Evidence for Allosteric Coupling between the Ribosome and Repressor Binding Sites of a Translationally Regulated mRNA†

Careen K. Tang and David E. Draper*
Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218
Received November 1, 1989; Revised Manuscript Received January 4, 1990

ABSTRACT: *Escherichia coli* ribosomal protein S4 is a translational repressor regulating the expression of four ribosomal genes in the α operon. In vitro studies have shown that the protein specifically recognizes an unusual mRNA pseudoknot secondary structure which links sequences upstream and downstream of the ribosome binding site for *rps*M (S13) [Tang, C. K., & Draper, D. E. (1989) *Cell 57*, 531]. We have prepared fusions of the *rps*M translational initiation site and *lac*Z that allow us to detect repression in cells in which overproduction of S4 repressor can be induced. Twenty-five mRNA sequence variants have been introduced into the S13–*lac*Z fusions and the levels of translational repression measured. Sets of compensating base changes confirm the importance of the pseudoknot secondary structure for translational repression. An A residue in a looped, single-stranded sequence is also required for S4 recognition and may contact S4 directly. Comparison of translational repression levels and S4 binding constants for the set of mRNA mutations show that nine mutants are repressed much more weakly than predicted from their affinity for S4; in extreme cases no repression can be detected for variants with unchanged S4 binding. We suggest that the mRNA contains functionally distinct ribosome and repressor binding sites that are allosterically coupled. Mutations can relieve translational repression by disrupting the linkage between the two sites without altering S4 binding. This proposal assigns to the mRNA a more active role in mediating translational repression than found in other translational repression systems.

Much of the regulation of gene expression that takes place in a cell is at the translational level. One of the better described translational control systems coordinates the synthesis of most ribosomal proteins in *Escherichia coli* with ribosomal RNA synthesis. Certain ribosomal proteins, if synthesized at rates higher than needed to assemble ribosomes, bind specifically to their own mRNAs to repress further ribosomal protein translation [reviewed by Nomura et al. (1984) and Lindahl and Zengel (1986)]. In the case of the α operon, accumulation of free S4 leads to specific repression of all four ribosomal proteins encoded by the operon, S13, S11, S4, and L17 (Yates et al., 1980; Thomas et al., 1987). (The α subunit of RNA polymerase is present on the same mRNA transcripts, but its synthesis is unaffected by S4.) The α mRNA leader sequence is required for translational repression to take place in vivo (Thomas et al., 1987), and specific interactions between the first 139 nucleotides of the α transcript and S4 protein have been detected and characterized in vitro (Deckman & Draper, 1985; Deckman et al., 1987). The S4–α mRNA interaction provides a well-characterized system for studying the mechanisms by which a protein recognizes specific RNA structures and represses translation.

Recently we prepared an extensive set of sequence variants in the S4 recognition site of the α mRNA and measured the affinity of each for S4 in vitro (Tang & Draper, 1989). The primary purpose of that study was to rigorously define the mRNA secondary structure recognized by S4. Formation of an S4–mRNA complex is only one aspect of translational repression, however; the mRNA must also be recognized by ribosomes, and S4 binding must affect some step of initiation complex formation. Additional mRNA features not needed for S4 recognition but necessary for its repressor function might be revealed if mRNA mutations are assayed for translation or translational repression.

In this paper we present an in vivo assay for translational repression using fusions of the α mRNA leader sequence with *lac*Z; these fusions are introduced into cells in which S4 overproduction can be induced. Translational repression of most of the mutants previously assayed for S4 binding has been measured in this system; a few additional sequence variants designed to test potential S4 contacts with nonhelical regions of the mRNA have also been prepared and tested. To our knowledge this is the first time the affinity of a protein for an mRNA can be correlated with its ability to carry out translational repression. The results confirm the importance of a previously deduced pseudoknot secondary structure. In addition, we find that some mRNA features not essential for S4 recognition are nevertheless needed to mediate translational repression.

Experimental Procedures

Construction of α–lacZ Fusions. In-frame fusions of the first 203 nucleotides of the α operon transcript (coding for the first 36 amino acids of S13) with *lac*Z were made in derivatives of a low copy number plasmid, pACYC177 (Chang & Cohen, 1978; Rose, 1988), to allow subsequent transformation of strains carrying the α operon on a high copy number plasmid. pACYC177 was first modified by replacing an *Ssp*I–*Pst*I DNA fragment, containing most of the ampicillin resistance gene, with an 18-bp synthetic DNA containing *Eco*RI and *Bgl*II restriction sites, to give pACSP. Sequence variants of the α leader sequence were obtained by site-directed mutagenesis of a M13mp18 derivative, M13α, which contains an α operon sequence extending from −16 to an *Sph*I site at +137, relative to the α promoter start (Tang & Draper, 1989). The α leader is present on an *Eco*RI–*Bgl*II restriction fragment of M13α; this was cloned into pACSP to give pACSPα. In another plasmid a *Pst*I fragment carrying the *lac*Z gene, derived from pMC1871 (Shapira et al., 1983), had been fused in-frame to the S13 gene at the *Pst*I site at position 203 of the α transcript; this fusion was also inserted in the amp^R gene

†Supported by NIH Grant GM29048 and a Research Career Development Award to D.E.D. (CA01081).

