Conserved Structures and Diversity of Functions of RNA-Binding Proteins

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In eukaryotic cells, a multitude of RNA-binding proteins play key roles in the posttranscriptional regulation of gene expression. Characterization of these proteins has led to the identification of several RNA-binding motifs, and recent experiments have begun to illustrate how several of them bind RNA. The significance of these interactions is reflected in the recent discoveries that several human and other vertebrate genetic disorders are caused by aberrant expression of RNA-binding proteins. The major RNA-binding motifs are described and examples of how they may function are given.

In eukaryotic cells, much of the regulation of gene expression is posttranscriptional. Messenger RNAs are produced in the nucleus from the primary transcripts of protein-coding genes, pre-mRNAs, or heterogeneous nuclear RNAs (hnRNAs) by a series of processing reactions that typically include capping, pre-mRNA splicing, and polyadenylation. mRNAs are then transported to the cytoplasm where the protein synthesis machinery is located, and the translation and stability of mRNAs are also subject to regulation. These processes are mediated by numerous RNA-binding proteins and by small RNAs as stable ribonucleoprotein (RNP) complexes (*1, 2*), and much progress has been made in the identification and elucidation of the functions of many of these components. The cloning of complementary DNAs (cDNAs) and the determination of amino acid sequences have led to the discovery of several conserved motifs in RNA-binding proteins (*3*) that have significant predictive value. The discovery of these motifs has led to a new appreciation of the prevalence of posttranscriptional regulation and has permitted assignment of function to several recently cloned genetic loci that are critical for development, and mutation of which gives rise to human genetic disease. Below we describe each of the major RNA-binding motifs and provide examples of how they may function.

The RNP Motif

The most widely found and best-characterized RNA-binding motif is the RNP motif (*4–6*), also referred to as RNA recognition motif (RRM) (*6, 7*), RNP consensus sequence (RNP-CS) (*4*), and consensus sequence RNA-binding domain (CS-RBD) (*4, 5*). It is composed of 90 to 100 amino acids which form an RNA-binding domain (RBD) that is present in one or more copies in proteins that bind pre-mRNA, mRNA, pre–ribosomal RNA (rRNA), and small nuclear RNAs (snRNA). Animal, plant, fungal, and bacterial cells contain RNP motif proteins in nearly all organelles where RNA is present (*2, 6*), which suggests that it is an ancient protein structure with important functions. The identifying feature of the RNP motif is the RNP consensus sequence (RNP-CS), which is composed of two short sequences, RNP1 and RNP2, and a number of other, mostly hydrophobic conserved amino acids interspersed throughout the motif (Fig. 1) (*4–6*). Some RNA-binding proteins lack canonical RNP1 and RNP2 sequences but contain other, well-conserved, structurally important residues, so that the overall structure of these domains is probably very similar to RNP motif RBDs (*4, 6, 8*).

The RNP motif is the only RNA-binding motif for which detailed structural information is available. The three-dimensional structures of the NH_2-terminal RBD of U1 snRNP A (U1 A) (*9, 10*) and the single RBD of hnRNP C have been determined and are very similar (Fig. 2) (*11*). The βαββαβ secondary structural elements of these RBDs form a four-stranded antiparallel β sheet packed against the two perpendicularly oriented α helices. Amino acids of RNP1 and RNP2 are juxtaposed on the two central β strands (β3 and β1) of the folded domain (*9–11*). These structures manifest two roles for conserved amino acids. Charged and aromatic side chains of RNP1 and RNP2 are solvent exposed and make direct contact with bound RNA, probably through hydrogen bonds and ring stacking (*9, 12, 13*). A second role is structural; the aromatic side chain at the last position of RNP1, for example, points to the interior of the folded domain and, along with other highly conserved hydrophobic amino acids in the two α helices, forms part of the hydrophobic core of the domain (*9–11*). These interactions are crucial for positioning the two α helices relative to each other and to the β sheet and constitute the major structural determinant of the RNP motif RBD (*9–11*). Other structural features include the pronounced right-handed twist of the β sheet, a very small antiparallel β sheet between α2 and β4 with a type I′ turn (*14*), and bulges in β1 and β4, all of which are likely to be common to most RNP motif RBDs (*6, 15*).

The structure of the RNP motif RBD shares features with several other RNA-binding proteins (*10, 16*) and even with proteins not thought to bind RNA. In fact, a prediction of RNP motif structure, based on similarity to acylphosphatase, was remarkably accurate (*17*). An RNP1 consensus sequence was found in the bacterial nucleic acid–binding cold shock protein (Csp) and, although the fold of Csp is different from the RNP motif RBD, the RNP1 segment forms the central strand of a three-stranded β sheet (*18*), as it does in the RNP motif RBD.

