TRAP (trp RNA-binding attenuation protein) is a tryptophan-activated RNA-binding protein that regulates expression of the trp biosynthetic genes by binding to a series of GAG and UAG trinucleotide repeats generally separated by two or three spacer nucleotides. Previously, we showed that TRAP contains 11 identical subunits arranged in a symmetrical ring. Based on this structure, we proposed a model for the TRAP-RNA interaction where the RNA wraps around the protein with each repeat of the RNA contacting one or a combination of two adjacent subunits of the TRAP oligomer. Here, we have shown that RNAs selected in vitro based on their ability to bind tryptophan-activated TRAP contain multiple G/UAG repeats and show a strong bias for pyrimidines as the spacer nucleotides between these repeats. The affinity of the TRAP-RNA interaction displays a nonlinear temperature dependence, increasing between 5 °C and 47 °C and then decreasing from 47 °C to 67 °C. Differential scanning calorimetry and circular dichroism spectroscopy demonstrate that TRAP is highly thermostable with no detectable changes in the structure between 25 °C and 70 °C, suggesting that the temperature dependence of this interaction reflects changes in the RNA. Results from circular dichroism and UV absorbance spectroscopy support this hypothesis, demonstrating that trp leader RNA becomes unstacked upon binding TRAP. We propose that the bias toward pyrimidines in the spacer nucleotides of the in vitro selected RNAs represents the inability of Us and Cs to form stable base stacking interactions, which allows the flexibility needed for the RNA to wrap around the TRAP oligomer.

The tryptophan biosynthetic genes of Bacillus subtilis are negatively regulated in response to the intracellular level of l-tryptophan by a tryptophan-activated RNA-binding protein called TRAP (trp RNA-binding attenuation protein; Ref. 1). TRAP regulates expression of the trp biosynthetic genes by binding to several RNA targets in a tryptophan-dependent manner. Two RNA binding sites for TRAP have been characterized, one located within the 204-nucleotide leader region preceding trpE (2–5) and the second in a segment of RNA overlapping the ribosome binding site of trpG (6). In both cases, TRAP binds to a series of GAG and UAG trinucleotide repeats generally separated by two or three spacer nucleotides (4, 5, 7). Recent experiments using synthetic RNAs have demonstrated that these trinucleotide repeats are crucial for TRAP binding, with GAG repeats being favored over UAG repeats (7, 8). This work also indicated that a spacer of two nucleotides is optimal for TRAP binding and that spacers containing A or U residues are preferred over Gs or Cs.

The crystal structure of TRAP complexed with l-tryptophan has recently been solved and refined to 1.8-Å resolution (5). TRAP is an 11-mer of identical subunits arranged in a symmetrical ring. The secondary structure of TRAP is made up of 11 7-stranded antiparallel β-strands from one subunit and three β-strands from the adjacent subunit. This structural arrangement generates an extensive subunit-subunit interface, which is a major stabilizing force of the oligomeric structure.

Based on the multiple repeats in the TRAP binding sites and the 11 subunits of the TRAP oligomer, we have proposed that RNA binds to TRAP by wrapping around the protein, with each trinucleotide repeat of the RNA contacting one, or a combination of two adjacent subunits of TRAP (5). We have previously shown that the affinity of TRAP for an RNA containing residues 36–92 of the trp leader (RNA 36–92) increased linearly as the temperature was raised from 5 °C to 47 °C (9). This result implied a strong unfavorable enthalpic contribution (ΔH°) to the binding free energy. Furthermore, the stability of the RNA/TRAP complex was found to be virtually insensitive to changes in ionic strength between 100 mM and 700 mM potassium glutamate, indicating that release of ions upon complex formation is not the driving force of the interaction.

