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Specific Interaction between Ribosomal Protein S4 and the α Operon Messenger RNA†

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ABSTRACT: The *Escherichia coli* ribosomal protein S4 is known to repress translation of its own gene and several other ribosomal protein (r-protein) genes in the α operon as part of a general mechanism coordinating the levels of rRNA and r-protein synthesis. Using a filter binding assay and RNA transcripts prepared in vitro, we have detected and quantitated specific interactions between S4 and α mRNA fragments. The main results are the following: (i) Only the α mRNA leader is required for specific recognition, with a small fraction of the binding free energy derived from sequences at the ribosome initiation site. (ii) 16S rRNA and α mRNA compete for binding to S4 with about the same affinity ($\approx 2 \times 10^7$ M^{-1}), suggesting that S4 utilizes the same recognition features in each RNA. (iii) Nonspecific binding of S4 to tRNA or other mRNA sequences is strongly salt dependent, while the specific S4–α mRNA affinity is nearly independent of salt. (iv) At physiological salt concentrations the nonspecific S4–RNA affinity (10^5–10^6 M^{-1}) is large enough to strongly buffer the free S4 concentration in vivo.

There is considerable evidence that specific interactions between ribosomal proteins and messenger RNAs are responsible for the close coordination of ribosomal protein and ribosomal RNA synthesis in bacteria. According to the autoregulation hypothesis [reviewed by Lindahl & Zengel (1982) and Nomura et al. (1984)], certain ribosomal proteins, if accumulated in excess over ribosomal RNA, bind to specific sites on their own mRNA to repress further translation of r-proteins.[1] Each r-protein operon codes for one such r-protein repressor specific for that operon. The repressor proteins all bind directly to ribosomal RNA early in ribosome assembly and presumably use the same RNA binding site to recognize both the ribosomal and messenger RNA sites. This transla-

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[1] Abbreviations: r-protein, ribosomal protein; RBS, ribosome binding site; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; kb, kilobase; bp, base pair; EDTA, ethylenediaminetetraacetic acid.

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tional regulation has been demonstrated by gene dosage experiments and by translational inhibition by specific ribosomal proteins in vitro (Dean & Nomura, 1980; Yates et al., 1980). Even though there is good evidence that at least nine r-protein operons autoregulate expression, only one demonstration of an r-protein–mRNA complex has been made (the L10–L7/L12 protein complex with the corresponding *rif* operon mRNA; Johnsen et al., 1982; Christensen et al., 1984), and no estimates of the binding strength or degree of specificity are available.

We have undertaken a study of ribosomal protein S4 binding to the α operon mRNA for two reasons. First, a study of the protein–RNA binding mechanism and thermodynamics is required to understand autoregulation. Second, the ribosomal RNA binding site for S4 encompasses nearly 600 bases (Zimmermann, 1980); the RNA features that S4 recognizes are not known. Comparing the structures of both the mRNA and rRNA S4 binding sites may suggest important recognition features shared by the two sites. In this paper we are concerned with isolating an mRNA fragment capable of specific S4 recognition and quantitating the S4–α mRNA and S4–16S rRNA affinities.

Materials and Methods

S4 Purification. Ribosomes were prepared from MRE600 cells (Grain Processing), and ribosomal proteins were extracted into acetic acid following the procedures of Hardy et al. (1969). About 300 mg of 70S ribosomal proteins in extraction buffer was dialyzed against two changes of 15% acetic acid, followed by two changes of 6 M urea, 50 mM potassium phosphate, and 0.5 mM dithiothreitol, adjusted to pH 5.6 with methylamine. The protein was then loaded onto a 150 × 21.5 mm cation-exchange column (TSK SP-5-PW, Bio-Rad). Proteins were eluted with a 400-mL linear gradient from 0 to 0.4 M KCl in the same buffer. Fractions containing S4 were pooled and rerun on a smaller column (75 × 7.5 mm) of the same type, with a shallower gradient. After rerunning the protein twice, its purity, estimated from a Coomassie Blue stained SDS gel, was about 95%. The protein was stored at −70 °C in the elution buffer. An extinction coefficient of 1.20 mg/(mL·cm) at 280 nm was used (Rhode et al., 1975). The molecular weight of S4 is 23 137 (Schiltz & Reinbolt, 1975).

