Secondary Structure Mapping of an RNA Ligand That Has High Affinity for the MetJ Repressor Protein and Interference Modification Analysis of the Protein-RNA Complex*

Alistair McGregor‡, James B. Murray §§, Chris J. Adams §§, Peter G. Stockley §, and Bernard A. Connolly¶

From the ‡Department of Biochemistry and Genetics, The University of Newcastle, Newcastle upon Tyne, NE2 4HH, United Kingdom and the §§Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, The University of Leeds, Leeds, LS2 9JT, United Kingdom

The secondary structure of an RNA aptamer, which has a high affinity for the Escherichia coli MetJ repressor protein, has been mapped using ribonucleases and with diethyl pyrocarbonate. The RNA ligand is composed of a stem-loop with a highly structured internal loop. Interference modification showed that the bases within the internal loop, and those directly adjacent to it, are important in the binding of the RNA ligand to MetJ. Most of the terminal stem-loop could be removed with little effect on the binding. Ethylation interference suggests that none of the phosphate groups are absolutely essential for tight binding. The data suggest that the MetJ binding site on the aptamer is distinct from that of the natural DNA target, the 8-base pair Met box.

Several genes involved in the biosynthesis of methionine in Escherichia coli are transcriptionally regulated by the methionine repressor, MetJ (1). The basic interaction occurs between a homodimer of the 12-kDa MetJ repressor subunits and an 8-base pair sequence that constitutes a Met box (2). The Met box is a tandem repeat that occurs between two and five times in natural operators and to which additional repressor dimers bind in a cooperative manner (3). The protein has a b-box topology where the β-strands from each subunit intertwine to form an anti-parallel β-ribbon, which lies in the major groove of the target DNA. Binding specificity is largely determined by hydrogen bonds between the side chains of lysine 23 and threonine 25 of the β-strands and 4 purine bases in each Met box. Many hydrogen bonds are also made between the protein and the DNA phosphate backbone, and there is evidence that the conformation of the backbone is also important for binding (4). Binding of the MetJ repressor to DNA is modulated by changes in the DNA consensus, a two Met box site interacting with two cooperatively bound holorepressors (3). Therefore, it is of interest to determine how such RNA aptamers interact with MetJ. Do they mimic the binding of the natural DNA Met box (as for the MS2 coat protein) or alternatively, do they represent novel solutions to the MetJ recognition problem? Inhibition of DNA binding proteins by RNA aptamers is likely to be of very general interest.

Recently, one of our laboratories reported the first x-ray crystal structure for an aptamer bound to the RNA bacteriophage MS2 coat protein (14). This structure revealed that the aptamer is bound to the natural RNA binding site of the protein, and although being based on differing primary and secondary structures, the aptamer is able to mimic most of the key recognition contacts of the natural ligand.

To extend our knowledge of the way in which aptamers interact with their protein targets, we have isolated tight binding RNA ligands for the E. coli MetJ repressor, a sequence-specific DNA-binding protein with no known RNA-binding function. Previously, we have shown that systematic evolution of ligands by exponential enrichment (SELEX) with degenerate DNA ligands results in isolation of tight binding DNA molecules based on the Met box consensus (16), a situation analogous to the isolation of RNA aptamers for the MS2 coat protein (9). However, a high proportion of the SELEX-RNA ligands isolated by binding to MetJ contained the consensus sequence 5′-UGC(AUACUCGU)3N14−16(G)UCAUGCAGCA-3′2, which is unrelated to the Met box DNA target. Remarkably, several of these ligands bind more tightly to an apo-MetJ dimer than does the DNA consensus, a two Met box site interacting with two cooperatively bound holorepressors (3). Therefore, it is of interest to determine how such RNA aptamers interact with MetJ. Do they mimic the binding of the natural DNA Met box (as for the MS2 coat protein with RNA ligands and MetJ with DNA ligands), or alternatively, do they represent novel solutions to the MetJ recognition problem? Inhibition of DNA binding proteins by RNA aptamers is likely to be of very general interest. DNA-binding proteins constitute a large group of potential drug targets, and RNA SELEX offers a route to generate tight binding inhibitors of such molecules.