0006-2960/90/0429-4434$02.50/0

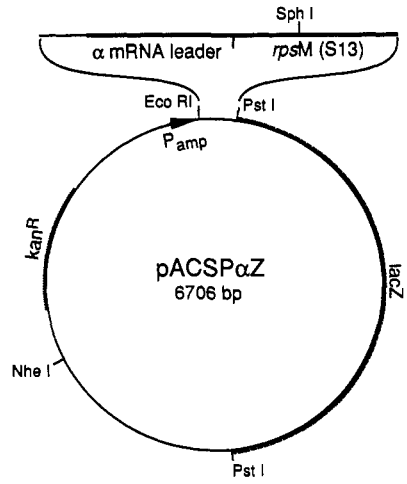

FIGURE 1: Plasmid containing a fusion between *lac*Z and the S13 gene. Restriction sites relevent to the plasmid construction are shown; see Experimental Procedures for a discussion. Sequences ultimately derived from the α operon are shown as a thick solid line in the expanded diagram.

of pACYC177. An *Sph*I–*Nhe*I fragment containing this fusion was then cloned into pACSPα to give the plasmid pACSPαZ, shown in Figure 1. Standard restriction digestion, ligation, and transformation methods were used to prepare all the plasmids, including the use of bacterial alkaline phosphatase to limit the ligation products to the ones desired (Maniatis et al., 1982). The constructs were all verified by extensive restriction digests.

Measurements of Translational Repression. Expression of S13–*lac*Z fusion proteins from all pACSPαZ derivatives was measured in JM105 [Δ(*lac pro*)*thi*1, *sup*E, F′*pro*AB, *lac*I^q, *ZΔM15*]; expression from some plasmids was also measured in JM109 (a *rec*A derivative of JM105) with similar results. The plasmid pNO2801, containing the entire α operon under control of the *lac*UV5 promoter and operator, was from the collection of M. Nomura. It is a derivative of pBR322 and confers ampicillin resistance (Thomas et al., 1987). Bacteria were grown at 37 °C in M9 minimal media (Maniatis et al., 1982) supplemented with thiamin and 25 μg/mL each of kanamycin and ampicillin. The β-galactosidase activity was assayed by following the hydrolysis of *o*-nitrophenyl galactoside at 420 nm. The protocol and unit calculation described by Miller (1972) was followed exactly. Cell lysis was achieved with 30 μL of 0.1% of SDS[1] and 60 μL of chloroform. Assay incubation times varied from 10 min to 17 h, depending on the β-galactosidase activity level; for times longer than 2 h control assays containing JM105(pNO2801) cells were included to correct for background *o*-nitrophenyl galactoside hydrolysis.

Repression was first measured by comparing the β-galactosidase units before and 30 min after addition of inducer (1 mM IPTG, final concentration). To obtain more accurate results, the total β-galactosidase activity was measured at five or six time points 5–75 min after IPTG addition to a growing culture. The least-squares slope of a semilog plot of the data was then taken as a measure of the rate of β-galactosidase synthesis.

S4 Binding Measurements. Mutants in the S4 repression site of the α mRNA were made in M13α and transferred to plasmids containing T7 promoters as described previously (Tang & Draper, 1989). Purification of S4 and nitrocellulose filter binding assays with ^{35}S-labeled RNA transcripts are described in detail elsewhere (Draper et al., 1988; Tang & Draper, 1989).

[1] Abbreviations: IPTG, isopropyl β-D-thiogalactoside; SDS, sodium dodecyl sulfate.

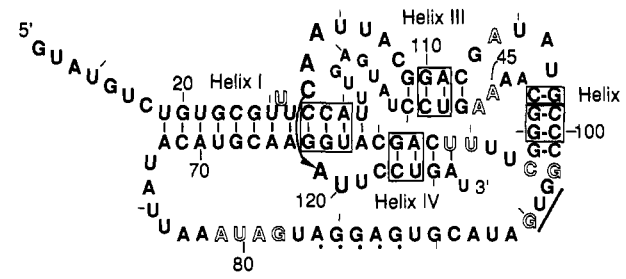

FIGURE 2: α mRNA secondary structure required for S4 recognition. Base pairing within boxes is supported by measurements of S4 affinity for compensating base changes (Tang & Draper, 1989); these base changes are listed in Table I. The helical segments are assigned numerals I–IV. Tic marks are located every five bases. Nucleotides in outline have been deleted or altered among the other sequence variants in Table I.