In this report, we continue the analysis of the RNA binding site for TRAP using in vitro selection (SELEX, for systematic evolution of ligand by exponential enrichment) of RNAs that bound specifically to tryptophan-activated TRAP from a pool of RNAs containing 25 positions with random nucleotides. All RNAs selected contained multiple GAG or UAG repeats, confirming the importance of these trinucleotide motifs. Surprisingly, >90% of the spacer nucleotides of the RNAs selected were pyrimidines (Us and Cs). In view of our model for the TRAP-RNA interaction, one possible explanation for the bias toward pyrimidines in the spacers might be their lesser ability to form base stacking interactions relative to purines (11). If so, this would suggest that base stacking interactions play a role in the TRAP-RNA interaction. In agreement with this hypothesis, we have shown that RNA 36–92 is stacked in solution and becomes unstacked when bound to TRAP. Further analysis

* This work was supported in part by National Science Foundation Grants MCB-9118654 and MCB-9603594. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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1 The abbreviations used are: TRAP, trp RNA-binding attenuation protein; SELEX, systematic evolution of ligand by exponential enrichment; PCR, polymerase chain reaction; oligo, oligonucleotide; SSB, single-stranded DNA-binding protein; ssDNA, single-stranded DNA.
2 P. Babitzke, personal communication.
showed that the temperature dependence of the TRAP-RNA 36–92 interaction is nonlinear, with a maximum at 47 °C. Since we have shown that the structure of the TRAP oligomer is stable at temperatures up to 67 °C, the nonlinear temperature dependence of the TRAP-RNA interaction likely reflects changes in the RNA structure at higher temperatures (i.e., unstacking of the bases). Therefore, we propose that the spacer nucleotides in the TRAP binding site play two roles: to allow the proper spacing of the trinucleotide repeats to interact with the binding sites on TRAP, and to maintain the flexibility needed for the trp leader RNA to wrap around the TRAP oligomer.

**EXPERIMENTAL PROCEDURES**

**Plasmodia and Strains—**Plasmodia used in this study were propagated in *Escherichia coli* JM107. Plasmid pTZ18U36–92 has been described previously (9). Plasmid pTZ18U2–64 contains residues +2 to +64 of the *B. subtilis* trp leader cloned downstream of the T7 promoter in pTZ18U (U. S. Biochemical Corp.). The insert was created by PCR using the ~20 Universal primer and trpl64 (5′-GCAAGCTTACCATTCATCTCTCAAC-3′) as primers, and pTZ18Udr4 (12) as template to generate a product containing bases 2–64 of the trp leader flanked by EcoRI and HindIII sites. Plasmid pCB1, which contains 11 repeats of the sequence 5′-GAGGAATTCAATTATAATA-3′, was a gift from the Price laboratory (9). Plasmid pUC118GAGUU or a hairpin ribozyme (pUC118GAGAU), and both were modeled on the system described in Price et al. (10). To generate an RNA containing the sequence 5′-GAGUU-3′ followed by 5′-GAGA-3′, respectively, cloned downstream of a T7 promoter. Each plasmid contains a fixed region to 1 mg/ml. For experiments involving L-tryptophan, both the sample and reference contained 1 mm t-tryptophan.

**Circular Dichroism—**Circular dichroism (CD) spectra were obtained on a JASCO model J-500C spectropolarimeter. TRAP was dialyzed into 50 mm potassium phosphate, (pH 7.4) and diluted to 1 mg/ml. Spectra were collected using 1-mm pathlength quartz cuvettes. For experiments at elevated temperatures, TRAP was equilibrated at 70 °C for 15 min prior to data acquisition. CD studies of trp leader RNA were performed using 1-cm pathlength cuvettes with 5 mm RNA 36–92. For RNA experiments containing TRAP and/or tryptophan, 5 μM TRAP and/or 50 μM t-tryptophan were included. Neither TRAP nor tryptophan show significant dichroism in this range (230–300 nm) and therefore make no contribution to the RNA spectra.

**RESULTS**

**RNA Binding Site for TRAP—**Two natural TRAP binding sites have been characterized and each contains multiple G/UAG trinucleotide repeats generally separated by two or three nucleotide spacers (4–5). Both of these sites must not only allow for efficient TRAP binding but must also function for proper gene regulation. To examine the sequence requirements of the RNA target of TRAP in the absence of this other selective influence, we used SELEX (10). RNAs that specifically bound to tryptophan-activated TRAP were selected from a pool containing 25 random positions. To follow the selection process, we measured the affinity of the selected RNA pools for TRAP using filter binding (9). We were unable to detect specific binding of the original random population of RNAs using TRAP concentrations up to 2 μM. By the fourth round of selection, the observed dissociation constant (Kd) was 40 nM, which improved to 20 nM in the 6th round and 10 nM in the 10th and 12th rounds. Since no improvement was seen between the 10th and 12th rounds, selections were stopped after 12 rounds.