Plasmids. A bacterial strain carrying the plasmid pNO1592 was provided by M. Nomura; it contains a 2.8-kb *Pst*I fragment of λ*spc*1, covering the 3′ end of the *spc* operon and the 5′ end of the α operon, cloned in the *Pst*I site of pBR322. A 213-bp DNA fragment from the α operon was prepared by digestion with nucleases *Alu*I and *Pst*I; this DNA was cloned between the *Sma*I and *Pst*I sites of pT7-1 (US Biochemicals) to give pT7AP3. The M13 phage derivative DWM3 was provided by R. Hayward; it contains a 262-bp *Sau*3A fragment starting immediately after the α gene termination codon and extending into the L17 coding region, cloned into the *Bam*HI site of M13mp9. This fragment was excised and recloned in pT7-1 (pT7DVM28). pNO2687 was also provided by M. Nomura; it contains a 212-bp *Hin*dIII–*Eco*RI DNA fragment covering the L11 gene ribosome binding site, cloned into pSP64 (Baughman & Nomura, 1984). All plasmids were propagated in HB101. Final plasmid purification steps included gel filtration on Sephacryl S500 (Pharmacia) and RPC-5 chromatography. No RNA was detectable in these preparations.

Preparation of RNA Fragments. T7 RNA polymerase was purified from the overproducer HMS174(pAR1219), obtained from F. W. Studier (Davanloo et al., 1984). Runoff transcripts were made in a buffer containing 20 mM Tris, pH 8.0, 8 mM $MgCl_2$, 6 mM dithiothreitol, 0.4 mM each of the nucleoside triphosphates, ≈5 μCi of adenosine 5′-(α-[^{35}S]thiotriphosphate) (Amersham, 600 Ci/mmol), and 4 μg of linear plasmid DNA in a 100-μL volume. Sufficient T7 RNA polymerase was added to obtain about 50% incorporation of the ATP in a 1-h incubation at 37 °C. Transcription with SP6 RNA polymerase (New England Nuclear) was carried out for 1 h at 37 °C in a different buffer (40 mM Tris, pH 8.0, 6 mM $MgCl_2$, 5 mM spermidine, 10 mM dithiothreitol, 0.4 mg/mL BSA, and the same triphosphate and DNA concentrations as for T7 RNA polymerase).

An RNA transcript was purified by first loading the transcription reaction onto a small reversed-phase column, using triethylamine as the ion pair reagent as recommended by the supplier (New England Nuclear "NENSorb" columns). After the column was rinsed with 1.0 mL of water, the RNA was eluted with 50% ethanol and precipitated by adding sodium acetate and additional ethanol. The RNA pellet was resuspended in 10 mM Tris, pH 7.6, and 1 mM EDTA and was phenol-extracted, followed by ether extraction and two ethanol precipitations. We found that the reversed-phase column was essential for obtaining reproducible filter binding results with low background retention of the RNA. The integrity of the RNA transcripts was checked by electrophoresing samples on 6% acrylamide–8.3 M urea gels in 0.1 M Tris–borate buffer with 2 mM EDTA. The RNA was examined either by autoradiography or by staining with ethidium bromide. Only the full-length RNA transcript was observed, unless the gel was heavily overloaded. Generally the plasmid DNA used for the transcription was not removed from the RNA preparations, since purifying the RNA from a gel did not alter the binding results.