RNA-binding studies indicate that three distinct structural elements of the RBD probably contact RNA: the β sheet, the loops connecting the strands of the sheet, and the contiguous NH_2- and COOH-terminal regions of the RBD (Figs. 1 and 2) (*7, 12, 13, 19, 20*). Highly conserved amino acids of RNP1 and RNP2, although crucial for RNA binding, probably do not distinguish between different RNA sequences. Major determinants of RNA-binding specificity reside in the most variable regions of the RNP motif, particularly in the loops and the termini. For example, the replacement of the amino acids connecting β2 and β3 of U1 A (loop 3) with the analogous loop of U2 B″ confers U2 B″ RNA-binding specificity to the hybrid protein (*19*). The RNA-binding specificities of U1 70K, U1 A, and hnRNP C are highly dependent on amino acids immediately COOH-terminal to their RNP motif (*7, 13, 20*). Taken together, these observations suggest that the β sheet of the RBD, which contains many of the most highly conserved residues of the domain, constitutes a general RNA-binding surface to which distinct

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and variable determinants of specificity are added. Furthermore, they provide a rationale for how different proteins of this family can bind such diverse RNA molecules specifically, even though they contain many highly conserved elements.

Overall, RNP motif proteins have a modular structure reminiscent of transcription factors; they can contain several RBDs and auxiliary domains rich in amino acids such as glycine, glutamine, proline, or RS dipeptides (*2, 5*). Many multi-RBD proteins (for example, hnRNP A1, PABP, U2AF^{65}, and SF2/ASF) require contiguous RBDs for wild-type RNA-binding specificity, and the amino acids that connect them (that is, the terminal regions of the RBDs) are often highly conserved between homologous proteins, which suggests that they may make sequence-specific RNA contacts (*21–23*). Thus, the overall RNA-binding properties of multi-RBD proteins are not simply the sum of activities of the individual RBDs. Some multi–RNP motif proteins can bind different RNA sequences simultaneously, a property first suggested by the independent conservation of individual RBDs (*4*). U1 A, for example, binds to U1 snRNA through its first RBD and to pre-mRNA sequences through its second RBD (*24*).

The structure of the RNP motif RBD when bound to RNA is nearly identical to the unbound structure (Fig. 1) (*13*). The exposed β sheet RNA-binding surface therefore engages RNA as an open platform rather than buries the RNA in a binding crevice (*13*). Bound RNA remains relatively exposed and potentially accessible for interaction with other RNA sequences or RNA-binding proteins (*13*). Consistent with this interpretation, many RNP motif proteins, and even a single RBD by itself, can promote annealing of complementary nucleic acids (*25, 26*). Such activity could dramatically influence overall RNA structure and may be akin to chaperone activity (*26*).

The presence of RNP motif proteins in different cellular compartments and the enormous diversity of their RNA ligands suggest that this family of proteins performs many different functions. RNA-binding experiments demonstrate that RNP motif proteins can bind RNA with a wide range of affinities and specificities. When bound to high-affinity binding sites [dissociation constant (K_d) ≈ 10^{-11} to 10^{-8} M] they can, like transcription factors, promote or hinder the formation of specialized complexes (for example, spliceosomes). U2 auxiliary factor (U2AF^{65}, with three RNP motif RBDs) binds to the branchpoint-polypyrimidine tract region at the 3′ end of introns and promotes binding of U2 snRNP to the pre-mRNA (*22*). Binding of U2 snRNP to pre-mRNA can also be negatively regulated by RNP motif proteins, such as those encoded by *Drosophila* Sex lethal (Sxl, with two RNP motif RBDs), that bind to pre-mRNA near the U2AF binding site and precludes U2AF binding (*27, 28*). Replacement of the auxiliary domain of Sxl with that of U2AF^{65} confers U2AF^{65} activity to the chimeric protein (*28*), further illustrating the modular nature of RNP motif proteins.

Interactions between stably bound RNP motif proteins could also profoundly affect RNA structure by bringing together disparate RNA sequences (*26, 29*). In other cases, RNP motif proteins bind RNA with high affinity to form a stable structure that functions as a ribonucleoprotein particle (for example, snRNP particles). Those RNP motif proteins in which there is an abundance of the protein over high-affinity binding sites (for example, the major hnRNP proteins) will also bind RNA with lower affinity (K_d ≈ 10^{-7} to 10^{-6} M) and less regard to sequence specificity (*23*). The likely functions of this mode of binding are to prevent the formation of higher-order RNA structures that might otherwise interfere with RNA-processing reactions (*30*) and to facilitate interactions with trans-acting factors (complementary RNAs and proteins) by protein-protein interactions and chaperone activity (*26, 29*). Furthermore, lower-affinity binding may also serve to reduce the dimensionality of diffusion, thus accelerating binding to high-affinity sites (*2*).

