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MINIREVIEW

The double-stranded RNA-binding motif, a versatile macromolecular docking platform

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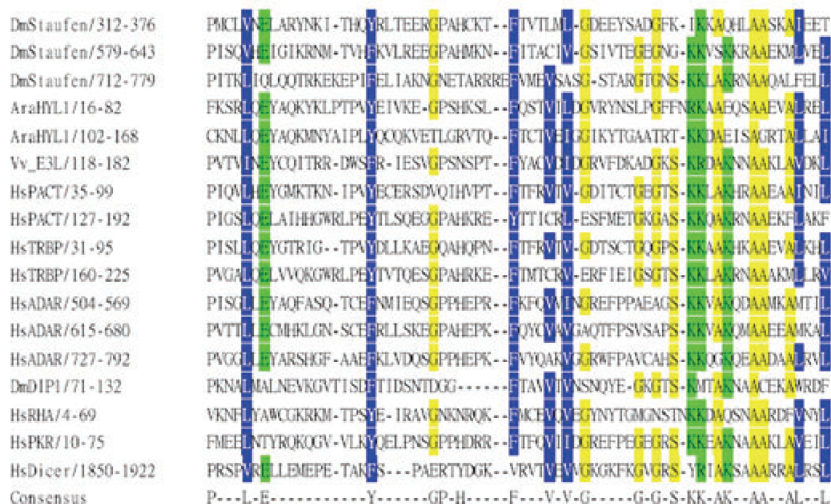
The double-stranded RNA-binding motif (dsRBM) is an αβββα fold with a well-characterized function to bind structured RNA molecules. This motif is widely distributed in eukaryotic proteins, as well as in proteins from bacteria and viruses. dsRBM-containing proteins are involved in processes ranging from RNA editing to protein phosphorylation in translational control and contain a variable number of dsRBM domains. The structural work of the past five years has identified a common mode of RNA target recognition by dsRBMs and dissected this recognition into two functionally separated interaction modes. The first involves the recognition of specific moieties of the RNA A-form helix by two protein loops, while the second is based on the interaction between structural elements flanking the RNA duplex with the first helix of the dsRBM. The latter interaction can be tuned by other protein elements. Recent work has made clear that dsRBMs can also recognize non-RNA targets (proteins and DNA), and act in combination with other dsRBMs and non-dsRBM motifs to play a regulatory role in catalytic processes. The elucidation of functional networks coordinated by dsRBM folds will require information on the precise functional relationship between different dsRBMs and a clarification of the principles underlying dsRBM–protein recognition.

Introduction

The double stranded RNA-binding motif (dsRBM) was first identified by comparing several regions of high sequence similarity within the staufen and *Xenopus laevis* RNA-binding protein A (XlrbpA) proteins against the protein sequence database [1] (Fig. 1). The chief function of this αβββα fold [2,3] is to bind structured RNA molecules [4], but other targets, mainly proteins, have also been identified. Indeed, a significant versatility exists in the role of the motif within the dsRBM-containing proteins found in the cytoplasm and the nucleus of eukaryotic cells as well as in bacteria and viruses [5]. These proteins are involved in processes ranging from RNA editing to protein phosphorylation in translational control, and some of these functions are summarized briefly in Table 1.

dsRBM-containing proteins possess a variable number of copies of the domains (up to a maximum of five in *Drosophila melanogaster* staufen) and can be

Abbreviations
dsRBM, double-stranded RNA-binding motif; ADAR1 and 2, dsRNA dependent adenosine deaminases 1 and 2; CTE, constitutive transport element; DIP1, disco interacting protein 1; GAG, group-specific antigen; HIV, human immunodeficiency virus; HYL1, human microsomal epoxic hydrolase; NC, nucleocapside; NF90, nuclear factor 90; NS1, non-structural protein 1; PACT, protein activator; PKR, RNA-dependent protein kinase; R2D2, two dsRBM-containing protein associated with Dicer2; RDE4, RNAi-deficient 4; RHA, RNA helicase A; TAR, transactivator RNA; TRBP, TAR RNA-binding protein; VA RNA, adenovirus associated RNA; Rnt1p, RNAseIII 1 protein; xlrbpA, *Xenopus laevis* RNA-binding protein A.