Transcription of pT7AP3 with T7 RNA polymerase yields an RNA with 25 bases at the 5′ terminus from the pT7-1 sequence, followed by 16 bases of the α promoter sequence and then the normal α mRNA transcript. Cutting the plasmid with the restriction enzymes *Rsa*I, *Hae*III, and *Hin*dIII yields transcripts of 110, 139, and 252 bases in total length, respectively. (Cutting with *Hin*dIII leaves 8 bases of the pT7-1 sequence at the 3′ terminus, following the *Pst*I site.) These RNA fragments will be referred to as "α110", "α139", and "α252". pT7DVM28 DNA was cut with *Pst*I to give a 308-base transcript containing 28 and 14 base sequences from pT7-1 at the 5′ and 3′ termini, respectively. This RNA is called "L17 RBS". pNO2687 DNA was cut with *Eco*RI to yield a 223-base RNA transcript, with 6 bases at the 5′ terminus derived from pSP64 ("L11 RBS").

Intact 16S rRNA was prepared by extracting total RNA from MRE600 cells, loading this onto a TSK DEAE-5-PW column (5 × 7.5 mm, Bio-Rad), and eluting with a gradient from 0.3 to 0.5 M NaCl in 0.1 M Tris, pH 7.6. The peak of 16S rRNA was ethanol-precipitated and run over the column a second time.

Filter Binding Assays. Protein and RNA were renatured separately before forming a complex: S4 at 37 °C for 30 min in 30 mM Tris, pH 7.6, 0.35 M KCl, 20 mM $MgCl_2$, and 15 mM β-mercaptoethanol and RNA for 5 min at 65 °C in 10 mM Tris, pH 7.6, 0.2 M NaCl, and 1 mM EDTA. After being cooled to room temperature, the RNA and protein were diluted into appropriate buffers for a final reaction volume of 50 μL and incubated at 37 °C for 10 min. The final salt concentrations in the reactions were either 30 mM Tris, pH 7.6, 8 mM $MgCl_2$, 140 mM KCl, and 20 mM NaCl ("MS buffer") or 30 mM Tris, pH 7.6, 20 mM $MgCl_2$, 350 mM KCl, and 20 mM NaCl ("HS buffer"). Each reaction contained (3–10) × 10^3 cpm ^{35}S-labeled RNA, corresponding to ≈10^{-9}

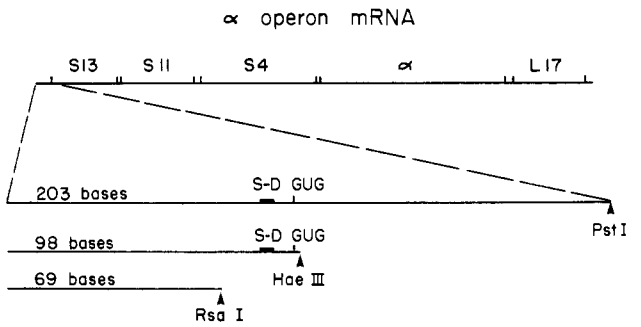

FIGURE 1: α operon mRNA, coding for five proteins. RNA sequences containing the α mRNA leader and used in this study are shown in the lower part of the figure. Three RNAs were prepared, all having the same 5′ terminus and ending at or near the indicated restriction site sequences. The actual transcripts prepared contain 41 additional nucleotides at the 5′ end, and the longest has 8 additional nucleotides at the 3′ end, to give total lengths of 110, 139, and 252 nucleotides. S–D and GUG indicate the positions of the Shine–Dalgarno sequence and initiation codon, respectively. The drawings are approximately to scale.

M RNA transcripts (stoichiometric titrations, as in Figure 2B, were done with RNA of ≈100-fold lower specific activity). The reaction was pipetted directly onto a 25-mm Millipore type HA 0.45-μm filter, previously degassed in the binding buffer, with slow suction. Filters were dried at 100 °C and counted with 3 mL of scintillation fluid. The data were corrected for the number of counts retained on the filter in the absence of protein (7–13% of the total radioactivity). Because the S4 protein is diluted from storage buffer containing 6 M urea, the binding assays were adjusted to contain a final concentration of 0.12 M urea, regardless of the amount of protein added. Increasing this to 0.24 M did not affect the binding affinity, but only lowered the maximum retention on the filters.