Important physiological roles for RNP motif proteins have been discerned from loss of expression or mutations in RNP proteins that result in developmental disorders in humans and other organisms. Chromosomal deletions of genes encoding RNP motif proteins result in azoospermia (reduced or complete lack of sperm production) in humans and *Drosophila*, indicating that some essential developmental regulation of spermatogenesis is posttranscriptional (*31*). The sex determination and maintenance pathway in *Drosophila* includes multiple gender-specific alternative splicing reactions that are regulated by RNP motif proteins (for example, tra2 and Sxl; see above) [reviewed in (*32*)]. The establishment of the dorsoventral axis of the *Drosophila* oocyte requires an hnRNP protein (hrp40) encoded by the squid gene to prop-

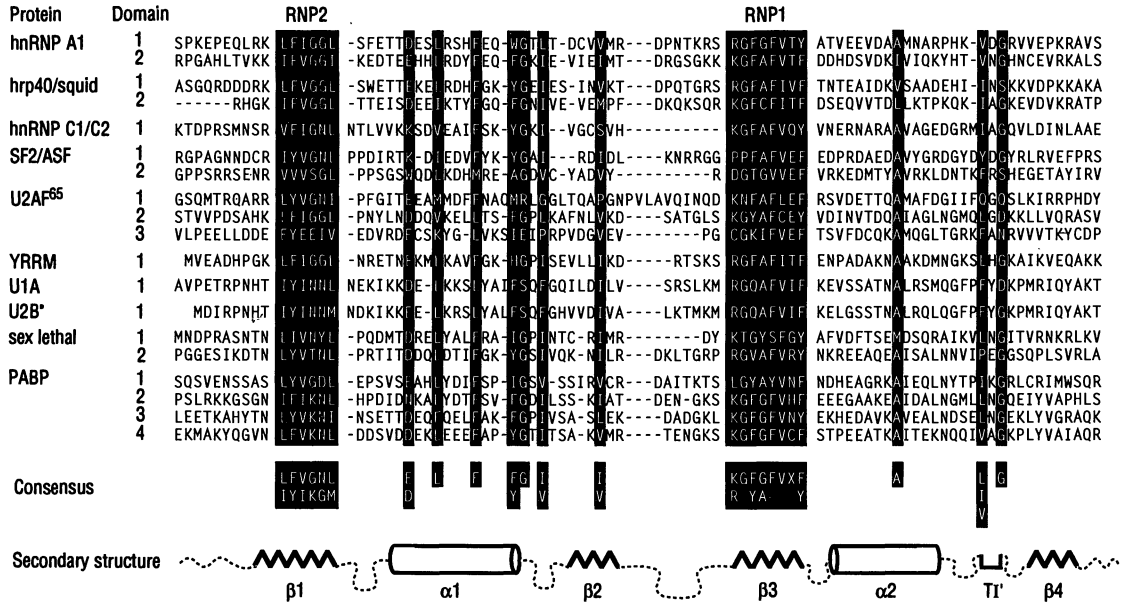

Fig. 1. The RNP motif. Amino acid sequences of the indicated (left) RNP motifs are aligned, and conserved residues are highlighted. The RNP1 and RNP2 consensus sequences are indicated at the top. The consensus RNP motif, as well as an alignment of the secondary structural elements [four β sheets (β1–4), two α helices (α1–2), and type I′ (TI′) tight turn] that constitute each RBD (using the structures of the hnRNP C and U1 A RBDs as a guide) are listed below (secondary structure). Note that the motif consensus sequence may not be accurately reflected in the small subset of proteins presented in each motif figure. Proteins listed are identified in Table 1 except U2 B″ (human U2 snRNP protein). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

erly localize the mRNA encoding dorsalizing morphogen gurken in the developing oocyte cytoplasm (*33*). Mutations in the *Drosophila* gene *elav* (with three RNP motif RBDs) affect the development of young neurons and reveal an essential role in the maintenance of mature neurons (*34*). For reasons not yet understood, RNP motif proteins, especially constituents of RNP complexes (for example, snRNP and hnRNP proteins), are common targets for autoimmune response, especially in individuals with systemic lupus erythematosus (*35*).

The Arginine-Rich Motif (ARM)