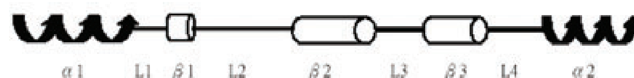


Fig. 1. (A) Sequence alignment for a set of dsRBMs along a cartoon for the basic αβββα fold (B) that dsRBM adopts. The alignment was generated by CLUSTALW [60] and manually optimized. The species of origin, name and residues numbers of the selected dsRBM sequences are listed in the left and the conserved small, charged and hydrophobic residues are highlighted in yellow, green and blue, respectively. (Dm, *Drosophila melanogaster*; Ara, *Arabidopsis thaliana*; Vv, vaccinia virus; Hs, *Homo sapiens*).

Table 1. A brief summary of the function of representative dsRBM-containing proteins.

dsRBP	Catalytic domain involved	Characteristic functions
RNaseIII/Dicer/Drosha [12–14]	RNase III domain[a]	dsRNA processing in RNAi/miRNA pathway
ADAR1 & 2 [10]	A → I deaminase domain	RNA editing
RHA [11]	DEXH helicase domain	Transcriptional coactivator
PKR [7–9]	Ser/Thr kinase domain	Translational control
TRBP/PACT/vvE3L [18–20]	None	PKR modulator
NF90 [17]	None	NFAT transcription factor
Staufen [15]	None	RNP localization
XlrbpA [16]	None	RNP localization and dsRNA annealing
R2D2/RDE4/HYL1 [23–25]	None	Components in RNAi/miRNA pathways

[a] Dicer also possesses a helicase-like domain.

grouped into two categories depending on whether the protein also harbours a catalytic domain [6]. A partnership with a catalytic domain is observed in the RNA-dependent protein kinase (PKR) that contains a serine/threonine kinase domain [7–9] and in the dsRNA-specific adenosine deaminases ADAR1 and 2 that covalently modify dsRNA substrates to convert adenosine residues to inosine [10]. The dsRBM is also found in RNA helicase A (RHA), which has a DEXH helicase domain for the unwinding of duplex of nucleic acids [11], and in RNaseIII and the related Dicer/Drosha enzymes of the RNAi/miRNA pathway, which carry an RNase domain for dsRNA cleavage and processing [12–14].

A second group of dsRBM-containing proteins do not carry an identifiable catalytic domain. Examples are the staufen and Xlrbpa proteins that play roles in RNP localization [15,16], the transcription-related nuclear factor 90 (NF90) family [17] and the three modulators of PKR activity, *trans*-activation region (TAR)-RNA-binding protein (TRBP), protein activator (PACT) and vaccinia virus E3L protein [18–20]. New additions to this group include the Disco interacting protein 1 (DIP1) [21,22] and the RNAi/miRNA pathways-related RDE4/R2D2/HYL1 proteins [23–25].

Initially this review will analyse the molecular basis of dsRBM recognition of structured RNAs. It will then describe the function that dsRBM performs in different proteins and explore the potential of this motif as a versatile platform for protein–RNA and protein–protein interactions. Finally we will discuss the role of multiple dsRBMs in regulating protein activity.

Recognition of the A-form RNA helix by the dsRBM

The dsRBM was originally associated with the ability to recognise double stranded RNA (dsRNA) [1]. However this ability is not universal to all dsRBMs. For example, only the first, third and fourth dsRBMs (dsRBM1, dsRBM3 and dsRBM4) of *Drosophila* staufen bind to dsRNA *in vitro* [26]. Furthermore, the dsRNA-binding affinity varies significantly within the ensemble of RNA-binding dsRBMs. In PKR, dsRBM1 is found to have higher *in vitro* dsRNA-binding affinity than dsRBM2 although optimal dsRNA-binding *in vivo* will need cooperation of the two domains [27]. An early sequence alignment study has divided the dsRBM domains into type A and type B. Type A (e.g. dsRBM1 of PKR) harbours conserved residues along the whole length of the domain, while in type B (e.g. dsRBM2 of PKR) conservation is limited to the short carboxy terminal region [1]. Although type A dsRBM seems to bind RNA with higher affinity than type B in most cases, this correlation is not absolute as the dsRNA-binding affinities of dsRBM1 and 2 of DIP1 have been shown to be opposite to those predicted [21].

Early analysis of RNaseIII targets showed that the dsRNA sequence is not crucial for recognition, although sequence variations have an effect on affinity in some cases [28,29]. dsRBMs also showed an absolute requirement for the presence of A-form helical conformation in their cellular RNA targets. Biochemical data on the length of the required helix are somewhat heterogeneous and place it somewhere between 12 and 16 base pairs [30,31]. The effect of disrupting this helix varies greatly depending on the nature of disruptions, with some leading to a total impairment of the binding [32] while others only result in a small decrease of affinity [33].