 Fifteen CDNA clones were sequenced from the 4th round of *in vitro* selection, 14 from both the 6th and 10th rounds, and 11 from the 12th round. All RNAs selected contained between 3 and 6 repeats/RNA (those with 6 repeats involved contributions from fixed flanking sequences). No RNAs were found that spe-
specifically bound to TRAP in a tryptophan-dependent manner, which contained less than three G/UAG repeats. The average number of G/UAG repeats per RNA was 3.6 in the 4th round RNAs, which increased to 4.5/RNA in the 6th round and 4.6/RNA in the 10th and 12th rounds. This finding is consistent with the improved affinity of the RNA pools from the later rounds for TRAP. Since there were no significant differences observed in the composition of the RNAs from the 6th, 10th and 12th rounds, further analyses were done on the combined set of 39 RNAs from these rounds (Table I). The sequences of 10 representative clones from this pool are shown in Fig. 1.

The ratio of G to UAG repeats in the combined set of selected RNAs was 1.8/1 (Table I), which is consistent with previous findings indicating that TRAP binds GAG repeats better than UAGs (7, 8). In addition, Clone 87, which contains 6 UAG repeats, bound TRAP with a $K_{obs}$ of 12 nM, whereas a clone containing 4 GAG repeats (Clone 83) bound TRAP with a $K_{obs}$ of 3 nM (Fig. 1), which again shows that GAG repeats bind TRAP better than UAGs.

We also analyzed the length and composition of the spacer nucleotides separating the trinucleotide repeats (Table I). Most (89%) of the G/UAG repeats were separated by 2 nucleotides, which is similar to the spacing seen in the natural TRAP binding sites and is consistent with other studies indicating that 2 nucleotide spacers are optimal (7). Although the exact sequence of the spacers does not appear to be critical, there was a great bias (88%) for pyrimidines in the spacers (Table I).

Based on our model of the TRAP-RNA interaction, with each repeat of the RNA interacting with one subunit of TRAP, it could be possible for more than one of these 3–6-trinucleotide repeat-containing RNAs to bind to a single TRAP oligomer. Therefore, we were concerned as to whether each RNA was selected based on individually binding to TRAP or whether each RNA was selected based on individually binding to TRAP and the sequences surrounding the trpG ribosome binding site. The trpG binding site also contains 1 AAG.

To examine the base stacking interactions present in a natural TRAP binding site, we examined the temperature-dependent hyperchromicity of an RNA containing bases +36 to +92 of the trp leader (RNA 36–92; Ref. 9). RNA 36–92 was studied since it contains all 11 G/UAG repeats but is not predicted to form any significant secondary structures (9), which have been shown to interfere with TRAP binding (7). When the UV absorbance of RNA 36–92 was monitored as a function of temperature, a broad hyperchromatic transition was observed (Fig. 2A). For comparison, we examined thermal denaturation of poly(A) (Fig. 2A), a process that has been well characterized as indicative of the disruption of base stacking interactions (21). Thermal denaturation of poly(A) also displays a broad non-cooperative transition, similar to that observed for RNA 36–92. Therefore we conclude that the observed hyperchromatic shift of RNA 36–92 is likely due to disruption of base stacking interactions.

We also examined base stacking in RNA 36–92 by circular dichroism (CD) spectroscopy (Fig. 2B). The presence of the strong maximum centered around 265 nm in the CD spectrum of RNA 36–92 at 25° is indicative of base stacking in single-stranded nucleic acids (22). The intensity of this maximum decreased when the spectrum was obtained at 80 °C (Fig. 2B), indicating that base stacking interactions are being disrupted. Together with the UV hyperchromicity results described above, these data demonstrate that RNA 36–92 is stacked in solution and does not appear to contain significant secondary structure.