Short (10 to 20 amino acids) arginine-rich sequences in viral, bacteriophage, and ribosomal proteins mediate RNA binding (Fig. 3). Other than the preponderance of arginine residues, there is little identity between ARM sequences (*36*), and the structure of the ARM regions of two proteins, Tat and Rev, are diverse. Most ARM proteins, therefore, probably do not share a common structure. Detailed structure-function information is available for human immunodeficiency virus (HIV) Rev, a regulatory RNA-binding protein that facilitates the export of unspliced HIV pre-mRNAs from the nucleus by an unknown mechanism (*37*). Rev binds with highest affinity ($K_d \approx 1 \times 10^{-9}$ M) to an internal bulged loop (Rev responsive element, RRE) found in all intron-containing viral mRNAs (*37–40*). Another HIV-encoded ARM protein, Tat, binds trans-acting responsive element (TAR, $K_d \approx 5 \times 10^{-9}$ M) (*41–44*) of HIV mRNAs and functions in transcription [reviewed in (*45*)]. Both of these ARM proteins are required for HIV replication [reviewed in (*46*)]. In vitro RNA-binding studies indicate that peptides containing the ARM region of Rev or Tat exhibit RNA-binding properties consistent with that of their complete proteins, but also that amino acids outside their ARM contribute to binding (*43, 47–54*). Peptides encompassing the Rev ARM (17–amino acid peptides) specifically bind RRE as an α helix, and at least six amino acids, including four arginines, are essential for specificity (*54*). In contrast, Tat ARM peptides are unstructured but adopt a stable (though noncanonical and uncharacterized) conformation upon binding TAR (*49*). Amino acids outside ARM are required for wild-type binding activity of other ARM proteins (*36*), indicating that a full understanding of the structure and RNA-binding properties of ARM domains will require consideration of the rest of the protein.

The RNA binding sites of ARM proteins are complex and consist of stem-loops (N proteins), internal loops (Rev), or bulges (Tat), and their structure, rather than particular sequence, may be the major binding determinant (*37, 39, 40, 42, 47, 49, 55, 56*). This is best illustrated by the Rev-RRE interaction. A critical component of RRE is an unusual G:G base pair (*40, 56*); however, this feature can be replaced with an isosteric A:A base pair, suggesting—if one considers the different protein-RNA hydrogen bonding potential of these base pairs—that an important determinant of high-affinity Rev binding is the RNA phosphoribose backbone (*56*). This type of interaction is also an important component of Tat binding (*50*), and in addition both Tat and Rev probably hydrogen bond with RNA bases (*40, 44, 57*). RNA-binding and modeling studies suggest that the bulged nucleotides of TAR and RRE distort the deep major groove of the RNA, thereby allowing access to required hydrogen bonding atoms (*40, 52, 57*). A further source of binding site complexity comes from protein-induced conformational changes in the RNA that, at least in the Tat-TAR case, involve RNA-RNA interactions (*57*). In summary, the RNA determinants of specific ARM protein binding include the phosphoribose backbone, bases, and RNA conformational changes.

These studies, as well as others described below, point to two general roles for arginine in RNA binding. First, the positive charge of arginine increases nonspecific affinity for RNA, thereby facilitating the search for high-affinity binding sites. In this context, the role of arginine may be to probe the local conformation of the RNA backbone, in search of the RNA determinants of high-affinity binding (*50, 52, 54*). A second function is to make specific hydrogen bonding networks with the RNA sugar-phosphate backbone and bases (*40, 43, 44, 50, 56*). This mode of RNA binding emphasizes the important role of RNA structure and may explain why arginine is the predominant basic residue of this motif; arginine has more potential hydrogen bonding atoms as compared with lysine and can therefore specifically accommodate a greater repertoire of RNA structures.

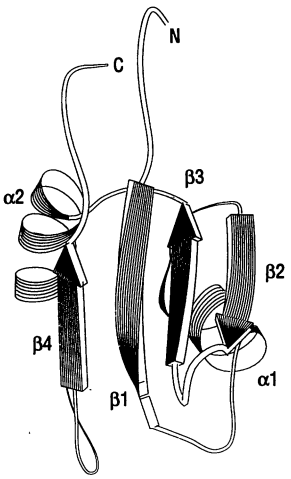

Fig. 2. Structure of the RNP motif RNA-binding domain of the pre–mRNA-binding protein, hnRNP C. Each arrow represents a β strand and the curled ribbons an α helix. This view emphasizes the four-stranded β sheet RNA-binding surface. The highly conserved RNP1 and RNP2 consensus sequences, which participate in making direct contact with the RNA, are juxtaposed on the central β3 and β1 strands, respectively. N, NH_2-terminus; C, COOH-terminus.

Protein	RNA-binding domain
HIV Rev	TRQARRNRRRRWRERQ
HIV Tat	ALGISYGRKKRRQRRRP
λ N	MDAQTRRRERRAEKQAQW
φ21 N	GTAKSRYKARRAELIAER
P22 N	GNAKTRRHERRRKLAIER

Fig. 3. The arginine-rich RNA-binding motif. The sequences of the minimal RNA-binding domains of the proteins listed are given with arginine residues highlighted. Amino acids outside each of the RBDs listed are also required for wild-type RNA-binding activity. Proteins listed are HIV Rev and Tat; and λ, φ21, and P22 bacteriophage antiterminator N proteins. See text and Table 1 for references.