The structure of the second dsRBM from XlrbpA bound to a non-physiological dsRNA molecule [34] provided a molecular insight in the dsRBM–dsRNA recognition process and a clue to the differences in dsRNA-binding capabilities of the variants of this motif. The XlrbpA protein binds across 16 RNA base pairs, interacting with successive minor, major and minor grooves (Fig. 2). The structure reveals that 2′OH groups and phosphate groups in the neighbouring minor and major grooves of the RNA duplex make contact with residues in the loops 2 and 4 of the αβββα fold. The contacts between side chains in the protein loops and the RNA described above are mostly water mediated, while the last RNA minor groove makes more heterogeneous contacts with

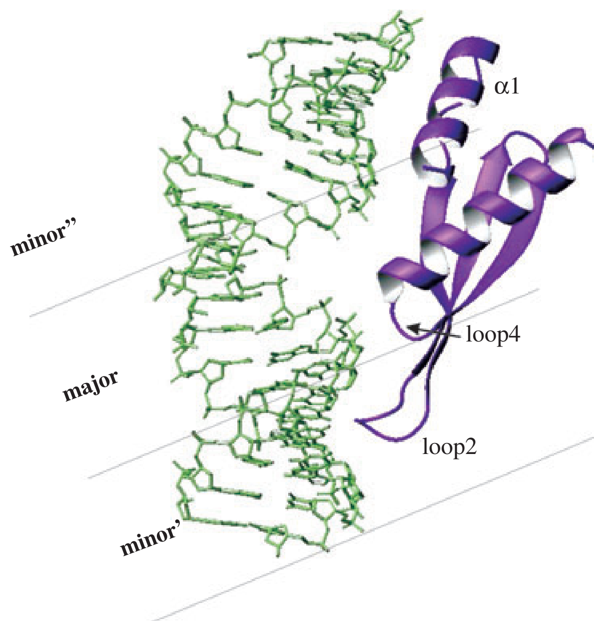


Fig. 2. MOLMOL ribbon representation [61] of the second dsRBM from *Xenopus laevis* RNA-binding protein A (magenta and grey) bound to a non-physiological dsRNA molecule (light green). Loop 2, loop 4 and helix 1 of the protein interact with three consecutive grooves on the RNA helix, that are here labelled as m (minor), M (major) and m (minor). The three regions of interaction are defined by black lines.

helix 1 of the fold, including few direct interactions with the RNA bases. Interestingly, the distance between loop 2 and loop 4 is constrained by their sandwiching of a conserved phenylalanine, and matches the spacing between the minor and major grooves along the RNA helix.

Two later structures of dsRBM–dsRNA complexes, the one of *Drosophila* staufen dsRBM3 in complex with an RNA hairpin [31] and the one of *Escherichia coli* RNaseIII dsRBM in complex with dsRNA [35], confirmed the existence of a common interaction pattern in dsRBM–dsRNA recognition. In both complexes, dsRBMs recognize the RNA A-form helix geometry via residues on loops 2 and 4, and this recognition is not related to direct reading of a specific RNA sequence as already observed in the XlrbpA–RNA interface. These amino acids are conserved within a number of type A dsRBMs [31,36] and their mutation impairs both RNA-binding [37–39] and protein function [31,39]. It is important to point out that the register of binding of RNaseIII to the RNA is different from the one of XlrbpA. In RNaseIII the dsRBM binds as a part of a large dimeric complex and rotates approximately one base pair along the RNA helix with respect to the register observed for

XlrbpA. In addition, the RNaseIII protein–RNA interface is more tightly packed than that of the XlbrpA–RNA. These observations indicate that the A-form RNA helices can be bound by two dsRBMs in similar but not identical fashion.

Furthermore, NMR and molecular dynamics studies show that a significant degree of flexibility exists in the staufen dsRBM–RNA interface. Initial NMR relaxation data detected high frequency motions in loops 2 and 4 of the bound staufen dsRBM3 protein [31]. This flexibility was rationalised in a molecular dynamics study by Castrignano and coworkers [40] where the positively charged lysine side chains of loops 2 and 4 do not make single, well defined interactions with the RNA groups, but rather switch between different polar interactions on a very fast timescale. The tolerance for non-identical positions of the negatively charged acceptor could explain the negligible effect of the small variations in the helix geometry associated with different sequences and possibly the tolerance observed towards small distortions caused by the introduction of unpaired nucleotides in the helix. The network of interactions mediating recognition may, however, not be able to accommodate more severe distortions that would then lead to loss of binding. The loss of dsRBM binding capability observed by *in vitro* assays would then be related to the severity of the distortion, as was originally postulated by Bevilacqua [33].