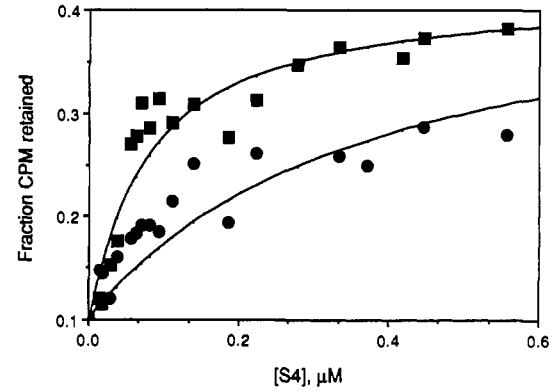

FIGURE 3: Titrations of variant α mRNAs with S4 protein. Retention of S4–[^{35}S]RNA complexes on nitrocellulose filters is shown. Lines are least-squares fits of binding isotherms to the data. (■) CKT13 RNA, K = 13.2 μM^{-1}, and filter retention efficiency of 0.43 (●) CKT 20 RNA, K = 2.63 μM^{-1}, and filter retention efficiency of 0.45.

RESULTS

S4 Recognition of the α mRNA. A pseudoknot secondary structure in the α mRNA sequence recognized by S4 was first suggested by the nuclease sensitivities of the RNA (Deckman & Draper, 1987), and this structure was confirmed by measuring S4 affinity for sets of compensating base changes in the mRNA (Tang & Draper, 1989). The complete secondary structure is shown in Figure 2. Each of the four helical segments shown was confirmed by showing that mutations in either strand weaken S4 binding, while a pair of mutations which restore the possibility of Watson-Crick base pairing also restore the binding to wild-type levels.

In an attempt to define sequences directly contacting S4, we made several mutations at sites outside the helical segments. Unusual codon usage suggested to us two nucleotides that might be essential for protein binding. The S13 gene uses GUG for an initiation codon, which is found in less than 10% of *E. coli* genes (Gren, 1984). However, a G95→A mutation did not alter the S4 binding (Table I). Isoleucine is encoded by a very rare AUA codon at 104–106; this is the only AUA of 59 Ile codons in the α operon (Bedwell et al., 1985) and is found 10–12 times less frequently than AUU or AUC in *E. coli* (Aota et al., 1988). A106→U changes this codon to AUU and does have a significant effect on the S4 binding (Figure 3 and Table I).

Since studies of the R17 coat protein (Wu & Uhlenbeck, 1987) and ribosomal protein L18 (Peattie et al., 1981; Christiansen et al., 1985) have suggested that these RNA

Table I: Translational Regulation of α mRNA Sequence Variants

RNA	base changes	K_{S4} μM^{-1} [a]	translation rate[b]	repression ratio[c]
wt [1–139 RNA]		12	43	1.97
CKT1	28CCA→GGU	3.0	46	1.48
CKT2	62UGG→ACC	1.8	32	1.21
CKT1/2	CKT1 + CKT2	13.0	65	2.03
CKT3	49GG→CC	1.5	42	0.99
CKT4	100CC→GG	1.2	23	1.08
CKT3/4	CKT3 + CKT4	13	30	1.52
CKT11	G102→C	1.6	51	1.02
CKT11/15	G102→C, C48→G	6.0	34	1.21
CKT12	G98→C	1.4	0.26	0.57
CKT16	C52→G	1.3	53	1.20
CKT12/16	CKT12 + CKT16	0.7	0.26	0.79
CKTΔ4	ΔA79–G82	11	30	1.18
CKT8	41CU→GG	17	34	1.12
CKT9	58AG→CC	9.9	37	1.18
CKT14	44AA→CC + 55UU→GG	5.6	25	1.48
CKT17	109AG→UC	1.4	230	1.25
CKT18	123CU→GA	6.7	1710	1.86
CKT21	41CU→GA	6.3	31	1.03
CKT22	58AG→UC	5.9	42	1.20
CKT17/21	CKT17 + CKT21	14	48	1.47
CKT18/22	CKT18 + CKT22	12	1680	0.96
CKT17/18	CKT17 + CKT18	1.8	1210	1.01
CKT13	ΔU26	13	21	1.72
CKT19	G95→A	12	6.9	1.81
CKT20	A106→U	2.8	35	1.07

[a] S4–mRNA binding affinities determined by filter binding assays. The last three entries (CKT13, CKT19, CKT20) are from this work; the remainder are taken from Tang and Draper (1989). Errors are about ±15% for values larger than ≈3 μM^{-1} and much larger for smaller values. [b] β-Galactosidase units in the absence of IPTG. Standard errors are about ±30%. [c] Ratio of the rate of β-galactosidase synthesis to the cell growth rate after induction of JM105(pNO2801, pACSPαZ) (harboring the indicated mutations in pACSPαZ) with 1 mM IPTG; see Experimental Procedures for details. Standard errors from duplicate and triplicate experiments are ±0.1 for ratios of ≈1.3–2.0 and ±0.05 for ratios near 1.0. Measurements of β-galactosidase synthesis in mutations CKT12 and CKT12/16 are very sensitive to corrections for background hydrolysis and scattering from residual cell debris and have much larger errors.

binding proteins recognize single-base bulges, a single U in the U_3 sequence at 25–27 was deleted. This had no effect on the S4 binding affinity (Figure 3 and Table I).

