Double-stranded RNA binding proteins and their substrates

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ABSTRACT
Double-stranded RNA (dsRNA) binding proteins (dsRBPs) pose unique questions in regard to how proteins interact with RNA. This article presents a general overview of these questions and also introduces specific dsRBPs that are studied by my laboratory.

INTRODUCTION
The various functions of RNA molecules in living organisms often require the RNA to fold into a precise shape. An RNA's shape allows it to be recognized by other biological molecules, such as proteins. An underlying assumption in my laboratory is that RNA molecules fold into two general types of shapes: compact, globular shapes (like protein enzymes), and linear shapes that are more extended, with fewer long range interactions. Examples of RNA molecules with a compact, globular shape are the well-characterized transfer RNAs (tRNAs) and catalytic RNAs like the Tetrahymena ribozyme; examples of RNAs with a more linear shape are those that consist predominantly of a double-stranded helix, such as the viroid RNAs that infect plants. Stem-loop structures such as the human immunodeficiency virus (HIV) trans-activating response (TAR) hairpin would also fall into this latter category.

Proteins that interact with RNA molecules of the "linear" shape category, have been loosely grouped together and called the double-stranded RNA (dsRNA) binding proteins (dsRBPs). Certain members of this class of proteins have been known for some time, such as RNase III and the dsRNA dependent protein kinase (PKR). Although specific in vivo substrates have been identified for some dsRBPs, in vitro they bind tightly to dsRNA of any sequence, but not other nucleic acids such as ssDNA, single-stranded DNA (ssDNA) or ssRNA. Consistent with the idea that there are two general shapes of RNA molecules, existing data indicate that dsRBPs will not bind to RNAs whose double-helical regions are buried by long range tertiary interactions.

dsRNA adenosine deaminase
One of the dsRBPs studied by my laboratory is the dsRNA adenosine deaminase (dsRAD, reviewed in 1). This enzyme is a dsRBP with unusual catalytic properties. Specifically, dsRAD converts adenosine (A) residues within dsRNA to inosine (I) residues using a hydrolytic deamination mechanism. Although originally observed in the frog Xenopus laevis, dsRAD has now been detected in organisms throughout the animal kingdom, including Caenorhabditis elegans, Drosophila melanogaster and mammals. Recently two RNAs have been proven to be biological substrates for dsRAD. As expected, both RNA substrates have linear shapes and consist of long double-stranded helices periodically interrupted by small bulges, loops and mismatches. For both of the identified substrates, a glutamate receptor mRNA (reviewed in 2) and hepatitis delta virus RNA (3), dsRAD appears to function as an RNA editing enzyme. (RNA editing is a way to control gene expression by changing the sequence of an RNA from that encoded by the DNA.) Both of the proven dsRAD substrates are messenger RNAs (mRNAs), and the A to I
conversions alter codons so that translation yields a different protein.

Like other dsRBPs, dsRAD will bind to dsRNA of any sequence in vitro. However, deamination by dsRAD does not occur randomly, and certain adenosines are preferentially converted to inosines (4). For example, dsRAD prefers to deaminate adenosines whose 5' neighbor is adenosine, uridine or cytidine over those whose upstream neighbor is guanosine. An unexpected observation is that the deamination preferences dsRAD exhibits in vitro can be observed in the patterns of editing found in its endogenous substrates. This observation suggests that if accessory proteins are involved in editing within the cell, they probably alter editing efficiency rather than its specificity.

Other dsRBPs and their substrates

By screening an expression library with radiolabeled dsRNA my laboratory has identified four cDNAs that encode previously unknown dsRBPs. Characterization of these dsRBPs led to the identification of two amino acid sequences ("motifs") that allow a protein to bind to dsRNA (5,6). One of these motifs is a variation of the well-characterized "zinc finger", a motif long known to be involved in binding to polynucleotides. The second motif is ~65 amino acids long and can be observed in many proteins previously shown to bind dsRNA, including RNase III, PKR and dsRAD. Several laboratories have observed the latter motif which is called the dsRNA binding motif (dsRBM). My laboratory has shown that proteins containing dsRBMs or the zinc finger motif will bind to dsRNA of any sequence, but not to dsDNA or single stranded nucleic acids.

For each of the dsRBPs studied in my laboratory we are conducting experiments aimed at determining biological function, and also more biophysical experiments aimed at determining the specific interactions that allow these proteins to bind dsRNA. The recognition of RNA molecules that consist predominantly of a double helix poses unique problems for a protein. Double helices of the type formed by DNA and RNA have two characteristic grooves, a major and a minor groove. Most of the functional groups that allow the sequence of a double helix to be deciphered by a protein are in the major groove. In contrast to the wide major groove of a DNA helix, the major groove of an RNA helix is too narrow for a protein to enter unless the helix is distorted, or interrupted by a mismatch, bulge or loop. Given the features of an RNA helix, it is not surprising that, at least in vitro, dsRBPs are not sequence specific.

The observation that dsRBPs are not sequence specific in vitro raises the intriguing question as to whether each dsRBPs has a unique substrate in vivo. If so, at what level does the discrimination occur? One possibility is that sequence specific interactions occur in vivo that have not yet been detected in vitro. Such interactions could occur, for example, in regions where the major groove is made more accessible by interruption of the helix by a bulge, mismatch or loop. Alternatively, as suggested by dsRAD’s deamination specificity, some dsRBPs may discriminate between potential endogenous substrates at a chemical step rather than upon binding.

Clearly some dsRBPs share substrates. Certain viruses produce dsRNA that elicits a cellular antiviral response by binding to and activating PKR. Upon binding dsRNA, PKR is autophosphorylated and in turn phosphorylates the initiation factor, eIF-2, resulting in the inhibition of protein synthesis. Reovirus and vaccinia virus prevent this host response by expressing other dsRBPs, which serve to occlude dsRNA from binding to PKR (7,8). Here the host and viral dsRBPs are clearly binding to the same dsRNA molecules. In my laboratory we have identified a protein factor in the cytoplasm of Xenopus oocytes that binds and protects cytoplasmic dsRNA from deamination by dsRAD (9). Our studies, as well as the work on viral dsRBPs, raise the possibility that dsRBPs carry out their specific biological functions through a complex interplay with other dsRNA binding proteins, all competing for the same endogenous substrates.
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REFERENCES