Selection of specific structured RNA targets

A crucial issue in the molecular understanding of dsRBM function is how the structural specificity for an RNA double helix is linked to the recognition of a precise cellular target. In some cases specificity could be achieved via the coordinated interaction of multiple copies of dsRBMs within a multidomain protein. Such an interaction could explain the cooperative dsRNA-binding observed for PKR and other multi-dsRBM-containing proteins. It is also possible that interaction with auxiliary domains may specify target recognition. For example, the first dsRBM of RHA needs to cooperate with a downstream disorder proline-rich domain to bind the retroviral constitutive transport element (CTE) RNA efficiently [41].

However, studies on a number of dsRBM-containing proteins [42–45] showed that a single dsRBM is sufficient to provide the protein with a clear specificity for target selection. As the primary sequences within an RNA helix seems an unlikely determinant of such specificity, the recognition of particular sequences or secondary structure elements flanking the helix could instead be the key for target specificity. Although the structure of the XlrbpA dsRBM2–RNA complex shows that dsRBM has the potential to span 16 base pairs of an RNA helix, uninterrupted helices of more than 10–11 nucleotides are very rare in RNA structures. This suggests that one of the recognition elements of dsRBM could interact with flanking non-helical structures within a duplex-containing RNA target. Such an interaction was observed for the first time in the structure of staufen dsRBM3 in complex with a non-physiological RNA hairpin and contributes substantially to binding affinity [31]. Helix 1 of the protein interacts with the UUCG tetraloop module and connects to its distorted minor groove (which provides a continuation for the minor groove of the RNA helix) via several direct contacts with the RNA bases. In contrast to what observed for the residues in loops 2 and 4, the sequence of helix 1 is not conserved in the dsRBM family or even within dsRBMs of the same proteins but is instead conserved between equivalent dsRBMs from different staufen homologues [45]. This pattern of conservation suggests that, *in vivo*, helix 1 could recognize non-helical secondary structure elements in either a structural or sequence specific way.

Two recent and independent studies of the interaction between *S. cerevisiae* Rnt1p dsRBM and the physiological target AGNN RNA hairpin [46,47] show that, similarly to what was observed in the staufen dsRBM3–dsRNA complex, helix 1 plays a pivotal role in Rnt1p target recognition. In the structure of the Rnt1p–RNA complex the first helix of the protein recognizes the geometry of the distorted minor groove in the AGNN RNA tetraloop (Fig. 3). Although contacts between the protein and RNA bases are made, recognition is not sequence but structure specific as the protein make contacts with the non-conserved bases of the tetraloop only [46]. The structure of the complex clarifies that the interaction of dsRBM helix 1 with non-helical secondary structure elements can provide the specificity of recognition required for the physiological interaction. Importantly, the precise positioning of helix 1 in the Rnt1p–RNA complex is modulated by an additional helix carboxy terminal to the ‘classical’ dsRBM fold (helix 3) that is here observed for the first time [47]. This novel observation shows that specificity of the helix 1–tetraloop interaction can be tuned by other elements of the protein. It is possible that a similar phenomenon of tuning is at the basis of the observed increased affinity for the RNA target when the dsRBM1 of RHA is extended to include a carboxy terminal proline-rich region [41].

The structural work of the past five years has identified a common mode of RNA target recognition by