Measurement of Repression in Vivo. To examine the ability of S4 to repress translation in vivo, a plasmid was constructed that contains the α mRNA leader sequence, including the first 36 codons of the S13 gene, fused in frame to the β-galactosidase gene (see Figure 1 and Experimental Procedures). A second plasmid with the intact α operon under control of the *lac*UV5 promoter, pNO2801 (Thomas et al., 1987), was used to raise the pool level of S4 in the cell. Cells containing both pNO2801 and the α operon–*lacZ* fusion plasmid pACSP–αZ were grown exponentially, and the β-galactosidase activity was measured. The cells maintain a constant level of β-galactosidase during growth, as indicated by the parallel growth and activity curves for uninduced cells in Figure 4. If inducer is added to the growing cells, the rate at which β-galactosidase activity accumulates decreases by a factor of 2 while the growth rate remains the same (Figure 4). This is the result expected from the increased level of S4 in the cells. Although the induced repression is not large, it is approximately the level expected from other experiments done on this system (see Discussion).

We attempted to obtain a higher level of repression by using a plasmid similar to pNO2801 but containing a deletion of the first 22 nt of the α operon transcript, pNO2824. This deletion reduces the S4 binding affinity and derepresses translation of the operon (Deckman et al., 1987; Thomas et al., 1987). In repeating the experiment of Figure 4 with cells containing pACSP–αZ and pNO2824, we found that adding inducer decreases the cell growth rate by nearly 2-fold. Since S4 overproduction must cause drastic changes in protein and ribosome synthesis rates in these cells, changes in β-galactosidase levels cannot be easily interpreted in terms of translational repression.

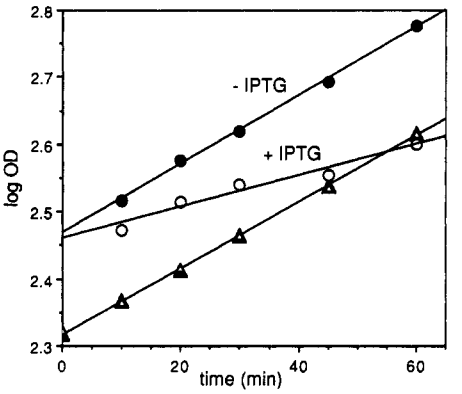

FIGURE 4: Response of β-galactosidase activity levels in cells to overproduction of S4 protein. An exponentially growing culture of JM105(pACYCαZ, pNO2801) was divided into two flasks and 1 mM IPTG added to one flask at zero time. (▲, △) OD_{600} (cell density) of uninduced and induced cultures, respectively. The doubling time of both cultures is 61 min. (●, ○) OD_{420} of the β-galactosidase assay for uninduced and induced cultures, respectively. The slopes of the least-squares fit lines are 0.00510 and 0.00232 min^{-1}, respectively.

The ratios of cell growth rate to β-galactosidase synthesis rate for a number of mutations in the α operon leader are compiled in Table I. We call this the "repression ratio" since it reflects the factor by which translation from the S13 ribosome binding site decreases when the S4 concentration increases. It is similar to measurements made for other autoregulated ribosomal protein operons (Thomas & Nomura, 1987; Parsons et al., 1988).

The Basic Pseudoknot Structure (Helices I and II) Is Required for Repression in Vivo. Two sets of compensating base changes have been made that define a pseudoknot mRNA secondary structure (helices I and II of Figure 2) recognized by S4: mutations CKT1, -2, -3, and -4 all disrupt potential base pairing and weaken S4 binding, while the double-mutants CKT1/2 and CKT3/4 restore base pairing and S4 binding (Tang & Draper, 1989). Similar compensatory behavior is observed in the repression assay (Table I), confirming the importance of this pseudoknot structure.

A third compensating base change, CKT11/15, provides additional evidence for base pairing in helix II. Helix II also has the potential of adding a fifth base pair between C52 and G98. Individual mutations at these two positions reduce the S4 affinity and translational repression, but the compensatory base change does not restore either activity. Either S4 specifically recognizes a C52–G98 base pair or the bases must be part of some other structure required for recognition.