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‡ Current address: Dept. of Chemistry, Indiana University, Bloomington, IN 47405.

¶ To whom correspondence should be addressed. Tel.: 44-191-222-7371; Fax: 44-191-222-7424; E-mail: b.a.connolly@ncl.ac.uk.

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were labeled, at their 5'-ends using [γ-32P]ATP (3000 Ci/mmol; Amer- 
sham) and T4 polynucleotide kinase (Amer sham Pharmacia Biotech) as 
described previously (19). Alternatively, the 3'-ends were labeled by the 
ligation of [5'-32P]Clp (3000 Ci/mmol; Amer sham Pharmacia Biotech) to 
the 3'-end using RNA ligase (Amer sham Pharmacia Biotech) (21). 
Labeled RNA was purified on a 5-50% glycerol gradient containing 7 M urea. RNA was eluted from the appropriate gel fragment by incubation in 0.1 M Tris/HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.1% (w/v) SDS for 12 h at 37°C. RNA was precipitated by the addition of 3 volumes of absolute ethanol. The pellet was collected by centrifugation, rinsed with absolute ethanol, and dried, before being resuspended in 50 μl of sterile water.

\textit{MetJ Repressor—MetJ repressor, purified as described (22, 23), was 
gift from Isabel D. Lawrenson (University of Leeds). It was supplied 
as an ammonium sulfate precipitate and stored at 4°C until required. 
Precipitates were dissolved in 100 mM sodium phosphate (pH 7) and 
dialyzed against 50 mM Tris/HCl, pH 8.0, 2 mM EDTA. The dialyzed 
solutions were used for Alloncide and DNA binding experiments.}

\textit{Enzymatic and Chemical Probing of RNA Secondary Structure— 
RNA labeled at either the 5'- or the 3'-end (~1 μg, ~1 × 10^6 cpm) was 
digested with RNase T1 (Boehringer Mannheim), RNase A (Sigma), or 
RNase U2 (Sigma) in 20 μl of 50 mM Tris/HCl, pH 7.0, 50 mM KCl, 
containing 6 units of tRNA (type X-SA, Sigma). Digestions were carried 
out at 37°C for 15 min. The cleaved products were analyzed on a 
denaturing 12% (w/v) polyacrylamide gel containing 7 M urea, and 
visualized by autoradiography or phosphorimaging (Fujifilm BAS 
1500). Quantitation was carried out using the PhosphorImager associ- 
ated software, TINA (Raytest Isotopenmeßgeräte GmbH).}

\textit{Diethyl pyrocarbonate (DEPC) modification of adenosine and 
guanosine was performed essentially as described in Ref. 24. Approxi-
}

\textit{Interference Analysis of MetJ Repressor-RNA Complexes—RNA was 
chemically modified with either DEPC, hydrazine, or ethylnitrosourea. 
Adenosine and guanosine were modified with DEPC as described above, 
using the denaturating buffer. Uridine bases were modified by 
dissolving an ethanol precipitate of labeled RNA (~6 pg, ~5 × 10^6 cpm) in 
10 μl of hydrazine hydrate (24). Following incubation in ice for 10 min, the reaction was stopped by the addition of 200 μl of 0.3 M sodium 
acetate, pH 3.8, and 750 μl of absolute alcohol. The RNA pellet was 
recovered by elution as described above. 25 units of tRNA (type X-SA, 
Sigma) were added to 10 μl of the DEPC-modified RNA and processed 
with RNase T1 (Boehringer Mannheim), RNase A (Sigma), or 
RNase U2 (Sigma) as described above. The RNase digestion was 
followed by centrifugation at 37°C for 30 min (native buffer) or 90°C 
for 2.5 min (denaturing buffer). The reaction was stopped with 75 μl of 
1 M sodium acetate, pH 4.5, and 750 μl of absolute ethanol. Following 
centrifugation the RNA pellet was dissolved in 200 μl of 0.3 M sodium 
acetate, pH 3.8, and precipitated with 600 μl of absolute alcohol. The 
precipitated RNA was rinsed with absolute ethanol and dried. DEPC-
modified RNA was then cleaved at the modified nucleotide by treatment 
with aniline (24). The fragmented products were examined by denatur-
ing gel electrophoresis, as above.}

