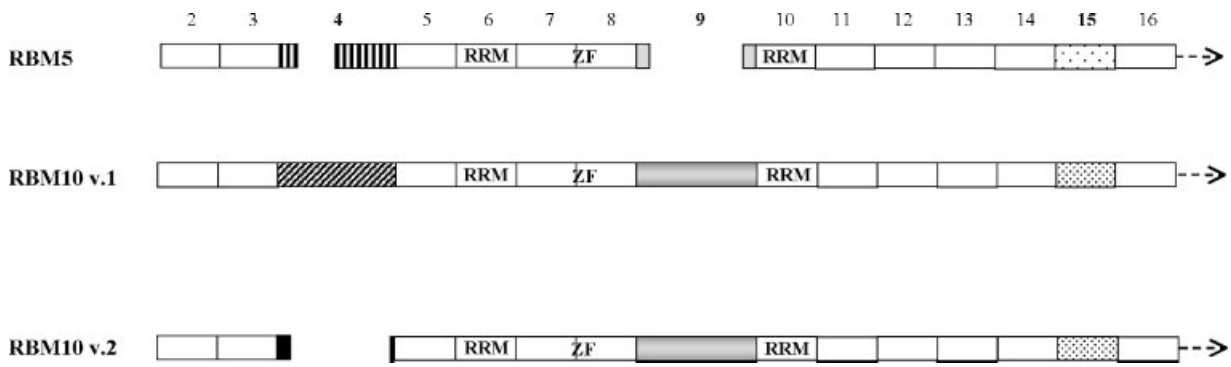

Fig. 5. RBM5 versus RBM10 homologies. Exons are represented by boxes, and are not drawn to scale. The different fills in exon 4 of each protein designates total lack of homology. Exon 9 sequence is identical between the two RBM10 variants, but lacking or limited within RBM5. Exon 15 sequence is identical between the two RBM10 variants but limited within RBM5.

RBM12

Partial RBM12 cDNA was cloned by Nagase et al. [1998] from a brain cDNA library, and by Stover et al. [2001] from a human colon carcinoma cell line cDNA library. Full-length RBM12 was cloned from a human acute monocytic leukemia cell line (THP1) cDNA library [Stover et al., 2001]. The gene maps to 20q11.2 and encodes a deduced protein of 932 amino acids with a molecular mass of 97 kDa. RBM12 mRNA expression has been observed in the T-84 (colon carcinoma), U-937 (histiocytic lymphoma), HL-60 (promyelocytic leukemia), and K-562 (chronic myelogenous leukemia) human cell lines [Stover et al., 2001]. RBM12 RNA is alternatively spliced in the 5′ untranslated region, resulting in two variants with identical open reading frames.

The putative protein contains two proline-rich regions and five RRMs, one RRM located at each end of the protein and three centrally. The RBM12 protein also contains a number of transmembrane domains, and nuclear and mitochondrial targeting sequences but no signal sequence. RBM12 exhibits calcium-dependant phospholipid binding properties and may function in membrane trafficking. Functional data on RBM12 have not been reported.

RBM15

RBM15 was cloned by two groups in 2001, as OTT [Mercher et al., 2001] and RBM15 [Ma et al., 2001]. The *RBM15* gene maps to 1p13. Alternative RNA splicing produces three variants: $RBM15_L$ (long, ~8.5 kb), $RBM15_S$ (short, ~4 kb), and $RBM15_{S+AE}$ (short plus alternative 111 bp exon, ~4 kb). Each of the RNA variants putatively encodes a distinct protein: long (957 amino acids), short (969 amino acids), and short plus alternative 111 bp exon (977 amino acids). Each protein contains three RRMs, a nuclear localization sequence and a Spen Paralog and Ortholog C-terminal (SPOC) domain [Ma et al., 2001; Mercher et al., 2001], and differ only in their 3′ alternative exon usage. The SPOC domain, which is a characteristic of the Spen protein family [Wiellette et al., 1999], is involved in cell-fate specification in a wide range of organisms [Ariyoshi and Schwabe, 2003]. This similarity between RBM15 and Spen family proteins suggests that RBM15 plays a role in chromatin organisation, HOX-regulated differentiation and/or extracellular signaling. *RBM15* also appears to play a role in acute megakaryoblastic leukemia, being one of two genes (the other being *MAL/MKL1*) that undergo recurrent translocation t(1;22)(p13;q13) in this disease [Mercher et al., 2001]. The resulting fusion protein is thought to mediate aberrant chromatin organization [Ariyoshi and Schwabe, 2003]. Functional characterization of, and RNA binding studies concerning, RBM15 have not been reported.