The RGG Box

The RGG box, initially identified as an RNA-binding domain in hnRNP U, is a 20– to 25–amino acid long RNA-binding motif typically found in combination with other types of RNA-binding domains (*58*). The motif is defined as closely spaced Arg-Gly-Gly (RGG) repeats interspersed with other, often aromatic, amino acids (Fig. 4). The minimal number of RGG repeats necessary for RNA-binding is not known; some proteins have as few as six (hnRNP A1), whereas others have up to 18 (yeast GAR1) (*59*). The high density of glycine and variations within the motif suggest that it is not a rigid protein structure, but spectroscopic analysis and molecular modeling of nucleolin RGGF repeats predict a helical β-spiral structure (*60*).

RGG boxes usually occur in proteins that also contain other types of RBDs. In nucleolin (with four RNP motifs and an RGG box), specific binding to pre-ribosomal RNA requires the four RNP motifs, and the RGG box region increases overall RNA affinity 10-fold (*61*). This suggests that RNA binding by the RGG box is relatively sequence nonspecific and that it facilitates binding by one or more RNA-binding domains. However, sequence-specific RNA binding by the RGG box should not yet be discounted because the RGG box region of hnRNP U, which is the only apparent RNA-binding element of this protein, can discriminate between different

RNA sequences (*58*). Specific RNA binding by ARM peptides further suggests that arginine-rich RNA-binding domains can bind RNA with specificity.

If an important role for arginine in the RGG box is to increase general RNA-binding affinity (solely as a result of positive charge density), it is not apparent why arginine should be used exclusively. One reason may be the greater hydrogen bonding potential of arginine as compared with lysine, but another clue may come from the fact that many RGG box–containing proteins contain the modified amino acid N^G,N^G-dimethylarginine (*62*). The significance of this posttranslational modification is not known, but one possible function could be to regulate the RNA-binding activity of RGG box proteins. Methylation would not affect the strong positive charge of the arginine side chain, but it could, by steric constraints, modulate RNA binding (*50*). Phosphorylation of adjacent residues is another potential mechanism for regulating RGG box RNA binding (*63*).

The KH Motif

The most recent addition to the collection of motifs in RNA-binding proteins, the K homology (KH) motif, was identified in the human hnRNP K protein (*64*). Protein family members include ribosomal S3 proteins from divergent organisms such as archaebacteria, the yeast alternative splicing factor, Mer1p, and several human RNA-binding proteins (*64, 65*) (Fig. 5). All KH motif proteins of known function are associated with RNA and many bind RNA in vitro (*65–67*). The definitive role of the KH motif in RNA binding is not known, but recent observations demonstrate that it is essential for RNA binding and probably binds RNA directly. Mutation of highly conserved residues impairs the binding of several KH motif proteins to single-stranded nucleic acids in vitro (*67*), and a mutation (underlined) in the core sequence, VIGXXGXXI, of one of the two KH motifs encoded by the fragile X mental retardation gene (FMR-1) causes fragile X mental retardation (*68*). Like many other RNA-binding motifs, KH motifs are found in one or multiple copies [14 copies in chicken vigilin (*65*)] and, at least for hnRNP K (three KH domains) and FMR-1 (two KH domains), each motif is necessary for in vitro RNA binding activity, suggesting that they may function cooperatively or, in the case of single KH motif proteins (for example, Mer1p), independently (*67*). Experiments with hnRNP K have demonstrated that this protein also binds single-stranded DNA (ssDNA) (*69*) and that its KH motifs are also required for this binding (*67*). The three-dimensional structure of the KH motif is not known.

The widespread presence of KH motifs in diverse organisms suggests that it is an ancient protein structure with important cellular functions. In yeast, the product of the MER1 gene contains a single KH motif and is required for developmentally regulated alternative pre-mRNA splicing (*70*). An exciting link between cognitive function and RNA-protein interaction was established by the recent discovery of two KH domains (and an RGG box) in the human fragile X mental retardation syndrome gene, FMR-1 (*66, 71*). This disorder, one of the most common human genetic diseases and the most common form of hereditary mental retardation, is usually caused by a loss of FMR-1 expression (as a result of the amplification of CGG repeats) (*72*). FMR-1 has been shown to be an RNA-binding protein (*66*), and a severely affected individual was described who expresses an FMR-1 protein with a mutation in a conserved KH motif residue (Fig. 5) (*68*). This mutant protein is impaired in RNA-binding properties (*67*), further strengthening the connection between RNA binding and FMR-1 function. A neural-specific protein with three KH motifs, Nova, is required for the development of the motor nervous system, and autoantibodies to Nova cause paraneoplastic opsoclonus-ataxia (*73*). The cognate RNAs for FMR-1 and Nova have not yet been identified.

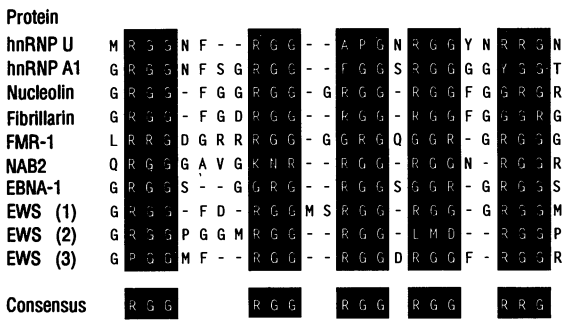

Fig. 4. The RGG box. Legend as in Fig. 1. Proteins listed are identified in Table 1 except EBNA-1 (Epstein-Barr virus nuclear antigen–1).