**Role of Base Stacking on the TRAP-RNA Interaction**—The model in which RNA wraps around TRAP implies significant structural changes in the RNA upon binding to TRAP. To investigate these changes, we examined the CD spectra of RNA 36–92 before and after binding to TRAP (Fig. 3). When an equimolar amount of tryptophan-activated TRAP was added to RNA 36–92, the intensity of the maximum at 265 nm decreased by over 60% (Fig. 3), demonstrating that the bases in the RNA become unstacked. This effect is dependent upon the RNA binding to TRAP, since addition of neither apoTRAP nor tryptophan alone induced significant changes in the RNA spectrum (data not shown). The absolute amount of this decrease is greater than that seen in thermally denatured RNA (Fig. 2B), suggesting that when bound to TRAP, the bases are separated by a greater distance than when the RNA is thermally dena-

### TABLE I

| Repeats | GAG | UAG | Spacers$^a$ | Pyrimidines | U | C | A | G |
|---------|-----|-----|------------|-------------|---|---|---|---|
| Selected in vitro$^b$ | 64$^c$ | 35$^c$ | 89% | 88% | 74 | 14 | 11.3 | 0.7 |
| Natural$^d$ | 70 | 25 | 80% | 50 | 35 | 15 | 40 | 10 |

$^a$ Spacers are defined as the nucleotides between two adjacent trinucleotide repeats, nt, nucleotides.

$^b$ 39 cDNA clones were analyzed from rounds 6, 10, and 12. These contained a total of 181 repeats consisting of 116 GAG, 64 UAG, and 1 CAG yielding an average of 4.64 trinucleotide repeats per RNA.

$^c$ Natural binding sites include the trpG ribosome binding site. The trpG binding site also contains 1 AAG.

$^d$ In calculating the ratio of GAG versus UAG repeats, repeats where the U in the first position came from nonrandom flanking sequence (such as clones 87 and 154) were not considered.
tured. In addition, a hyperchromatic shift in the UV spectrum of RNA 36–92 occurs upon binding to TRAP (data not shown), and the magnitude of this shift was 3-fold greater than that observed for thermally denatured RNA.

Effects of Temperatures on the TRAP-trp Leader RNA Interaction—In light of the indication that base stacking interactions play a role in the TRAP-RNA interaction, we examined the affinity of TRAP for RNA 36–92 at temperatures up to 67 °C (Fig. 4). As we have shown previously (9), the affinity of TRAP for RNA 36–92 increased as the temperature was raised from 5 °C to 47 °C with \( K_{\text{obs}} \) decreasing from 3.6 nM to 0.07 nM (Fig. 4). As the temperature was raised above 47 °C, the affinity of TRAP for \textit{trp} leader RNA gradually decreased; however, at 67 °C \( K_{\text{obs}} \) was still 1 nM.

The decrease in affinity of the RNA-TRAP interaction at temperatures above 47 °C could be due to reduced affinity of TRAP for L-tryptophan at these temperatures, resulting in TRAP not being fully activated to bind RNA. TRAP binds L-tryptophan cooperatively with an \( S_0.5 \) of 5 \( \mu \)M at 37 °C and 100 \( \mu \)M at 60 °C (data not shown), suggesting that TRAP should be fully activated in our assays containing 1 mM tryptophan. Moreover, TRAP had identical affinity for RNA 36–92 in the presence of 1 mM or 10 mM L-tryptophan at 52 °C and 67 °C, confirming that tryptophan is not limiting at these temperatures (data not shown).

A second possible explanation for the decrease in the affinity of the RNA-TRAP interaction at temperatures above 47 °C could be due to reduced affinity of TRAP for l-tryptophan at these temperatures, resulting in TRAP not being fully activated to bind RNA. TRAP binds l-tryptophan cooperatively with an \( S_0.5 \) of 5 \( \mu \)M at 37 °C and 100 \( \mu \)M at 60 °C (data not shown), suggesting that TRAP should be fully activated in our assays containing 1 mM tryptophan. Moreover, TRAP had identical affinity for RNA 36–92 in the presence of 1 mM or 10 mM l-tryptophan at 52 °C and 67 °C, confirming that tryptophan is not limiting at these temperatures (data not shown).
the high percentage of β-strands (>60%) seen in the crystal structure (5), the CD spectrum of TRAP displays a strong minimum at 215 nm (Fig. 5). The spectrum remained virtually unchanged at temperatures up to 70 °C (Fig. 5), indicating that the secondary structure of TRAP does not change significantly within this temperature range.