\textit{Analysis of a RNA-MetJ Repressor Complex—The RNA-MetJ 
repressor complex was treated with DEPC, hydrazine, or ethylnitrosourea. 
Adenosines and guanosines were modified with DEPC as described above, 
using the denaturating buffer. Uridine bases were modified by dissolving an ethanol precipitate of labeled RNA (~6 pg, ~5 × 10^6 cpm) in 
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were labeled, at their 5'-ends using [γ-32P]ATP (3000 Ci/mmol; Amer-
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FIG. 1. A, the sequence and predicted secondary structure of the RNA 
ligand used in this study. B and C, the truncated RNA ligands, in which 
parts of the sequence shown in A were replaced with a hexaethylene 
glycol linker.

Here a number of techniques have been employed both to 
determine the solution structure of the highest affinity RNA 
ligand and to probe the nature of the interaction between the 
two macromolecules. In particular we have attempted to deter-
mine the regions of the RNA aptamer that are involved in 
binding to the protein. Similar approaches have been used to 
good effect to study the inhibition of HIV-I reverse tran-
scriptase with RNA ligands (17). In the absence of high reso-
lution structural data for the RNA-protein complex, these stud-
ies provide a useful insight into how the aptamer complex 
differs from the DNA operator complex with the same protein.

\textit{Experimental Procedures—Oligoribonucleotide Synthesis 
and Purification—RNA was synthe-
}
with 0.1 M Tris/HCl, pH 9.0 (25). The cleaved products were visualized by denaturing gel electrophoresis as described above.

**Filter Binding Assays**—Filter binding assays were performed as described previously (6). The MetJ protein was serially diluted in 50 mM Tris/HCl, pH 7, 50 mM KCl and incubated with approximately 20 nM RNA for 10 min at 37 °C. Samples were filtered through 0.45-μm nitrocellulose filters (Whatman) and rinsed with 2 × 400 μl of binding buffer. Following drying, 5 ml of Ecoscint A (National Diagnostics) was added, and the samples were counted in a liquid scintillation counter. The K_d was estimated as the protein concentration at which half-maximal binding of the RNA occurred.

**Circular Dichroism of RNA and MetJ Repressor-RNA Complexes**—Circular dichroism spectra were obtained using a Jobin Yvon CD6 Dichrograph with the cell (path length 10 mm) thermostatted at 37 °C, unless stated otherwise. Samples (0.15 ml) were prepared in 50 mM Tris/HCl, pH 7.0, 50 mM KCl. An RNA concentration of 1 μM was used, with the protein concentrations quoted in the legend of Fig. 9.

**UV Cross-linking of MetJ Repressor with RNA Containing 4-Thiouridine**—Cross-linking of the MetJ repressor to RNA containing 4-thiouridine was carried out as described previously (26). Samples contain-
ing 7.5 μM MetJ repressor dimer and approximately 50 nM radiolabeled oligoribonucleotide in 50 mM Tris/HCl, pH 7, 50 mM KCl, were incubated at 37 °C for 10 min prior to irradiation, at 350 nm, for 10 min. The oligoribonucleotide shown in Fig. 1A was used, but with the uridines at positions 20, 23, 35, and 36 replaced with 4-thiouridine. Potential protein-RNA cross-links were analyzed by SDS-polyacrylamide gel electrophoresis.