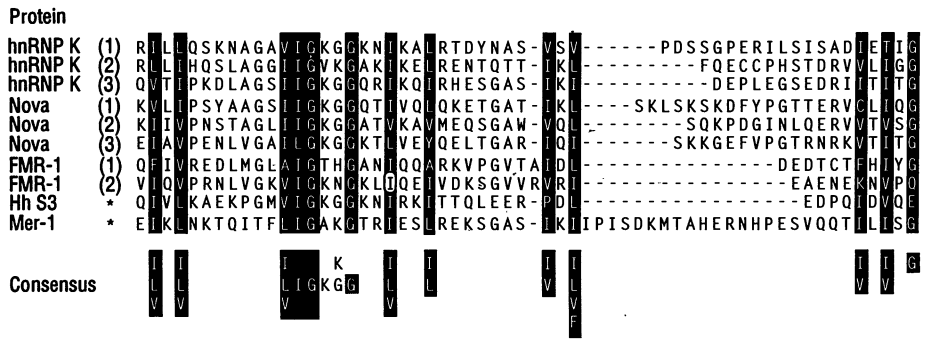

Fig. 5. The hnRNP K homology (KH) motif. Legend as in Fig. 1. The circled isoleucine in repeat 2 of FMR-1 is mutated to asparagine in a severely affected patient (*68*). Proteins listed are human hnRNP K; Nova, paraneoplastic opsoclonus–ataxia-associated Ri antigen; FMR-1, fragile X–mental retardation gene product; Hh S3, *Halobacterium halobium* S3 ribosomal protein; Mer-1, *S. cerevisiae* alternative pre-mRNA splicing factor. See text and Table 1 for references.

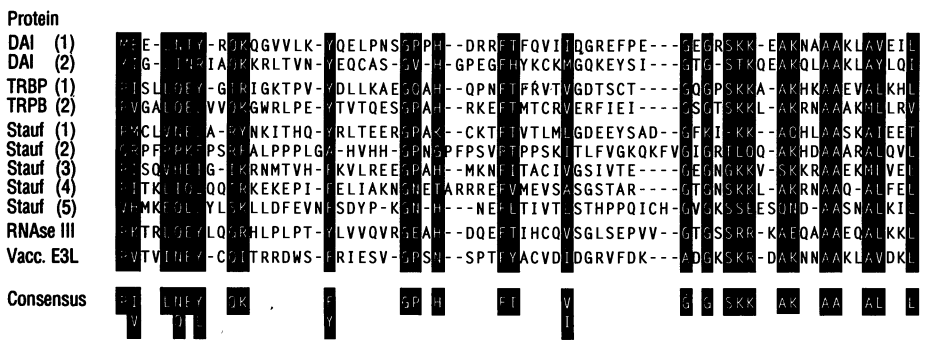

Fig. 6. The double-stranded RNA-binding motif. The legend is the same as for Fig. 1. Proteins listed are identified in Table 1 except TRBP (human HIV-1 TAR-binding protein), Stauf (*D. melanogaster staufen* gene product), and *Escherichia coli* RNAse III.

The Double-Stranded RNA-Binding Motif (DSRM)

A growing list of RNA-binding proteins have in common one or more copies of an approximately 70–amino acid region that binds double-stranded RNA (dsRNA) (*74–76*). Conserved positions, including many basic (both arginine and lysine) and hydrophobic amino acids (Fig. 6), are scattered throughout the DSRM; the motif will likely need to be refined in the future because only a relatively small number of DSRM proteins are currently known. RNA-binding experiments demonstrate that mutations of nearly each of the conserved residues (Fig. 6) affect RNA binding (*74–78*), and genetic data argue that at least some DSRM proteins bind unique RNA sequences. Because DSRM proteins do not bind dsDNA, it will be informative to compare the manner in which they bind dsRNA to that of proteins that bind dsDNA (dsRNA forms A helices and dsDNA forms B helices).

DSRM proteins are involved in diverse cellular functions and provide further examples of posttranscriptional gene regulation by RNA-binding proteins. In the developing *Drosophila* oocyte, the gene product of staufen (five DSRMs) is required to properly localize bicoid and oskar mRNAs, an activity that is essential for anterior-posterior axis formation (*79*). An important component of the response of mammalian cells to viral infection is the interferon-induced protein kinase (DAI) [reviewed in (*80*)]. This enzyme contains two DSRMs, binds dsRNA, and shuts off host protein synthesis when activated by dsRNA (viral RNA). In an apparent attempt to abrogate this host response, some viruses (for example, vaccinia and rotavirus) encode DSRM proteins that block the activation of DAI (*74, 76, 81*). Gene expression can also be up-regulated by DSRM proteins; a cellular protein containing two DSRMs binds the TAR RNA element of HIV mRNAs and may influence Tat-mediated activation (see above) (*77*).