To characterize further the effects of temperature on TRAP, we used differential scanning calorimetry (Fig. 6). Between 15 °C and 70 °C, no significant thermodynamic changes were seen in TRAP either in the absence or presence of 1 mM l-tryptophan. The difference in the absolute heat capacities between the two samples represents the heat capacity of tryptophan. Above 70 °C, two major thermal transitions occur. The first has a midpoint of 90 °C in both samples and is reversible, as judged by scanning a sample that was previously heated to 95 °C (data not shown). The second transition has a midpoint of 103 °C in the absence of tryptophan and >105 °C in the presence of tryptophan. TRAP is irreversibly denatured at these temperatures. These experiments suggest there is little change in the structure of TRAP between 15 °C and 70 °C. Therefore, it does not appear that the decreased affinity of TRAP for trp leader RNA at temperatures between 47 °C and 67 °C can be attributed to thermal denaturation of TRAP.

Role of the Spacer Nucleotides in TRAP Binding—Previous studies have suggested that the residues separating the trinucleotide repeats in the TRAP binding site do not make specific contacts with the protein in the TRAP-RNA complex (4, 7) but instead are important for proper spacing of the GAG and UAG repeats (4, 5, 8). To test this hypothesis, we generated several RNAs containing artificial TRAP binding sites consisting of 11 GAG repeats separated by various two nucleotide spacers. We characterized the affinities of these RNAs for TRAP at temperatures between 37 °C and 62 °C. The first RNA (GAGUU)11 contained UU spacers, the second (GAGAA)11 had AA spacers, and the third RNA (GAGAU)11 contained AU spacers. The temperature dependence of TRAP binding to each of these RNAs was found to be nonlinear between 37 °C and 62 °C (data not shown). Direct comparison of the affinities of these RNAs for TRAP at temperatures below 47 °C was not possible since (GAGUU)11 forms a stable secondary structures at these temperatures, which has been shown to interfere with TRAP binding (7), while neither (GAGAA)11 nor (GAGAU)11 appear to form any significant secondary structures at these temperatures (data not shown). However, above 47 °C, TRAP binds all three RNAs with nearly equal affinities (data not shown), consistent with the proposal that the spacer nucleotides are not forming specific contacts with TRAP.

DISCUSSION

Two TRAP binding sites have been characterized in B. subtilis. One site, located within the leader region of the trpED-CFBA operon, contains 11 trinucleotide repeats (7 GAG and 4 UAG), each separated by two or three nucleotide spacers (4, 5). The second site, located 5′ of trpG (4, 6), contains 9 trinucleotide repeats (7 GAG, 1 UAG, and 1 AAG) with spacers up to 8 nucleotides long. Recent footprinting experiments have demonstrated that TRAP contacts all nine of these repeats, including...
the AAG repeat. The RNAs that we selected in vitro based on their ability to bind TRAP all contain multiple GAG and/or UAG repeats confirming the importance of these trinucleotides for TRAP binding. Moreover, as the affinity of RNAs from successive rounds of selection improved, the average number of G/UAG repeats/RNA increased. The ratio of GAG to UAG repeats in the in vitro selected RNAs was approximately 2:1 (Table I) suggesting TRAP binds GAGs better than UAGs. This finding is consistent both with the bias seen in the natural TRAP binding sites and with the results of Babitzke et al. (7, 8), who showed that RNAs containing GAG repeats bound TRAP significantly better than UAG containing RNAs. Most (89%) of the trinucleotide repeats in the RNAs selected in vitro were separated by two nucleotide spacers (Table I). This finding is also consistent with previous results (8) showing that two nucleotide spacers are optimal for TRAP binding. The natural TRAP binding sites also reflect this bias, with 70% of the spacers containing two nucleotides. However, the trp leader RNA also contains three 3-nucleotide spacers and the trpG binding site contains two rather long spacers of up to 8 nucleotides. The identities of the nucleotides in the spacers of the natural TRAP binding sites are relatively random, though there is a bias toward As in both binding sites and a preference for Us in the trp leader. In total, if the spacers from both natural sites are considered, there are equal numbers of pyrimidines and purines (Table I). In sharp contrast, there was a very strong bias (88%) toward pyrimidines in the spacer nucleotides of the RNAs we selected in vitro with Us favored over Cs; G residues are underrepresented in both in vitro selected RNAs and the natural TRAP binding sites (Table I).