Additional Base Pairing within the Pseudoknot Structure. Two more sets of compensating base changes define additional base pairing within the pseudoknot structure. CKT17, CKT21, and CKT17/21 show compensatory behavior supporting the existence of helix III. Helix III apparently inhibits translation: CKT17 increases the β-galactosidase levels measured in the absence of S4 overproduction by 5-fold, while the double-mutant CKT17/21 has a normal translational level. It may be that helix III has some direct interaction with the ribosome binding site which weakens ribosome binding.

Helix IV is disrupted by mutations CKT18 and CKT22. Both mutations have rather weak effects on S4 binding and

Table II: S4-Induced Repression of S13 Translation[a]

one copy α operon regulated	2.0[b] →	multiple α operon copies regulated
0.5–0.7[c] ↓		2.9[d] ↑
one copy α operon unregulated	[1.0] ↔	multiple α operon copies unregulated

[a] Translation from the S13 ribosome binding site in cells with different combinations of effective α operon gene dosage and translational regulation are compared. Unregulated translation was due to either a mutation in S4 (*c*) or a deletion in the α operon leader (*d*). Mutiple copies of the α operon were obtained by induction of the plasmid pNO2801 carrying the α operon under lacUV5 promoter control. Numbers indicate ratios of S13 synthesis rate per α operon copy under two different conditions; the arrow points to the denominator of the ratio. [b] This study. [c] Olsson and Isaksson (1979); Jinks-Robertson and Nomura (1982). [d] Thomas et al. (1987).

show some repression by S4 in vivo. However, the double-mutant CKT18/22 shows no repression, even though its S4 binding affinity is not detectably different from that of wild type. This result implies that helix IV participates in a more complex structure which is required for repression and cannot tolerate the compensatory base change.

CKT8 and CKT9 are alternate mutations at the same locations as CKT21 and CKT22, respectively. Even though these mutations also have weak effects on S4 binding (CKT8 actually has a slightly enhanced S4 affinity), they reduce translational repression substantially. The unexpected finding that some mutations can reduce repression without disrupting S4 binding is considered further under Discussion.

Mutations in Nonhelical Regions of the mRNA. The effects of several other mutations on repression are easily understood in terms of their S4–mRNA binding affinities. Thus, deletion of the bulged U26 (CKT13), introduction of an AUG initiation codon (CKT19), and changes in an A–U-rich region (CKT14) have small effects on S4 binding and correspondingly small effects on repression. A106→U (CKT20), which substantially reduces S4 binding, also abolishes regulation. However, CKTΔ4, which removes four bases upstream of the Shine–Dalgarno sequence, has no effect on S4 binding but a major effect on repression.

Discussion

Translational Repression of the α Operon. Several studies examining the translational repression of α operon genes have appeared and are compared in Table II. Some mutations in the S4 protein relieve translational repression; comparison of α operon protein synthesis rates between these strains and wild-type cells (left column of Table II) shows that r-protein synthesis from the α operon is normally repressed by 30–50% (Olsson & Isaksson, 1979; Jinks-Robertson & Nomura, 1982). The synthesis of α operon proteins in cells overproducing α mRNA transcripts (via pNO2801 induction) has also been measured; the effective increase in gene dosage should elevate the pool levels of S4 sufficiently to cause additional repression of translation. Deletions in the mRNA leader that disrupt helix I increase the rate of S13 translation by 2.9-fold under these conditions (right column of Table II). Our studies measure the decrease of S13–*lac*Z fusion protein synthesis upon induction of the same pNO2801 plasmid (top row of Table II). If we assume that S13 synthesis per gene copy is independent of the copy number in the absence of translational regulation (bottom row of Table II), then Table II implies that the repression ratio we measure should be (2.9)(1.0)(0.5–0.7) $\approx$ 1.5–2.0, which is the observed value (Table I). These studies therefore give a consistent picture of translational repression levels in the α operon.

It is surprising that only small changes in translational repression levels are detected in these experiments. The pool of ribosomal proteins in the cell is 1–3% of the ribosome concentration when single chromosomal copies of the ribosomal protein genes are present (Gausing, 1974). Induction of pNO2801 more than doubles the rate of S4 synthesis measured in a 15-min pulse, so that the steady-state pool concentration of S4 could be as much as 100% of the ribosome concentration, i.e., a 30–100-fold increase. However, the 2–3-fold increase in repression caused by pNO2801 induction requires only a 3–4-fold increase in S4 pool concentrations (Draper, 1988). The actual steady-state level of S4 has not been measured in cells in which S4 is overproduced, so it is possible that protein turnover accounts for the low repression levels measured. Ribosomal proteins synthesized in excess tend to be degraded rapidly (Fallon et al., 1979; Olsson & Isaksson, 1979), and in fact S4 mutations that relieve translational repression probably do so because pools of these proteins do not accumulate detectably (Olsson & Isaksson, 1979). If rapid S4 turnover is the correct explanation for the small changes in repression, then protein turnover is as important as autoregulation in matching α operon ribosomal protein synthesis with ribosomal RNA synthesis.