RESULTS AND DISCUSSION

Secondary Structure of the RNA Ligand—The sequence and the predicted secondary structure of the SELEX RNA ligand used in this study is shown in Fig. 1. It consists of a stem-loop (terminal loop) and an internal loop (27). To test this prediction experimentally, we have mapped the RNA ligand using ribonucleases and with DEPC. As shown in Fig. 2, the terminal loop was most sensitive to cleavage by the single-strand specific nucleases RNase A (C/U-specific) and RNase U2 (A-specific). A number of nucleotides within and directly adjacent to the internal loop were also cleaved by the nucleases, but to a lesser degree than those in the terminal loop. These include A6/U7/A8 (5'-labeled strand) and C33/C38/C41 (3'-labeled strand). A few internal loop nucleotides, which would be expected to be cleaved, if single-stranded, were either not affected or were very poorly cut by the nucleases examined. Nucleotides in this category were C9/U10/C11 (5'-labeled strand) and A34/U35/U36/A39 (3'-labeled strand). The nucleotide C29, which is predicted to be in a 2-nucleotide bulge in the main stem, was also susceptible to cleavage by RNase A. Consistent with most of the guanosines being double-stranded, RNase T1 (G-specific) did not cleave well, including position G37 within the internal loop.

Comparison of DEPC modification (which modifies the N7 position of adenosine and, to a lesser extent, that of guanosine) of the RNA at 37 °C and 90 °C showed marked differences, with greater modification under denaturing as compared with native conditions, for two of the three adenosines (A31/A42), predicted to be in double-stranded regions (Fig. 2). The four adenosines (A6/A8/A34/A39) which are predicted to be in the internal loop, were also modified to a greater degree in the denatured oligonucleotide compared with the native one. In contrast, little difference in reactivity was observed for the two adenosines (A21/A22) in the terminal loop. The nucleotide A24 also behaved in this manner. Although predicted to be in a double-stranded region, this nucleotide is immediately adjacent to the terminal loop.

The results are summarized in Fig. 3 and are in broad agreement with the predicted structure. The terminal loop, which closes the stem-loop, has properties consistent with its being single-stranded. The regions predicted to be double-stranded map as a duplex when probed with nucleases or DEPC. The most interesting region is the internal loop. A number of nucleotides within this region, that would be expected to be ribonuclease-sensitive, were either not cleaved or were cut significantly more slowly than those in the terminal loop. Similarly, several nucleotides have a diminished reactivity to DEPC in the native compared with the denatured RNA. This suggests that the internal loop cannot be considered as...
single-stranded but must contain some degree of stable secondary structure, limiting the accessibility of both ribonucleases and DEPC. It is conceivable that some of the bases on opposite sides of the internal loop form non-canonical base pairs. In addition bases on the same strand may be strongly stacked.

Mapping Contacts between Bases in the RNA Ligand and the MetJ Repressor—

Interactions between the bases in the RNA ligand and the MetJ repressor have been determined by binding-interference analysis. DEPC has been used to probe the role of adenosine and guanosine, and hydrazine has been utilized for uridine. The results for DEPC are shown in Fig. 4. To map the entire RNA sequence, it was necessary to use both 3'- and 5'-end labeling. A similar quality autoradiogram (not shown) was obtained using hydrazine. Scans of both the DEPC and hydrazine autoradiograms are shown in Fig. 5, and the results are summarized in Fig. 6. Both DEPC and hydrazine caused notable interference at a number of bases. Modification of bases within, and directly adjacent to, the internal loop significantly reduced the binding of the RNA ligand to MetJ. Modification of the eight bases in and around the terminal loop had no effect on binding. Modification of the bases in the intervening stem that connects the internal and the terminal loops had a mildly detrimental effect on binding. This strongly suggests that it is the internal loop region of the RNA ligand that is responsible for tight binding to the MetJ repressor.