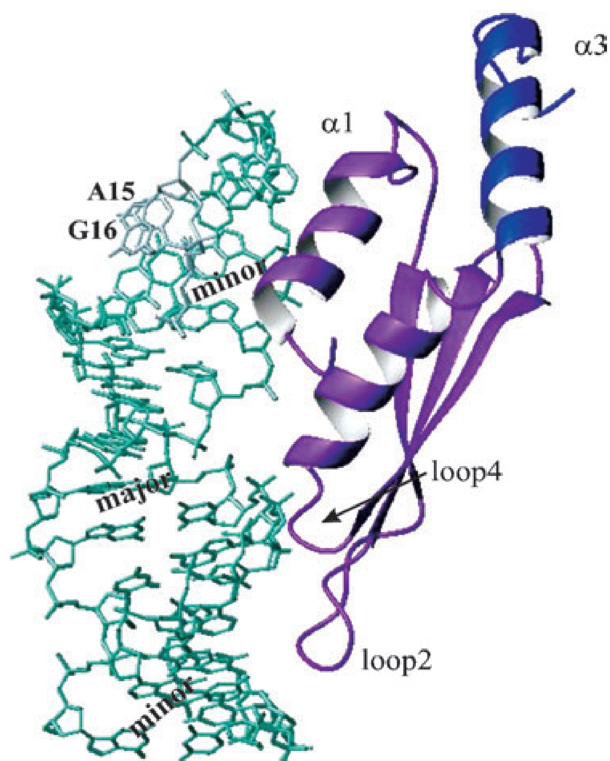


Fig. 3. MOLMOL ribbon representation [61] of the structure of the *S. cerevisiae* Rnt1p dsRBM (magenta, blue and grey) in complex with its physiological RNA target (blu/green and grey). Recognition of the RNA A-form helix is mediated by contacts between by loops 2 and 4 and the RNA minor and major grooves, as observed for the xlrbpA structure (Fig. 2). Helix 1 interacts with the minor groove in the RNA tetraloop. Helix 1 is oriented by a third helix (helix 3, blue) so to achieve the largest possible surface of interaction with the minor groove in the RNA tetraloop. Within the tetraloop, the conserved A15 and G16 nucleotides (grey) do not contact the protein: recognition of the tetraloop is structure rather than sequence dependent.

dsRBMs and dissected this recognition into two functionally separated interaction modes. These are the interaction of residues of the protein loops 2 and 4 with 2′OH and phosphate groups of sequential minor and major grooves of the RNA helix, and the interaction of helix 1 with helical or non-helical secondary structure elements presenting a minor groove type surface. An outstanding question is whether interactions described above are common to all dsRBM–RNA complexes and if the conclusions on the role of helix 1 drawn from the study of four dsRBM–RNA complexes can be extended to all other RNA targets and hence be used to identify suitable target sites within large RNAs. It is also important to define whether the tuning of the position of helix 1 can be achieved by structural elements of a different domain or even from a different protein. If this is the case, the ability of dsRBM to bind dsRNA could be modulated directly by intermolecular protein–protein interactions, thus adding an additional regulatory layer to dsRBM–RNA recognition.

Interaction with non-dsRNA partners

Although dsRNA-binding is the defining feature of dsRBMs, other macromolecular partners have been identified. In staufen, dsRNA and protein binding functions are clearly separated, as dsRBMs 1, 3 and 4 show (to a different degree) RNA-binding capability, while dsRBM5 (and possibly dsRBD2) acts as a protein–protein interaction motif. However RNA and protein binding are not necessarily mutually exclusive. In addition to dsRNA-binding, the isolated dsRBMs of PKR are found to form a heterodimer with a full length PKR via dsRBM–dsRBM interaction [48]. This intrinsic dsRBM–dsRBM interaction is thought to be responsible for the inactivation of PKR and is separated from the dsRNA-dependent dimerization required for PKR autophosphorylation and activation [49]. Similar protein–protein interactions are also observed in the PACT and TRBP proteins whose dsRBMs are capable of forming dsRNA-independent heterodimers with the dsRBMs of PKR and modulating its activity [50,51]. In addition, dsRBM–dsRBM interactions are also thought to be responsible for the observed PKR–ribosome and NF90–RHA association *in vivo* [6]. However, the nature of these dsRBM interactions is not well characterised and the study of PKR is complicated by the existence of an extra dimerisation site downstream of the dsRBMs [48].

dsRBMs interact not only with other dsRBMs but also with different protein domains either intra or intermolecularly. The dsRBM2 of PKR has been shown to interact with the kinase domain of PKR itself to convert PKR into an inactive form by blocking the accessibility of its protein substrate [20]. A similar interaction has also been shown between dsRBM and the catalytic domain of RNAse III [35]. Interactions with non-catalytic domains are also known. Recently, the dsRNA-binding incompetent dsRBM5 from *Drosophila* staufen was shown to interact with the protein Miranda, which mediates protein and RNA localisation in the developing nervous system [52]. In addition, the human homologue of the *Drosophila* staufen was demonstrated to participate in the viral particle assembly of HIV by binding to the NC domain of HIV Gag protein in an RNA-independent way mediated by its dsRBM3 [53]. The same protein was also reported to bind influenza NS1 protein

although the staufen domain responsible for this interaction was not defined [54].