EVOLUTIONARY ASPECTS OF THE RBM GENES

Of the ten RBM family members described here, two are located on chromosome 1 (*RBM15*:p13, *RBM8A*:q12), two are clustered on chromosome 3 (*RBM5*:p21.3, *RBM6*:p21.3), two are on chromosome 11 (*RBM4*:q13, *RBM7*:q23.1-.2), one is on chromosome 20 (*RBM12*:q11.21), two are located on the X chromosome (*RBM3*:p11.23, *RBM10*:p11.23), and one maps to the Y chromosome (*RBMY*). It has been suggested that both *RBM7* on chromosome 11 and *RBM5* on chromosome 3 are retroposon derivations of *RBMY* [Elliott, 2000] on the Y chromosome and *RBM10* [Timmer et al., 1999a] on the X chromosome, respectively. *RBM7* functions during meiosis, when the X and Y chromosomes are transcriptionally inactive, thus retroposition may have resulted from ancient selective pressure. *RBM6* is presumed to have arisen from a gene duplication and retroposition of *RBM5* [Timmer et al., 1999a].

Apart from the related RRMs, the structural similarities within the RBM proteins are far from conserved, as shown in Figure 3. This lack of homology hinders functional predictions. A number of the RBM proteins (e.g., RBMY, RBM4, RBM7, and RBM8) appear to play a role in RNA splicing. For instance, RBMY has been shown to interact with a number of proteins (see Table I), each known to be involved in RNA splicing; RBM4 plays a role in splice site selection; RBM7 interacts with two members of the splicosome, SAP145 and SRp20, and; RBM8, as part of a protein complex, is deposited by the splicosome on spliced mRNA, and aids in targeting mutant RNA molecules for nonsense-mediated decay. Still others (e.g., RBM5, RBM6, and RBM10) are predicted to play a role in RNA splicing, based on the presence of conserved functional motifs such as the RRM

domains and the D111/G-patch [Aravind and Koonin, 1999]. Therefore, despite the fact that RBM3 plays a role in mediating apoptosis, RBM12 is involved in membrane trafficking and RBM15 is important in cell fate determination, it cannot be ruled out that all three of these proteins may be found to perform these functions in association with an RNA splicing event.

The structural—and potentially functional—relationship between RBM5 and RBM6 is rather intriguing. RBM5 is alternatively spliced, a phenomenon that appears to centre around the retention or excision of the RRM motif in exon 6. RBM6 is also alternatively spliced, and although it is exon 5 that experiences the alternative splicing, exon 5 of RBM6 and exon 6 of RBM5 both harbor the core RRM RNP-1 sequence, which has 52% identity between the two proteins. The preservation of gene sequence upon duplication, in conjunction with the conservation of alternative splicing mechanisms, suggests a functional relatedness between the alternative splice products.

RBM5 is predicted to have arisen from a gene duplication and retroposition of *RBM10* [Timmer et al., 1999a]. As seen in Figure 5, the *RBM10* gene encodes two different RBM10 isoforms, variant 1 and variant 2. Homology between either RBM10 variant and RBM5 is absent in exons 4 and 9, and limited in exon 15 of RBM5. RBM10 would therefore be an interesting candidate for apoptosis studies, as it could help to determine whether the ability of RBM5 to enhance death receptor-mediated apoptosis is restricted to the regions common to both proteins.

Homologues for each RBM gene have been identified in a number of species, as outlined in Table II.

TABLE II. RBM Homologues

Gene	Organism	Homologous Gene
RBMY1A1	Mouse	RbmY1a1
RBM3	Mouse	Rbm3
	Rat	Rn.18057
	Pig	Ss.3311
	Cow	Bt.9571
	Frog	XI.8151
	C. elegans	T12D8.2
	Maize	AAIP[a]
RBM4	Mouse	Rbm4
	Fruit fly	lark
RBM5	Mouse	Rbm5
	Pig	Ss.4601
	Cow	Bt.11059
	Frog	MGC68576
	Fruit fly	CG4887
	C. elegans	TO8B2.5[b]
RBM6	Mouse	Rbm6/def-3
	Rat	Rn.4198
	Pig	Ss.6914
RBM7	Mouse	1500011D06Rik
	Rat	Rn.22366
	Frog	X1.7972
	Zebrafish	Dr.13662
	C. elegans	Y37D8A.21
RBM8	Mouse	Rbm8
	Rat	Rn.37716
	Cow	Bt.6706
	Frog	XI.3667
	Fruit fly	Tsu
	C. elegans	R07E5.14
RBM10	Mouse	Rbm10
	Rat	S1-1
	Pig	Ss.13034
	Cow	Bt.3851
	Fruit fly	CG4896
RBM12	Mouse	Rbm12
RBM15	—	—

Data detailed in http://bioinfo.weizmann.ac.il/cards.
[a]Derry et al. [1995].
[b]Lerman and Minna [2000].

RNA BINDING PROTEINS AND APOPTOSIS

Proteome analysis of Jurkat T cells resulted in the identification of 21 different proteins whose expression was altered during Fas-mediated apoptosis. Notably, 15 of the 21 were RNA-binding proteins, and 12 of the 15 were involved in RNA splicing [Thiede et al., 2001]. These results demonstrate the importance of RNA processing regulation in apoptosis.