The importance of the internal loop was confirmed using two truncated RNA ligands. The deletions remove (a) the terminal loop and 2 base pairs from the stem and (b) the terminal loop, 5 base pairs from the stem, and the two single stranded cytosines (Fig. 1). In both cases the deleted bases were replaced by a hexaethylene glycol chain to substitute as a hairpin loop (28). The binding affinity of the two shortened RNA molecules to the MetJ repressor were determined using a filter binding assay (Fig. 7). Neither deletion markedly affected the binding of the RNA to MetJ. $K_d$ values (assessed as the protein-dimer concentration at which one-half of the counts were retained on the filter) of between 2 and 4 $\times 10^{-7}$ M were seen for both the deletions and the parent RNA ligand. A control RNA, consisting of the first 21 nucleotides of the parent RNA, did not bind to MetJ. These results clearly demonstrate that the initial, synthetic SELEX RNA fragment used does in fact bind tightly to MetJ as expected from the original SELEX experiment. Furthermore, we confirm that the internal loop of the

![Fig. 5](http://www.jbc.org/)

![Fig. 6](http://www.jbc.org/)
RNA ligand encompasses the high affinity site for the MetJ repressor, in agreement with the binding-interference analysis. Although the terminal loop is not directly involved in binding it is necessary to allow the reversal of the RNA strand and hence the formation of the internal loop.

Mapping Contacts between Phosphates in the RNA Ligand and the MetJ Repressor—Ethylnitrosourea, which ethylates the non-esterified oxygen atoms of backbone phosphates in nucleic acids, was used to probe the role of the RNA phosphate groups in binding to the MetJ repressor. No single phosphate was found to be critical for interaction with the MetJ repressor, i.e. there was no phosphate group, which when ethylated, strongly inhibited the binding of MetJ. This is shown in Fig. 8, which shows scans of the interference autoradiograms.

The role of RNA phosphates in binding to MetJ has been further evaluated by the determination of $K_d$ values at various salt concentrations using a filter binding assay (data not shown). If interactions between negatively charged phosphates and positively charged amino acid side chains are important, the $K_d$ would be expected to increase with increasing ionic strength (29). In 10 mM Tris/HCl, pH 7.0, with KCl concentrations of 10, 25, 50, and 100 mM, identical $K_d$ values of $1.2 \pm 0.3 \times 10^{-7}$ M were obtained. The dissociation constant was marginally higher at KCl concentrations of 150 and 200 mM, $2.3 \pm 0.5 \times 10^{-7}$ and $6.3 \pm 1 \times 10^{-7}$ M, respectively. Interestingly, although the data at 100–200 mM salt fitted a typical protein-ligand binding isotherm, those at 10–50 mM KCl were notably steeper, suggesting that some form of cooperativity occurs. One possible explanation is that MetJ dimers may

![Graph showing RNA-MetJ repressor complex binding](image)

**Fig. 7.** The interaction of RNA ligands with MetJ as assessed by filter binding. □, intact RNA (structure A in Fig. 1); ○, RNA with 6 bases replaced by a hexaethylene glycol spacer (structure B in Fig. 1); ▲, RNA with 16 bases replaced by a hexaethylene glycol spacer (structure C in Fig. 1). The line along the bottom of the graph (solid squares) is a control RNA consisting of nucleotides 1–21 (top half) of the intact RNA. The individual data points shown are the average of three readings. The error in the averaged measurement was between 5 and 10%. Each binding isotherm was determined four times to give $K_d$ values.

**Fig. 8.** Densitometry of the interference data found for MetJ using ethylnitrosourea to ethylate RNA phosphates. A, 5′-labeled RNA; B, 3′-labeled RNA. —, RNA that was gel-shifted by MetJ protein; —, RNA that failed to shift in the presence of MetJ protein. 19/18 is the phosphate between bases 18 and 19 in Fig. 1, etc. Some peaks are indicated merely for the purpose of identification.
dissociate at low concentrations in low salt buffers. Although it has been shown that thermally denatured MetJ protein will readily reassociate (30), we have no evidence for this dissociation. However, the related Arc repressor from bacteriophage P22 does dissociate into unfolded monomers with a dissociation constant of $5 \times 10^{-6}$ M (31, 32). Alternatively, salt-dependent conformational changes of the RNA ligand could be involved, although the salt concentrations examined here had no effect upon the circular dichroism spectra of the RNA (not shown). Steep binding curves have been observed for the binding of MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3). Because of these complexities caution is needed in the interpretation of how the MetJ protein to its DNA target (3).