The differences, particularly in the composition of the spacers, seen between our in vitro selected RNAs and the natural TRAP binding sites most likely reflect the regulatory functions of the natural TRAP binding sites in vivo. The sequence of the trp leader RNA must not only allow for TRAP binding but also form the appropriate secondary structures required for proper regulation of the trp operon (1). trpG RNA has been shown to bind TRAP with a lower affinity than trp leader RNA in vitro (4). This reduced affinity is consistent with the dual role of the amidotransferase encoded by trpG in both tryptophan and folic acid biosynthesis (23, 24), allowing for an appropriate level of trpG expression in the presence of excess tryptophan. Therefore, in both the trp leader RNA and in trpG RNA, the sequences of the TRAP binding sites appear to reflect the need for both TRAP binding and proper gene regulation.

Footprinting studies have shown that in trp leader RNA, the spacer nucleotides are not protected by TRAP binding and this was interpreted to indicate that the spacer nucleotides do not directly contact TRAP (4). When we performed RNA binding assays with three artificial TRAP binding sites with various spacer nucleotides similar to those previously (7), at temperatures where these RNAs should be unstacked, TRAP binds to all of these RNAs with nearly identical affinities (data not shown). This result also indicates that the spacer nucleotides do not play a direct role in TRAP binding. However, recent results (7) suggest that the composition of the spacers does have an effect on TRAP binding. RNAs containing either As or Us as the spacer nucleotides were found to bind TRAP better than those containing Cs or Gs in the spacers. While these results (7) reflect the bias seen in the natural TRAP binding sites, they differ from our in vitro selection results described above. This discrepancy could be due to the high concentrations of TRAP (5.0 nM) and RNA (2.5 nM) used in the filter binding experiments of Babitzke et al. (7). Under these conditions, relatively large differences in the affinity of these RNAs for TRAP would not be observed. Furthermore, these studies used an RNA containing the sequence 5'-GAGCU-3' repeated six times and we have found that similar RNAs containing homopyrimidine spacers form stable secondary structures at the temperatures used in these studies (data not shown). Since TRAP binds only single-stranded RNAs (7–9), the conclusion of Babitzke et al. (7) that Cs in the spacers are inhibitory to TRAP binding may instead reflect the tendency of this RNA to form secondary structures. Therefore, we propose that the spacer nucleotides do not contact TRAP but instead have an indirect role in TRAP binding related to their ability to form base stacking interactions, since we have demonstrated that trp leader RNA becomes unstacked when bound to TRAP (Fig. 3).

The affinity of the TRAP-RNA interaction displays a nonlinear temperature dependence between from 5 °C to 67 °C with a maximum at 47 °C (Fig. 4). Results from differential scanning calorimetry and circular dichroism spectroscopy indicate that the decrease in the affinity of the TRAP-RNA interaction above 47 °C cannot be attributed to thermal denaturation of TRAP. Furthermore, preliminary NMR data, to be published elsewhere, confirms that the structure of TRAP is virtually unchanged between 30 °C and 60 °C. Several models have been proposed to explain a nonlinear temperature dependence in protein: nucleic acid interactions: 1) the heat capacity model proposed by Record and co-workers (25, 26) and 2) the coupled equilibria model proposed by Ferrari and Lohman (27).