S4 Recognition of α mRNA. The in vivo results confirm the importance of at least helices I–III for S4 recognition of the α mRNA. The fact that complementary base changes in helix IV do not give a regulated mRNA does not rule out the existence of this helix in a complex with S4 but suggests that we have not yet detected all the mRNA structures which are required to form a repressible mRNA. A triple helical interaction between helix IV and another sequence is a possibility; the compensatory base change might retain base pairing required for S4 recognition but disrupt additional hydrogen bonding required for bound S4 to affect translation. The observations that the two complementary mutants CKT17 and CKT21 have much different effects on S4 binding, repression, and translational efficiency also suggest that a more complex structure than a simple duplex is involved at helix III. Freedman et al. (1987) have described a similar situation in which a compensating base change in a regulated mRNA restores translational regulation but not transcriptional regulation or translational efficiency.

The loop containing A106 is very accessible to T_2 nuclease (Deckman & Draper, 1987) and is therefore unlikely to be involved in any tertiary structure. Since an A106→U change weakens S4 binding, this position is a good candidate for a nucleotide directly interacting with S4. An AUA sequence in a loop of 16S rRNA is also important for S4 recognition (J. Vartikar and D.E.D., unpublished observations).

Correlation of Repressor Binding and Translational Repression. The mRNA target sites for a number of translational repressors have now been defined by sets of mutations [Springer et al., 1986; Thomas & Nomura, 1987; Parsons et al., 1988; see also reviews by Draper (1989) and by Gold (1988)]. However, the data presented in Table I allow the first comparison of a repressor binding affinity to the in vivo effectiveness of the repressor. As we have noted under Results, some of the mutations seem to reduce repression out of proportion to their effects on repressor binding. This finding is illustrated in Figure 5, which plots the repression ratio as a function of S4–mRNA binding affinity. The curve has been calculated by assuming only that repression is proportional to the extent of S4 binding at equilibrium, i.e.

$$\text{degree of repression} \propto K_R[R]/(1 + K_R[R])$$

where K_R and [R] refer to the repressor–mRNA binding

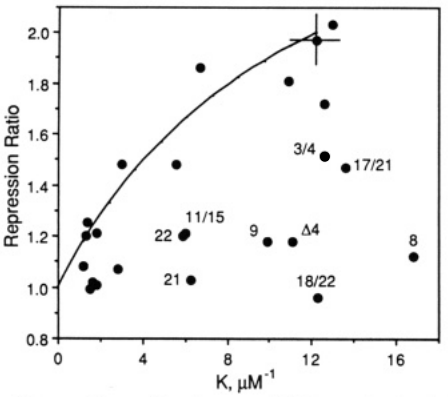

FIGURE 5: Repression ratios for α mRNA mutants plotted as a function of S4 binding affinity. Data are taken from Table I; only CKT12 and CKT12/16 have been omitted. Error bars for the wild-type sequence are indicated. The curve has been calculated by presuming that repression is proportional to the extent of S4 binding and that S13 translation in uninduced cells is already repressed by 50%. Mutants from Table I that deviate significantly from this curve are labeled.

affinity and the free repressor concentration, respectively. This proportionality is true for all translational regulation mechanisms that involve equilibrium binding of repressor to mRNA or mRNA–ribosome complexes (Draper, 1988). (The curve in Figure 5 has been calculated by assuming that translation is 50% repressed in the absence of added inducer, but the shape of the curve is very insensitive to this value.) Many of the mutants fall within experimental error of the curve, but a number are repressed much less than predicted. The latter group, indicated in Figure 5, involve helices II–IV as well as the deletion near the ribosome binding site, CKTΔ4.

The unexpected behavior of these mutants has implications for the structure of the α mRNA. A translationally regulated mRNA must have binding sites for both ribosomes and repressor, and the two binding sites must be coupled in some way so that bound repressor may inhibit ribosome initiation. In most translationally regulated mRNAs this coupling is a consequence of overlapping binding sites for repressor and ribosomes. Thus, the T4 *reg*A protein, T4 gene 32 protein, and R17 coat protein all interact directly with mRNA sequences between the Shine–Dalgarno sequence and initiation codon (Winter et al., 1987; Gold, 1988), so that repressor and ribosome binding should be mutually exclusive. This simple mechanism cannot take place in the α mRNA, since mutants that fall below the curve in Figure 5 have disrupted the coupling between ribosomes and S4 repressor without substantially altering either the ribosome or S4 binding sites.[2] We are therefore led to propose the model shown in Figure 6A, which shows S4 acting as an allosteric effector of translational initiation. In this view, mutations may disrupt translational regulation either by weakening the repressor binding affinity or by altering the linkage between the repressor and ribosome binding sites (Figure 6B). In an allosteric mechanism the mRNA plays an active role in mediating the interaction between repressor and ribosomes, in contrast to the more passive role needed when repressor and ribosomes recognize overlapping binding sites on the mRNA.