Conformation of the RNA Ligand, Both Free in Solution and Bound to MetJ—Circular dichroism data (Fig. 9) are consistent with the RNA being largely double-stranded and in an A-form conformation. The spectrum of the free RNA shows a large positive $\Delta \varepsilon$ at 263 nm and a small negative peak at 295 nm, typical of an A-form helix. Heating the RNA caused a decrease in the intensity of the main peak and a shift to 271 nm, consistent with the conversion of the RNA to a single strand (33). This is consistent with the mapping data presented above, which show that the internal loop cannot be considered single-stranded, but must have some element of structure. Thus, with the exception of the single-stranded terminal loop, almost all of the RNA ligand appears to exist as an A-form duplex. However, the exact structure of the internal loop may deviate somewhat from classical A-duplex parameters. When the RNA ligand binds to the MetJ protein, there is hardly any change in the circular dichroism spectrum of the nucleic acid (Fig. 9), especially above 240 nm where almost all ($\geq 90\%$) of the intensity is due to the nucleic acid. This suggests that there are no large conformational changes to the RNA after binding to the protein.

Photocross-linking of 4-Thiouridine-containing RNA to MetJ—It has previously been shown that both oligodeoxynucleotides containing 4-thiothymidine and oligoribonucleotides containing 4-thiouridine can be used to photocross-link DNA- and RNA-binding proteins (26, 34). We had hoped to use this approach to map the regions of MetJ in contact with the RNA ligand. Unfortunately, substitution of several uridines by 4-thiouridine, in the oligoribonucleotide shown in Fig. 1A, and subsequent 350 nm irradiation of these oligoribonucleotides, in the presence of MetJ, failed to generate any cross-links (data not shown). Considering the data above, this is hardly surprising for the uridines (positions 20 and 23) in the terminal loop. The failure of 4-thiouridines, within the internal loop (positions 35 and 36), to cross-link was disappointing, particularly as modification of uridines 35 and 36, with hydrazine, interferes with MetJ binding (Figs. 5 and 6). However, we have previously shown that the proximity of 4-thiouridine to a protein is not necessarily sufficient for cross-linking to occur (26). Filter binding (not shown) demonstrated that replacement of uridines 20, 23, 35, and 36 with 4-thiouridine either did not affect binding or reduced it to a small extent, such that significant amounts of MetJ-RNA complex was formed at the concentrations used for photocross-linking.

CONCLUSION

Although there is a great deal of interest in RNA ligands selected to bind particular target molecules with high affinity, there is, as yet, little structural information on the nature of the RNA-target complexes. In the last few years several structures have been determined for selected RNAs bound to small molecular weight ligands such as ATP, using NMR spectroscopy (35). More recently the first high resolution crystal structure for a selected RNA-protein complex has been determined (14). Additionally, low resolution methods, mainly interference and protection modification combined with base substitutions, have been used to map the interfaces between proteins and selected, high affinity, RNA ligands (17).

Here we have characterized an RNA ligand of a natural DNA-binding protein, the E. coli methionine repressor, MetJ. The wealth of structural data on the wild-type MetJ system, including crystal structures for the apo-, holo-, and repressor-operator complexes (4, 5), suggest that this is a good model in which to study the details of aptamer-protein recognition and to compare how the same protein can interact with both RNA and DNA. So far MetJ has proven to be relatively straightforward to crystallize, and hopefully it will be possible to obtain an x-ray structure for this protein in complex with an RNA aptamer. Additionally the small size of MetJ, a homodimer of subunit molecular mass 12 kDa, suggests that NMR spectroscopy might be useful, and the Leeds group already has extensive NMR assignments of the repressor protein. The secondary structure of the free RNA has been probed using ribonucleases and DEPC, and the data largely support the secondary structure predictions shown in Fig. 1. However, the internal loop region of the RNA ligand, shown in Fig. 1 as single-stranded, is