Based on studies of the lac repressor-DNA interaction, Record and co-workers proposed that a large negative change in the standard molar heat capacity (ΔCp°) is the primary source of the observed nonlinear temperature dependence (25, 26). This model predicts that the ΔH° and ΔS° of the association process vary in parallel as a function of temperature, resulting in a ΔG° that is nearly temperature-independent. At the temperature where Kobs is maximal ΔH° = 0 (Tobs), and at the temperature where ΔG° is maximal ΔS° = 0 (Tobs). According to this model, the major source of a ΔCp° is removal of nonpolar surfaces from the aqueous environment upon complex formation, otherwise known as the hydrophobic effect. Therefore, they suggest that the major driving force of the lac repressor-DNA interaction is the removal of nonpolar surfaces from the aqueous environment with the concomitant release of H2O upon complex formation. Recent studies have shown that the interaction of the splicosomal protein U1A with its RNA stem-loop target also displays a nonlinear temperature dependence (28, 29). Moreover, it has also been proposed that a ΔCp° is the source of the nonlinear temperature dependence in this interaction.

The second model describes the thermodynamics of the interaction between E. coli single-stranded DNA-binding protein (SSB) and poly(dA) (27). In this system, the temperature dependence of SSB binding to poly(dC) or poly(dT) is linear while binding to poly(dA) displays a nonlinear temperature dependence. To explain these results, the authors proposed that SSB only binds to fully unstacked nucleic acids. Since the unstacking of poly(dA) has a positive change in enthalpy (∆H) and the binding of SSB to unstacked poly(dA) has an negative enthalpy change (∆H), the observed temperature dependence represents the thermodynamic coupling of poly(dA) unstacking to SSB-poly(dA) binding. At low temperatures, poly(dA) is stacked and binds SSB relatively poorly. As the temperature increases, poly(dA) becomes unstacked and SSB binding improves until a temperature is reached where ∆Hunstacking = ∆Hbinding; this temperature represents the inflection point in the van’t Hoff plot. Above this temperature, poly(dA) is fully unstacked and the SSB-poly(dA) interaction decreases, reflecting the ∆H of this interaction.

3 P. Flynn, J. Wand, and P. Gollnick, unpublished results.
Based on the insensitivity of the TRAP-trRNA interaction to ionic strength, we previously proposed that the association of TRAP with trp leader RNA is driven by the release of ordered H$_2$O molecules upon complex formation (9). If this hypothesis is correct, the nonlinear-temperature dependence observed for the TRAP-trp leader RNA interaction could be explained by the $\Delta C_p$ model proposed by Record and co-workers (25, 26). However, this model requires that the structures of the protein and the nucleic acid, both alone and in complex with each other, do not change as a function of temperature (27). While our studies have demonstrated that the structure of TRAP remains virtually unchanged in the temperature range studied, trp leader RNA is clearly undergoing a temperature-dependent structural change (27). Unstacking of the bases in the RNA is consistent with the disruption of base stacking interactions (22, 23). Unstacking of the bases in solution (Fig. 2; Refs. 21–22 and 30) and the gene 5 protein from the bacteriophage fd (35, 39) have similarities between TRAP and gp32, whose structure has been solved with a large increase in the average base to base distance, indicative of the disruption of base stacking interactions (22, 23).

Intriguingly, these three residues fall on a circle with the TRAP oligomer (5). The mechanism of interaction, suggesting that the coupled equilibria model proposed by Ferrari and Lohman (27) may better describe the thermodynamics of the TRAP-trp leader RNA interaction. trp leader RNA, like other single-stranded nucleic acids, is stacked in solution (Fig. 2; Refs. 21–22 and 30). When bound to TRAP, the structure of this RNA changes dramatically (Fig. 3) with a large increase in the average base to base distance, indicative of the disruption of base stacking interactions (22, 23).

We thank Jerry Koudelka, Peter Flynn, Barry Hurlburt, and John Otridge for critical review of the manuscript and David Draper, Jerry Koudelka, and Min Yang for many useful discussions. We are grateful to Joshua Wand for use of the differential scanning calorimeter, Robert Straubinger for use of the circular dichroism spectrophotometer, Mark Erhardt for help with the differential scanning calorimetry experiments, and Paul Babitzke for sharing results prior to publication. We are also indebted to Xiao-ping Chen for excellent technical assistance and Jack Keene for generously donating the N25 oligo used in our in vitro selection experiments.

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