Other RNA-Binding Domains

A small number of RNA-binding proteins, including retroviral nucleocapsid proteins, RNA polymerases, and yeast RNA-binding proteins, contain sequences (appropriately spaced cysteine-histidine residues) that relate these proteins to the zinc finger family of DNA-binding proteins. A generalized zinc finger–knuckle motif can be written as $CX_{2-5}CX_{4-12}C/HX_{2-4}C/H$ (in which X represents any amino acid). The best characterized sample is TFIIIA, a nine–zinc finger protein that binds both the 5S rRNA gene and 5S RNA. The middle three fingers are primarily responsible for RNA binding, and there is no obvious difference between their amino acid sequences when compared to the predominant DNA-binding zinc fingers (three NH_2-terminal fingers) (*82*). Furthermore, a highly related 5S RNA-binding protein (42Sp43) with the same modular structure as TFIIIA does not reveal any obvious amino acid differences that might explain its restricted nucleic acid–binding activity (*83*). The amino acid sequences of several eukaryotic transcription factors (Y-box proteins) are related to the bacterial cold shock domain (*20, 84*), and these proteins may have dual roles as DNA- and RNA-binding proteins (*85*). It remains to be seen how widespread this motif, whose structure is reminiscent of the RNP motif RBD, is in eukaryotes.

Table 1. Physiological roles of RNA-binding proteins.

Motif	Proteins	Function/disorder	Reference
RNP motif	hnRNP A1	mRNA biogenesis	(*2*)
	hnRNP C1/C2	mRNA biogenesis	(*2*)
	mRNP PABP	Translation/mRNA stability	(*89*)
	snRNP U1A	Pre-mRNA splicing/polyadenylation	(*24, 90*)
	$U2AF^{65}$	Pre-mRNA splicing	(*22*)
	SC35	Pre-mRNA splicing	(*91*)
	SF2/ASF	Pre-mRNA splicing	(*92*)
	Sex lethal	Alternative pre-mRNA splicing	(*32*)
	CStF	Polyadenylation	(*93*)
	YRRM	Azoospermia	(*31*)
	Hrp 40/squid	Dorsoventral axis formation	(*33*)
Arginine-rich	HIV Rev	HIV (pre-) mRNA export	(*37–40, 54–56*)
	HIV Tat	Transcription trans-activator	(*41–45, 47–50, 52, 57*)
	Bacteriophage N proteins	Transcription antitermination	(*36*)
RGG box	hnRNP U	mRNA biogenesis	(*58*)
	Nucleolin	rRNA biosynthesis	(*94*)
	EWS	Ewing's sarcoma translocation	(*95*)
KH motif	FMR-1	Fragile X mental retardation syndrome	(*66, 67, 71*)
	Mer-1	Alternative pre-mRNA splicing	(*70*)
	Ribosomal S3	Translation	(*64, 65*)
	hnRNP K	mRNA biogenesis	(*69*)
dsRNA-binding	Staufen	Anterior/posterior axis formation	(*79*)
	DAI kinase	Translation regulation	(*75, 80*)
	Vaccinia E3L	Translation regulation	(*81*)
Zinc finger/knuckle	TFIIIA	Pol III transcription/5S rRNA transport?	(*82*)
	NAB2	mRNA biogenesis	(*96*)
Cold shock domain	Csp	Transcription	(*18*)
	mRNP p54, p56	Transcription/mRNA storage	(*84, 85*)
Metabolic enzymes	IRE-binding protein (aconitase)	Translation/mRNA stability	(*86*)
	Thymidylate synthetase	Translation regulation	(*87*)

Finally, it should be noted that some functionally related RNA-binding proteins have conserved amino acid sequences that have so far not turned out to be as widespread as the motifs discussed above. Most tRNA synthetases, for example, have motifs that distinguish related groups of synthetases and whose amino acids directly contact RNA. Along this same line of reasoning, several metabolic enzymes bind RNA specifically, affecting translation or mRNA stability (for example, aconitase and iron response element RNA, and thymidylate synthetase and its own mRNA), and these interactions could serve to regulate certain aspects of metabolism (86–88). It can be expected that with more information additional motifs will be discovered.

Summary and Perspectives

The RNA-binding motifs described here provide extremely useful tools for recognizing and classifying RNA-binding proteins. Few representative structures of RNA-binding motif proteins are available, so it is not yet possible to outline principles by which a particular protein binds RNA. Obviously, knowledge of the structures of other RNA-binding domains and especially RNA-protein complexes will be essential for understanding these interactions, and many such studies are underway.