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9}
\caption{Circular dichroism spectra of the RNA. A, circular dichroism spectra of the RNA containing 46 nucleotides (Fig. 1A) at 37 °C (solid line) and at 90 °C (dashed line). B, spectra of RNA with increasing concentrations of MetJ. RNA alone, solid line; $5.5 \times 10^{-7}$ M MetJ (dimer), dotted line; $5.5 \times 10^{-6}$ M MetJ (dimer), dashed line. The spectrum of the free protein was subtracted from the last two spectra. In each case the RNA concentration was $10^{-6}$ M. Above 240 nm the protein spectrum was $\leq 10\%$ the intensity of that of the RNA giving a correction of $\leq 10\%$. Below 240 nm the intensities of both spectra became comparable and so accurate subtraction was not possible. This probably accounts for any deviation between the three spectra below 240 nm.}
\end{figure}
Analysis of a RNA-MetJ Repressor Complex

clearly structured in some way. Binding interference studies and the use of sequence variants of the RNA have demonstrated that this structured internal loop, and the regions immediately flanking it, are the critical elements in tight binding to the MetJ repressor.

The exact details of the interaction between the two macromolecules will require x-ray or NMR structural information, and the experiments described here have helped to define a minimal RNA target for structural studies. The most likely binding location for the SELEX RNA ligand is the DNA binding site. MetJ inserts two β-strands into the major groove of its DNA target, and this element is used to generate specificity. Although the large major groove of B-DNA can accommodate the β-ribbon, it would not fit into the smaller major groove of A-form double-stranded RNA. It is possible that the internal loop forms a loose, double-stranded structure, with either the major or minor grooves being large enough for binding of the β-ribbon. The nucleotides in and directly adjacent to the internal loop were found to be highly conserved during the SELEX procedure, consistent with the important role of this region in tight binding. Indeed, the degree of conservation is surprisingly high, and a few minor differences, such as single nucleotide substitutions, might be expected. With the Met box DNA recognition sequence a number of substitutions can be readily accommodated, and only a small number of bases from each strand are directly contacted (4). We propose that most, if not all, of the bases in the internal loop of the RNA ligand play critical roles either by making hydrogen bond contacts to MetJ or maintaining structural integrity. Thus many more contacts are likely to be made between the MetJ and the RNA bases compared with the bases of the DNA operator. The finding that the RNA ligand undergoes little conformational change on binding to MetJ contrasts with many other examples where protein binding results in significant conformational changes to the RNA ligands (36, 37).

Many protein-nucleic acid complexes are stabilized by large numbers of contacts to the phosphodiester backbone. Thus each MetJ dimer contributes 19 direct hydrogen bonds to phosphate groups when binding DNA (4). However, with the MetJ-RNA interaction, protein-phosphate contacts seem to play, at most, a very minor role. Phosphate ethylation failed to identify important phosphate groups for the interaction, implying that charge neutralization is not a major component of the RNA-protein affinity. Similar weak ionic dependences have been observed for physiologically important RNA-protein interactions, such as the binding of ribosomal protein L11 to its target within 23 S rRNA (15).

In summary it is clear that MetJ is capable of tight and specific binding to its natural DNA target, the Met box, and to a selected RNA ligand. The nature of the interaction between the protein and these two nucleic acids is, however, very different. With DNA there are only a few interactions with the bases and multiple contacts to phosphates. With RNA interactions the bases dominate and the phosphates play only a minor role. The RNA aptamer therefore appears to be a novel solution to the MetJ recognition problem.

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Alistair McGregor, James B. Murray, Chris J. Adams, Peter G. Stockley and Bernard A. Connolly

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