How does the protein binding capability of dsRBM compare to its RNA-binding ability? The interaction between *E. coli* RNAseIII dsRBM and the catalytic domain of the same protein is loose at best, and does not provide much information on the possible local determinants of recognition. A similar lack of interdomain contacts is observed in the structure of the two dsRBM domains of PKR [55]. The limited information available does not allow the drawing of a conclusion on the existence of common themes in dsRBM–protein recognition. In fact, dsRBMs recognise structurally different protein targets and it seems unlikely that the same surface could mediate the interaction with domains of very different structure, although it is conceivable that dsRBMs–dsRBMs interactions are mediated by a common surface. Furthermore, the sequence conservation within protein-binding dsRBMs is lower than that observed for RNA-binding dsRBMs, hinting that different residues are involved in the interactions.

Interestingly, dsRBMs have the capability to bind not only protein and RNA but also DNA molecules. The first 250 amino acids of RHA contain two dsRBMs and a novel dsDNA binding activity has also been located within this region [41]. It is not clear how this nucleic acid binding protein can accommodate both the A-form dsRNA and B-form dsDNA, although a mutagenesis study suggests that their binding may involve two sets of distinct but overlapped residues involving the dsRBM1 and its extended carboxy domain. Also, a variation of the dsRBM fold, a ββββα platform, has been shown to harbour the DNA binding activity for integrase on a different surface from the one used in RNA-binding by dsRBM [56].

More functions for dsRBMs?

Although the main function of dsRBMs seems to be the recruitment of RNA (or protein) molecules to a multidomain protein, this relatively small motif has also been shown to serve other roles. Recently, the full length XlrbpA and its isolated dsRBM domain, as well as the dsRBM1 of PKR, have been shown to possess RNA strand annealing activity [27,57]. Such activity does not depend on RNA-binding as it can be uncoupled from the latter. These data are consistent with the discovery that the dsRBMs from PKR are capable of straightening the bulged RNA [58], and may also help to explain some of the observed cooperative dsRNA-binding effects of tandem dsRBMs. Perhaps a chaperone-like activity for those dsRBMs defective in RNA and protein binding could facilitate strand annealing and refolding of unqualified RNA substrates for proper recognition by a nearby dsRNA-binding dsRBM.

Additionally, tandem dsRBMs such as the ones found in PKR and RHA can regulate catalytic activity within the same protein. PKR is a vital component of the cellular antiviral mechanism and binding to dsRNA (synthesized in large quantities in viral infection) can result in protein dimerisation, and subsequent autophosphorylation and activation [49]. The PKR kinase activity is inhibited by virally encoded RNA and protein inhibitors such as VA RNA_I of adenovirus and dsRBM containing protein E3L of vaccinia virus [20,59], and is also tightly regulated by cellular factors such as the dsRBM-containing protein activator, PACT. and inhibitor, TRBP [50,51]. This network of interactions is based on several dsRBM–dsRBM, dsRBM–kinase and dsRBM–dsRNA interactions and has at its core a regulatory unit formed by the tandem dsRBMs of PKR. Similarly, a complex interplay occurs among the three dsRBM domains of the RNA editing enzyme ADAR1, its RNA targets and the nuclear shuttling machinery, with the masking of a nuclear localisation activity embedded in dsRBM3 by dsRBM1 upon interaction with a target RNA [45]. In contrast, the functional activity of RHA is less explored than that of PKR but the capability of its dsRBM to recognise different class of macromolecules (DNA, RNA and protein) makes it a good candidate for a regulator of nucleic acids metabolism. The effect of distinct ligand binding on the helicase activity of RHA remains to be examined but highlights the potential of this domain as a nucleic acid-responsive regulator. The data on three different systems clarifies that the dsRBM is not just a protein fold for dsRNA recognition, but is indeed a versatile macromolecule docking scaffold.

Conclusions

The dsRBM motif harbours the important capability to recognise the basic element of RNA structure, the A-form helix, in very diverse structural contexts. As we gain further understanding of the role that large structured RNAs play in inherited and infectious pathologies and in the generality of post-transcriptional regulatory processes, the dissection of the ground rules of this recognition becomes increasingly important. Initial results have been obtained using a combination of structural, biochemical and functional information. However, recent work has made clear that dsRBMs can recognise non-RNA targets and can act in combination with other dsRBMs and

non-dsRBM motifs. The understanding of the multi-component interactions underlying this complex processes will require both information on the precise functional relation between different domains and a clarification of the principles underlying dsRBM-protein recognition.

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