The recent discoveries of RNA-binding motifs in numerous and diverse RNA-binding proteins underscore the plethora of RNA-protein interactions along the pathway of gene expression. Much progress has been made in elucidating the functions of RNA-binding proteins (Table 1). They can facilitate or hinder the formation of specialized complexes at particular sites on the RNA by protein-protein interactions. They can also directly modify RNA structure, either locally (the conformation of bound RNA) or globally (RNA secondary and tertiary structure). These activities can have important and far-reaching consequences, including influence on the interaction of sense RNA with antisense RNAs and on RNA catalysis. In addition, RNA-binding proteins can serve as structural components and form stable RNP particles, and they may serve to transport and localize RNAs. Genetic disorders caused by mutations in RNA-binding proteins provide valuable insight for new experiments that are certain to expand our understanding of their functions. Finally, one of the exciting conclusions to emerge is that RNA-protein interactions constitute the foundation of the essential pathways of mRNA biogenesis, animal development, sexual differentiation, and at least one aspect of human intelligence.

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Arctic Ocean Gravity Field Derived From ERS-1 Satellite Altimetry

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The derivation of a marine gravity field from satellite altimetry over permanently ice-covered regions of the Arctic Ocean provides much new geophysical information about the structure and development of the Arctic sea floor. The Arctic Ocean, because of its remote location and perpetual ice cover, remains from a tectonic point of view the most poorly understood ocean basin on Earth. A gravity field has been derived with data from the ERS-1 radar altimeter, including permanently ice-covered regions. The gravity field described here clearly delineates sections of the Arctic Basin margin along with the tips of the Lomonosov and Arctic mid-ocean ridges. Several important tectonic features of the Amerasia Basin are clearly expressed in this gravity field. These include the Mendeleev Ridge; the Northwind Ridge; details of the Chukchi Borderland; and a north-south trending, linear feature in the middle of the Canada Basin that apparently represents an extinct spreading center that "died" in the Mesozoic. Some tectonic models of the Canada Basin have proposed such a failed spreading center, but its actual existence and location were heretofore unknown.

Persistent sea ice has kept surface research ships out of much of the Arctic Ocean. As a consequence, our understanding of Arctic Ocean Basin geology and geophysics lags well behind that of the other major ocean basins (*1*). On the basis of relatively abundant aeromagnetic surveys (*2*, *3*) plus a limited amount of surface data (*4*), an uncertain tectonic history has been constructed for the oceanic Arctic Basin (*5*). Over ice-free oceans, data from satellite radar altimeters have been used with great success (*6*, *7*) to determine marine gravity fields. These fields can help in determining the tectonic history of the ocean floor (*8*). Previous altimeter missions have covered areas below 72°N, but the ERS-1 satellite provides observations up to 82°N covering a large area of the Arctic Basin for the first time. The Arctic Basin is generally divided into two sub-basins: the Eurasia Basin and the Amerasia Basin, separated by the Lomosonov Ridge. The Amerasia Basin, whose tectonic history is particularly uncertain, is mostly covered by ERS-1 altimeter observations of sea ice. The presence of sea ice introduces unacceptable errors into the height estimate that is computed on board the satellite (*9*). In this article we present a gravity field for the Arctic Ocean, derived from data that have been corrected for on-board errors by reprocessing. We further validate our gravity field using surface observations and discuss the tectonic implications.

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Tectonic History

Knowledge of the tectonic history of the Arctic Basin is key to understanding the role of the Arctic Ocean in past climate change. An accurate Arctic tectonic history is also needed to ensure accurate global tectonic models and to understand the geology of the Arctic continental margins (*10*).

The Arctic Basin is generally divided into two parts, each with a distinct plate-tectonic history: The smaller Eurasia Basin, which lies between Eurasia and the Lomonosov Ridge, was formed by sea floor spreading on the Arctic (Nansen) mid-ocean Ridge likely beginning 55 million years ago (Ma) (*11*). The Amerasia Basin (Fig. 1) represents the remaining two-thirds of the Arctic Basin, which probably formed in late Neocomian to Late Cretaceous time (~130 Ma) (*3*, *4*), and has an uncertain tectonic history (*2*, *5*). Of the many tectonic models proposed for the early development of the Amerasia Basin, the most widely held involve arctic Alaska rifting away from the Canadian arctic islands in the Mesozoic and rotating to its present position. This rotation is thought to have been accompanied by sea floor spreading that produced the ocean crust of the southern and central Canada Basin. However, the locus or axis of such sea floor spreading had not been found. Taylor and colleagues (*3*), on the basis of aeromagnetic profiles, speculate that an extinct spreading axis was located roughly in the middle of the southern Canada Basin. However, data from aeromagnetic surveys have since been considered inadequate to resolve this problem. Another possible source of the spreading is the Mendeleev–Alpha Cordillera Ridge (*2*).