[2] If repression takes place because of direct competition between ribosome and repressor binding, an mRNA mutation may relieve translational repression by increasing the affinity of ribosomes for the mRNA while leaving the repressor affinity unchanged. The competition between ribosomes and repressor is then shifted in favor of ribosomes, and repression will be less effective (Draper, 1988). In a series of mutations prepared in the L1 operon, there is an inverse correlation between translation rate and repression ratio, as predicted by this model (Thomas & Nomura, 1987). Although a few of the mutations listed in Table I have substantially altered levels of translation, there is no correlation with repression ratio, and this mechanism cannot account for all of the mutants that fall below the curve in Figure 5.

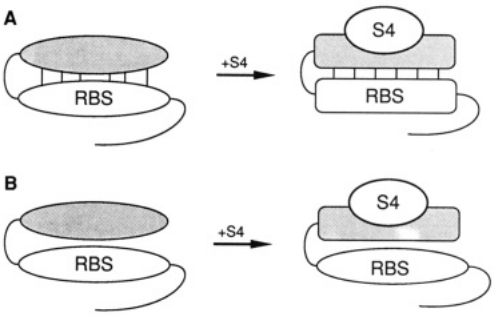

FIGURE 6: Allosteric model for S4 repression of translation. (A) The α mRNA leader is proposed to contain two separate binding sites for S4 repressor (shaded oval) and ribosomes (RBS, ribosome binding site). The sites are physically linked by RNA secondary or tertiary interactions (vertical lines). Binding of S4 to the α mRNA induces a conformational change in both the repressor and ribosome binding sites, which alters the rate of translational initiation. (B) Mutations that disrupt the linkage between the S4 and ribosome binding sites render translation independent of S4 binding, while leaving the S4 binding site intact.

Another operon that may use an allosteric mechanism to carry out translational repression is *rif*, which is regulated by ribosomal protein L10. There is convincing evidence that L10 recognizes an mRNA structure ~150 nt upstream of the ribosome binding site (Climie & Friesen, 1987). Presumably, the repressor and ribosome sites are coupled via mRNA secondary and tertiary structure, as proposed here for the α mRNA.

It was originally suggested that an autoregulated ribosomal protein mRNA would mimic the structure of the rRNA site recognized by the regulatory ribosomal protein (Nomura et al., 1984), and later work has shown that there are striking similarities between the mRNA and rRNA secondary structures recognized by some ribosomal protein repressors (Draper, 1989). We extend this original suggestion by speculating that the function of some ribosomal proteins is to perturb rRNA structure in specific ways [see the discussion in Vartikar and Draper (1989) of likely S4 effects on 16S rRNA conformation]. If this is the case, an mRNA might also mimic the rRNA conformational flexibility and take advantage of protein-induced conformational changes to evolve an allosteric mechanism for translational repression. In this way the regulatory protein–mRNA complex may be a more profound imitation of the protein–rRNA complex than the simple structural similarity originally envisioned.

Effects of α mRNA Mutations on Translational Efficiency. The location of the ribosome binding site on a single-stranded linker within the pseudoknot structure might limit ribosome accessibility, with the consequence that disruption of the pseudoknot structure would increase translational efficiency. The mutations CKT17 and CKT17/21 in helix III have this sort of effect on translation. It is surprising that none of the mutants in helices I and II shows significant change in the S13–*lac*Z fusion protein translational level of uninduced cells; the C–G-rich helix II, in particular, might be expected to be a very stable block to initiating ribosomes. However, we do not know what the stability of a pseudoknot structure like this is; perhaps helix II is too weak in the absence of S4 to affect translation. It is also possible that melting of the structure is not a rate-limiting step for translating ribosomes.

Several mutations do have drastic effects on the translation of the fusion protein; between CKT12 and CKT18 there is a 6000-fold range. Two of the mutations are worth remarks.

Replacement of the GUG initiation codon with AUG reduces translational efficiency by 6-fold. In other cases that have been studied, GUG has been either 4-fold less efficient than AUG (Munson et al., 1984) or comparable to AUG (Shinedling et al., 1987). Why an AUG codon should be less efficient in this case is not at all clear. G98→C (CKT12) decreases the rate of fusion protein synthesis more than 100-fold. Statistical analysis of ribosome binding sites has shown that some sequences are heavily favored in the second codon position (Gold, 1988). Looman et al. (1987) have measured *lacZ* translation rates with all possible second codons, and the same change caused by CKT12, GCC → CCC, reduces *lacZ* synthesis about 3.5-fold in their system. We doubt this small sequence preference can account for the drastic reduction in translation observed in the α mRNA. These mutations show that the structure surrounding a ribosome binding site may have an enormous influence on gene expression, which has potentially interesting implications for the mechanisms of translational initiation and repression in this mRNA. Further interpretation of these data will have to await an analysis of the effects of the mutations on ribosome binding and mRNA half-life.