It has been known for some time that RNA binding proteins can participate in the regulation of apoptosis. KH-containing RNA binding proteins with suspected roles in regulating apoptosis include TIA-1 and TIAR (which also both contain three RRMs) [Beck et al., 1996; Forch et al., 2000; Forch and Valcarcel, 2001], the *Drosophila* proteins Kep1, SAM [Di Fruscio et al., 1998], and FMR1 [Wan et al., 2000], the p53 target gene *MCG10* [Zhu and Chen, 2000], and the mammalian homologue of *E. coli* Ras-like GTPase, ERA [Akiyama et al., 2001]. RGG-box containing RNA binding proteins HSV type 1 early and leaky-late inhibit apoptosis in human HEp2-cells [Aubert et al., 2001]. The RGG-box containing protein SAF-A/hnRNP-U has both RNA-binding and scaffold-binding functions; apoptosis results in caspase cleavage of the scaffold binding domain, and disrupts scaffold binding, but has no effect on RNA binding [Gohring et al., 1997]: therefore, although SAF-A is an RNA-binding protein and an apoptosis regulatory molecule, the two functions appear to be unrelated. In addition, although an

RNA binding protein may contain more than one type of RNA-binding motif, e.g., FMR1, containing both KH and RGG domains, all domains may not be required for apoptosis regulation [Wan et al., 2000].

TIA-1 regulates apoptosis, at least in part, by regulating alternative splicing of the Fas receptor [Forch et al., 2000]. Binding of TIA-1 to the weak 5′-splice site in intron 5 of Fas facilitates binding of U1 snRNP and differential splice site selection. This results in the retention of exon 6, and membrane-bound receptor, which is capable of signaling apoptosis: excision of exon 6 leads to the production of a soluble receptor that acts as an inhibitor of apoptosis.

Two of the RBM proteins discussed above, RBM3 and RBM5, have been shown to play a role in the modulation of apoptogenic signaling. The 17 kDa RBM3 protein suppresses apoptosis triggered by the presence of polyglutamine tracts, and the 17 kDa alternative splice variant of RBM5, RBM5Δ6, suppresses death receptor-mediated apoptosis. On the other hand, the 100–120 kDa full-length RBM5 protein enhances death receptor-mediated apoptosis. With high sequence conservation to RBM5, RBM10, and to a lesser extent RBM6, are predicted to also have apoptotic modulatory capabilities.

The observation that both RBM3 and RBM5Δ6 suppress apoptosis raises the question as to whether their apoptosis modulatory capability is related to their function as RNA binding proteins. And if the latter is the case, does this suggest that other RBM family members may, too, be important regulators of apoptosis?

SEQUENCE COMPARISONS AS AN AID IN THE PREDICTION OF APOPTOTIC REGULATORY FUNCTION

The function of many of the RBM proteins remains undefined, which is precisely why they have been temporarily classified by the HUGO Gene Nomenclature Committee as RBM proteins, a category based on structure and not function. The purpose of this review is to bring all the information together regarding this emerging family, and to examine their characteristics from the viewpoint of RBM5, a known apoptosis modulator. With the recent identification of RBM3 as a protein with apoptotic suppressive function, it is intriguing to speculate that the RBM family may represent a novel family of apoptosis regulators. What has emerged is a picture that has too many gaps to allow significant conclusions. Based on evolutionary considerations and significant homologies, however, it appears likely that, at the very least, RBM6 and the two isoforms of RBM10 will be found to have apoptotic modulatory ability. Indeed, RBM5, RBM6, RBM10, and perhaps RBM3 may be members of an RRM "subfamily" having apoptotic related functions. Within the RBM proteins, primary sequence analysis does not help to predict a common, similar, or overlapping apoptotic function. This is largely due to the fact that the regions of RBM3 and RBM5 that are responsible for the modulation of apoptosis are presently unknown. Whether or not the ability to modulate apoptosis is even related to the ability of the protein to interact with RNA is unknown. These studies are crucial to our understanding of the potential of the RBM proteins as apoptosis modulators.

CONCLUDING REMARKS

The assignment of certain genes by the HUGO Gene Nomenclature Committee to the "RBM" designation is based on the presence of at least one RRM within the putatively encoded protein of a gene for which little else is known. A plethora of proteins that contain an RRM sequence is not designated "RBM," largely due to the fact that they generally have well described functions that are more accurately reflected in their name. Perhaps, as more knowledge of the individual RBM genes described in this review is gained, each will have yet other, more functionally descriptive designations. Certainly, for some of these genes, such as RBM3, RBM5, RBM6, and RBM10, this may reflect a combined RNA binding and apoptotic modulatory role, which might be best suited by a more descriptive designation such as DARRM, for Death-associated RRM.

It is the intention of this review to bring together a number of seemingly related but disparate proteins and look at them in relation to RBM5, to see if viewing them, or RBM5, from a different perspective might give some insight into either RBM5 as an RNA binding protein or other members of the RBM family as potential apoptotic regulators. Hopefully, this review has stimulated the reader to pursue new avenues of investigation on these, as yet, very undefined

proteins and to address key structure/function issues that remain unresolved at the present time.

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