To estimate binding constants from the data, the maximum retention was calculated by plotting the reciprocal of the counts retained as a function of the reciprocal of the protein concentration for the five or six points taken at the highest protein concentrations; the y intercept is the maximum retention. This was between 45% and 50% of the total RNA for titrations with low RNA concentrations. For the stoichiometric titrations, 70% of the RNA could be retained on the filter. A simple computer program was used to display a family of hyperbolic binding curves superimposed on the data, and the best fit was selected by eye. The accuracy of the data did not warrant more elaborate procedures.

Results and Discussion

The α Operon. A map of the α operon is shown in Figure 1; the entire sequence has been published recently (Bedwell et al., 1985). There are four ribosomal protein genes and the gene coding for the α subunit of RNA polymerase. In vivo overproduction of S4 represses the synthesis of the remaining three r-proteins (Dean & Nomura, 1980; Nomura et al., 1980), while in vitro experiments have demonstrated an effect of S4 only on S13, S11, and S4 synthesis (Yates et al., 1980). The first 202 bases of the operon are required for S4 repression and presumably contain the S4 recognition site (Nomura et al., 1980). It is not clear how the α gene escapes regulation by S4, while genes on either side are regulated. Meek & Hayward (1984) have proposed that translation of the first three genes is coupled and regulated by an S4 binding site at the 5′ terminus, while a second S4 binding site just preceding the L17 gene affects only that gene. Support for this hypothesis comes from the observation that similar secondary

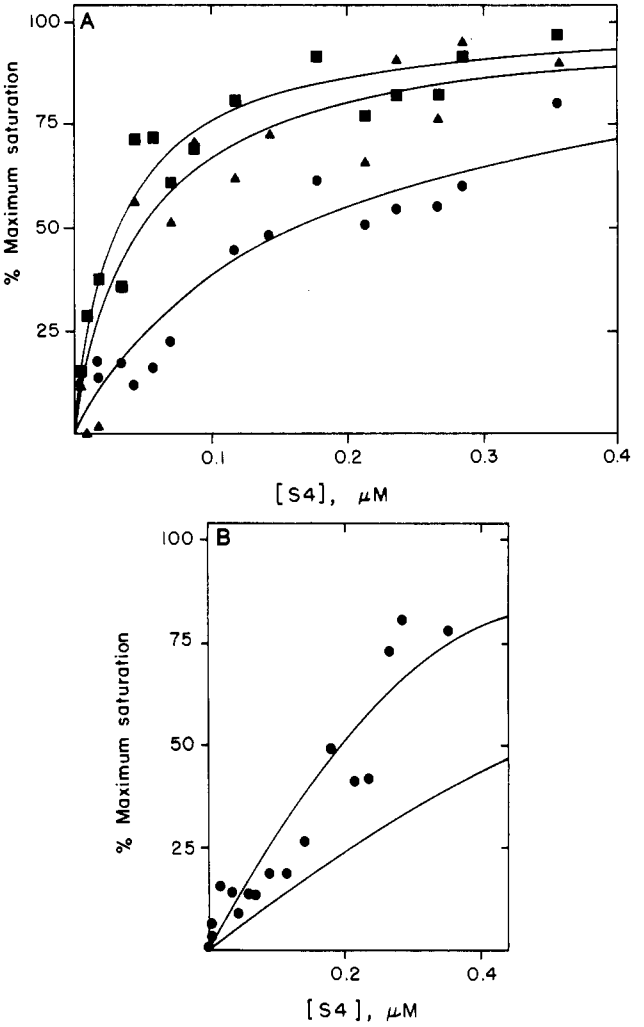

FIGURE 2: Titration of α mRNA fragments with S4 protein in MS buffer. Filter binding assays were performed as described under Materials and Methods. (A) Titration of α252 (■), α139 (▲), or α110 (●); protein is always in large molar excess over RNA. Curves drawn are calculated by using binding constants of 3×10^7 M^{-1} (α252), 2×10^7 M^{-1} (α139), and 6×10^6 M^{-1} (α110). (B) Stoichiometric titration of the α139 RNA with S4. The upper curve shown was calculated by assuming full protein activity, a 1:1 ratio of S4 to RNA in the complex, and a binding constant of 2×10^7 M^{-1}; for the lower curve 50% activity of S4 in binding is assumed.

structures can be drawn for a region of the 16S rRNA presumed to bind S4 and for the ribosome binding sites of the S13 gene and L17 gene (Nomura et al., 1980; Meek & Hayward, 1984).