References

Aota, S., Gojobori, T., Ishibashi, F., Maruyama, T., & Ikemura, T. (1988) *Nucleic Acids Res. 16*, r315–r402.

Bedwell, D., Davis, G., Gosink, M., Post, L., Nomura, M., Kestler, H., Zengel, J. M., & Lindahl, L. (1985) *Nucleic Acids Res. 13*, 3891–3903.

Chang, A. C. Y., & Cohen, S. N. (1978) *J. Bacteriol. 134*, 1141–1156.

Christiansen, J., Douthwaite, S. R., Christensen, A., & Garrett, R. (1985) *EMBO J. 4*, 1019–1024.

Climie, S. C., & Friesen, J. D. (1987) *J. Mol. Biol. 197*, 371–381.

Deckman, I. C., & Draper, D. E. (1985) *Biochemistry 24*, 7860–7865.

Deckman, I. C., & Draper, D. E. (1987) *J. Mol. Biol. 196*, 323–332.

Deckman, I. C., Draper, D. E., & Thomas, M. S. (1987) *J. Mol. Biol. 196*, 313–322.

Draper, D. E. (1988) in *Translational Regulation of Gene Expression* (Ilan, J., Ed.) pp 1–26, Plenum Press, New York.

Draper, D. E. (1989) *Trends Biochem. Sci. 14*, 335–338.

Draper, D. E., Deckman, I. C., & Kean, J. M. (1988) *Methods Enzymol. 164*, 203–220.

Fallon, A. M., Jinks, C. S., Yamamoto, M., & Nomura, M. (1979) *J. Bacteriol. 138*, 383–396.

Freedman, L. P., Zengel, J. M., Archer, R. H., & Lindahl, L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6516–6520.

Gausing, K. (1974) *MGG, Mol. Gen. Genet. 129*, 61–75.

Gold, L. (1988) *Annu. Rev. Biochem. 57*, 199–233.

Gren, E. J. (1984) *Biochimie 66*, 1–29.

Jinks-Robertson, S., & Nomura, M. (1982) *J. Bacteriol. 151*, 193–202.

Lindahl, L., & Zengel, J. M. (1986) *Annu. Rev. Genet. 20*, 297–326.

Looman, A. C., Bodlaender, J., Comstock, L. J., Eaton, D., Jhurani, P., de Boer, H. A., & van Knippenberg, P. H. (1987) *EMBO J. 6*, 2489–2492.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Miller, J. (1972) *Experiments in molecular genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Munson, L. M., Stormo, G. D., Niece, R. L., & Reznikoff, W. S. (1984) *J. Mol. Biol. 177*, 663–683.

Nomura, M., Gourse, R., & Baughman, G. (1984) *Annu. Rev. Biochem. 53*, 75–177.

Olsson, M. O., & Isaksson, L. A. (1979) *MGG, Mol. Gen. Genet. 169*, 271–278.

Parsons, G. D., Donly, B. C., & Mackie, G. A. (1988) *J. Bact. 170*, 2485–2492.

Peattie, D. A., Douthwaite, S., Garrett, R. A., & Noller, H. F. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 7331–7335.

Rich, A., & RajBhandary, U. L. (1976) *Annu. Rev. Biochem. 45*, 805–860.

Rose, R. E. (1988) *Nucleic Acids Res. 16*, 356.

Shapira, S. K., Chou, J., Richard, F. V., & Casadaban, M. J. (1983) *Gene 25*, 71–82.

Shinedling, S., Gayle, M., Pribnow, D., & Gold, L. (1987) *MGG, Mol. Gen. Genet. 207*, 224–232.

Springer, M., Graffe, M., Butler, J. S., & Grunberg-Manago, M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4384–4388.

Tang, C. K., & Draper, D. E. (1989) *Cell 57*, 531–536.

Thomas, M. S., & Nomura, M. (1987) *Nucleic Acids Res. 15*, 3085–3096.

Thomas, M. S., Bedwell, D. M., & Nomura, M. (1987) *J. Mol. Biol. 196*, 333–345.

Vartikar, J. V., & Draper, D. E. (1989) *J. Mol. Biol. 209*, 221–234.

Winter, R. B., Morrissey, L., Gauss, P., Gold, L., Hsu, T., & Karam, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7822–7826.

Wu, H.-N., & Uhlenbeck, O. C. (1987) *Biochemistry 26*, 8221–8227.

Yates, J. L., Arfsten, A. E., & Nomura, M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1837–1841.