Binding of S4 to α mRNA Fragments. We first compared the binding of S4 to three different RNA fragments, all containing the α mRNA 5′ terminus and extending various lengths into the mRNA leader and S13 coding region (see Figure 1). Protein–RNA complexes were formed in a moderate ionic strength buffer (MS buffer: 8 mM Mg^{2+}, 190 mM monovalent cations) to approximate in vivo ionic conditions [found by Kao-Haung et al. (1977) to be ≈0.2 M ionic strength]. The two longest fragments (α139 and α252) bind with identical affinity, 2.5×10^7 M^{-1} (Figure 2 and Table I). Removing another 29 nucleotides from the 3′ end (α110 RNA) reduces this affinity by about 5-fold. We show below that all of these binding interactions are specific and conclude that the S4 recognition site requires sequences just 5′ to the S13 initiation codon, but not 3′ to it.

The titrations shown in Figure 2A were done with a large excess of protein over RNA. If only a small fraction of the

Table I: S4–RNA Association Constants[a]

RNA	K (μM^{-1}), MS buffer	K (μM^{-1}), HS buffer
α110	5	4
α139	25	nd
α252	25	20
L17 RBS	5.5	<0.5
L11 RBS	7	0.7
$tRNA^{Phe}$	0.3[b]	<0.05[b]
poly(A)–poly(U)	<0.01[b]	nd[c]
16S rRNA	nd[c]	17[b]

[a] Binding constants are the average of two or three determinations and are reproducible to within a factor of 2. [b] Binding constant determined by competition with α139 or α252 RNA, assuming one S4 binding site per competitor. [c] Not determined.

protein is able to bind the RNA tightly, the binding constants we measure will be correspondingly low. To check for this possibility, the titration of α139 was repeated with a stoichiometric amount of RNA (Figure 2B). A curve drawn by using the affinity constant calculated from titrations at low RNA concentrations and assuming full S4 activity fits the data well. Assuming only 50% of the protein is active does not account for the data. Therefore, our affinity constant measurements are not biased by partially active protein preparations.

Nomura et al. (1980) noted a set of sequence and structure homologies between a potential secondary structure in the α mRNA and a hairpin in the 16S rRNA. The proposed secondary structure covers the S13 gene ribosome binding site and requires sequences 3′ to the initiation codon. α139 RNA terminates one nucleotide after the initiation codon and cannot contain the proposed secondary structure. Since α139 RNA contains the full S4 recognition site, we conclude that the noted homologies are coincidental. Using computer search programs, we have found other potential stable secondary foldings with some homologies to 16S rRNA helices, but experimental work on the α mRNA structure is necessary before drawing any conclusions about S4 recognition features.

Specificity of S4 Binding. To see if the S4 binding with α mRNA is specific, we tested several other RNAs for binding under the same conditons. One is the L11 ribosome binding site, demonstrated by Baughman & Nomura (1984) to contain the target site for translational repression by protein L1. S4 does not affect translation at this site in vitro (Yates et al., 1980). The L11 RBS RNA binds to S4 in MS buffer with significant affinity, 7×10^6 M^{-1} (Table I), but 5-fold weaker than α252 or α139 RNA. We also found significant competition between yeast $tRNA^{Phe}$ and α139 RNA for S4 binding in MS buffer, with a nonspecific affinity of 3×10^5 M^{-1}. Only the synthetic polynucleotide poly(A)–poly(U) gave an insignificant level of binding ($K < 10^4$ M^{-1}).

These experiments demonstrate that S4 prefers binding to the α mRNA over other RNAs, but with an unexpectedly small degree of specificity. Since ribosome assembly in vitro requires high salt concentrations (Traub & Nomura, 1969), we measured S4–α mRNA binding in a buffer similar to the ribosome reconstitution buffer (HS buffer: 20 mM Mg^{2+}, 400 mM monovalent cations) to see if specificity is enhanced. Electrostatic interactions should be weakened in higher salt, and in fact $tRNA^{Phe}$ and L11 RBS bind an order of magnitude more weakly (Figure 3 and Table I). The surprising result was that α252 RNA did not show significant changes in binding affinity. Evidently S4 binds RNA in two different modes: nonspecific binding has a large electrostatic component, reflected in a strong salt dependence, while specific recognition is approximately independent of salt.[2] Notice that

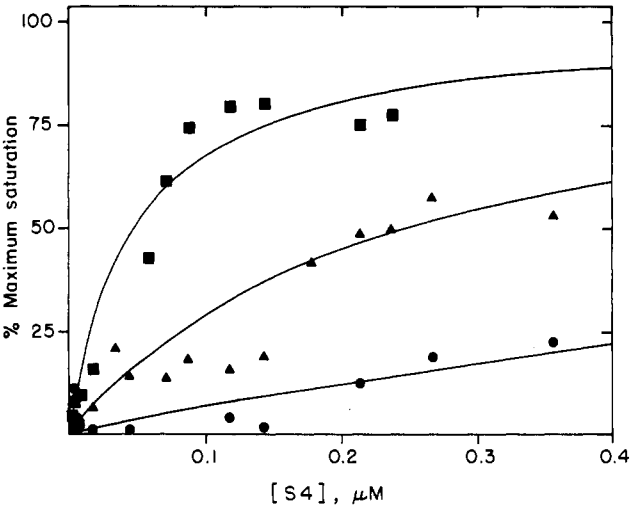

FIGURE 3: Titration of α252 (■), α110 (▲), and L11 RBS (●) RNAs with S4 in HS buffer. Curves are drawn by using binding constants of 2×10^7 M^{-1} (α252), 4×10^6 M^{-1} (α110), and 7×10^5 M^{-1} (L11 RBS).

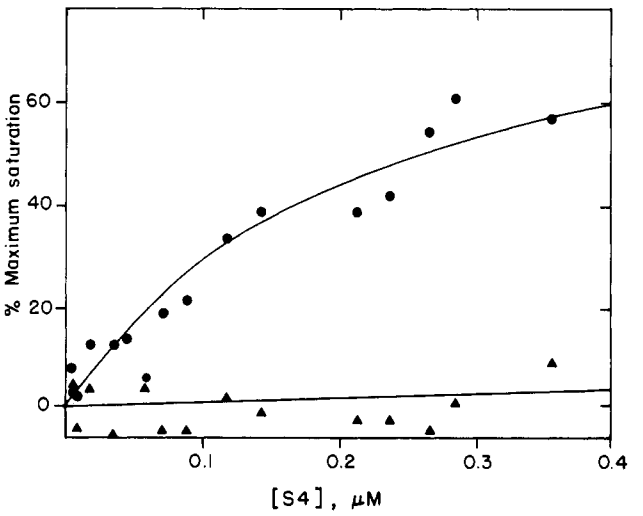

FIGURE 4: Titrations of L17 RBS RNA with S4 in MS buffer (●), $K = 4.5 \times 10^6$ M^{-1}, and HS buffer (▲), $K = 10^5$ M^{-1}.

α110 RNA, which showed the same affinity for S4 as L11 RBS RNA in MS buffer, also binds in the "salt-independent" mode and shows specificity for S4 in HS buffer. Most of the S4 affinity for α mRNA must derive from interactions with the α110 RNA sequence.

Does S4 Bind Specifically at the L17 Ribosome Binding Site? An RNA fragment containing the L17 ribosome binding site was also prepared and tested for S4 binding. In MS buffer significant binding is observed ($K = 5.5 \times 10^6$ M^{-1}), but binding is at least an order of magnitude weaker in HS buffer (Figure 4). Since this salt-dependent binding parallels the behavior of the nonspecific L11 RBS RNA, we conclude that S4 does not bind the L17 RBS specifically. This binding rules against separate regulation by S4 at this site. Bedwell et al. (1985) have ruled out differential transcription of the L17 gene

[2] Although it is possible to estimate the number of ion pairs involved in a protein–nucleic acid complex from the salt dependence of the binding constant, the presence of Mg^{2+} complicates the analysis (Lohman et al., 1980). It is also possible that the changing salt perturbs the apparent S4–α mRNA binding affinity by affecting the RNA conformation. For these reasons the absence of salt dependence in our measurements is difficult to interpret. More detailed studies of the salt dependence at low Mg^{2+} concentrations, which may give a more reliable estimate of the ionic contribution, are in progress.

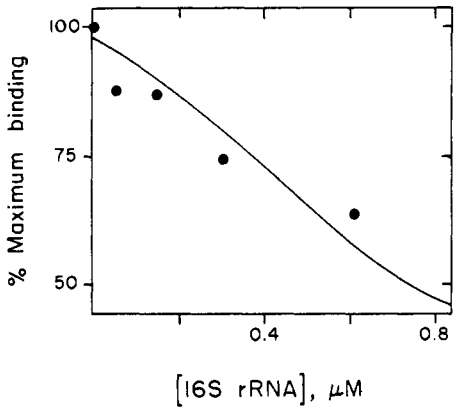

FIGURE 5: Competition for S4 binding to the α139 RNA in HS buffer. The curve is drawn by presuming S4 binds to α252 with $K = 2 \times 10^7$ M^{-1} and to 16S rRNA with $K = 1.7 \times 10^7$ M^{-1}.

as a regulatory mechanism, which leaves the mechanism that coordinates L17 synthesis with the other α operon r-proteins a puzzle.

Comparison of mRNA and rRNA Binding Strengths. Competition between α252 RNA and unlabeled 16S rRNA for binding S4 in HS buffer is shown in Figure 5. We had some difficulty carrying out competition reactions at very high tRNA or 16S rRNA concentrations (>0.5 mg/mL), as >20% of the labeled RNA was retained on the filter in the absence of protein, apparently caused by aggregation of the RNA. We were therefore limited to less than 0.5 μM 16S RNA in the reactions and could observe no more than about 50% competition (Figure 5). The same nucleotide concentration of tRNA gave no significant competition (data not shown). Using the affinity constant measured by Schwarzbauer & Craven (1981) for the S4–16S rRNA complex in ribosome reconstitution buffer, 1.7×10^7 M^{-1}, and the measured S4–α252 RNA affinity (Table I), we predict a competition curve in reasonable aggrement with the data (Figure 5). Thus S4 recognizes the α mRNA and the 16S rRNA with about the same affinity and may make use of the same recognition features in each.

Does Equilibrium Competition Account for in Vivo r-Protein Pool Sizes? The actual extent to which S4 binds the α mRNA and represses translation in vivo depends on a number of parameters: the concentrations of free S4 and free ribosomes, the affinities of each for α mRNA, and the concentrations of free 16S rRNA and nonspecific RNA and their affinities for S4. Competing reaction rates may be important as well as competing equilibria. von Hippel & Fairfield (1983) have discussed the equilibrium case and point out that the extreme cooperativity of ribosome assembly effectively makes the S4–16S rRNA affinity very large. Thus free S4 will accumulate and start to bind α mRNA only after all the 16S rRNA in the cell is titrated. Measurements of r-protein pool sizes have been made at different cell growth rates (Gausing, 1974), and are on the order of 1 μM. The concentration of S4 available for binding α mRNA will be reduced from this pool concentration by any nonspecific binding. We can make an order of magnitude estimate of the free S4 concentration by assuming (1) that the effective in vivo ionic strength of a cell is ≈0.2 M (Kao-Huang et al., 1977), roughly that of our MS buffer, and (2) that tRNA is the most abundant RNA free to compete with α mRNA for S4 binding. Since tRNA binds S4 with an affinity of $\approx 10^5$ M^{-1} in MS buffer and reaches an in vivo concentration of ≈100 μM at moderate growth rates (Kjeldgaard & Gausing, 1974), the predicted order of magnitude of the free S4 concentration is 0.1 μM. Thus nonspecific binding is an important factor in determining the level of r-protein required for autoregulation. Since concentrations of mRNA, tRNA, and different ions are a function of cell growth conditions (Kjeldgaard & Gausing, 1974; Roe & Record, 1985), the level of nonspecific binding is expected to vary with growth conditions as well. The observation that r-protein pool sizes increase with growth rate may reflect, in part, different levels of nonspecific binding.

To predict the degree of translational repression expected from 0.1 μM free S4, it is necessary to know how effectively S4 competes with ribosomes for initiation of translation. Only one experiment in the literature bears on this: Yates et al. (1980) observed nearly complete repression of translation by 1 μM S4 in an in vitro r-protein synthesis system using 1 μM ribosomes. We estimate that the free S4 concentration was ≈0.5 μM, which predicts a 90–95% degree of α mRNA saturation in the absence of ribosomes. S4 must therefore compete very effectively with ribosomes, and our estimate of 0.1 μM free S4 in vivo predicts a significant degree of translational repression. This agrees with the autoregulation proposal, which contends that translational repression significantly reduces r-protein expression from the maximum capacity of the cell, especially at low growth rates when relatively few ribosomes are being synthesized (Nomura et al., 1984). Our estimates are clearly only order of magnitude, and more quantitative measurements of the degree of α mRNA repression induced by S4 in vitro and in vivo need to be made before we can rule out the possibility that other factors influence the level of α operon expression.

CONCLUSIONS

Our binding studies indicate that there is a specific interaction between S4 and the leader sequence of the α mRNA. Bases just preceding the initiator codon and contained within the presumed ribosome binding site are required for full binding strength but contribute only a small fraction of the total binding free energy. In contrast, most other translational repressors recognize a limited sequence or structure containing the initiation codon or Shine–Dalgarno sequence [e.g., the R17 coat protein (Krug et al., 1982) and the T4 *regA* protein (Karam et al., 1981)]. An exception is the L10–L7/L12 repressor of the L10 operon, which binds over 100 bases upstream of the L10 initiation codon and is thought to induce a large structural transition in the mRNA (Christensen et al., 1984). It is likely that a similar indirect mechanism will be required to account for S4 repression of α mRNA translation, rather than direct competition for the ribosome recognition sequences.

The α mRNA leader and 16S rRNA compete for binding to S4 with about the same affinity, even though the two RNAs appear unrelated in sequence and in potential overall secondary structures. An intriguing possibility is that the RNAs may have quite different foldings which present the same three-dimensional array of recognition features to S4. Efforts are under way in this laboratory to deduce the α mRNA secondary structure and nucleotides required for S4 binding.

Since S4 (and most ribosomal proteins) is rather basic, fairly strong, nonspecific electrostatic binding to RNA is expected in low salt buffers. A conclusion of this study is that nonspecific binding is strong enough under physiological salt conditions to compete effectively with specific binding. We note that the L10–L7/L12 complex also binds only an order of magnitude more weakly to nonspecific mRNAs as to the target L10 mRNA (Christensen et al., 1984). The importance of nonspecific binding for explaining in vivo levels of the repression of the lac operon by lac repressor has been pointed

out (Kao-Huang et al., 1977). In the same way, the "buffering" of free ribosomal protein concentrations by a large excess of nonspecific sites may be of general importance in explaining the levels of translational repression in r-protein synthesis.

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