Proc. Natl. Acad. Sci. USA
Vol. 75, No. 6, pp. 2741–2745, June 1978
Biochemistry

Mutations of bacteriophage T7 that affect initiation of synthesis of the gene *0.3* protein

(mRNA–rRNA interaction/Shine–Dalgarno model/initiator AUG/RNA sequence analysis/ribosome binding)

JOHN J. DUNN, ELIZABETH BUZASH-POLLERT, AND F. WILLIAM STUDIER

Department of Biology, Brookhaven National Laboratory, Upton, New York 11973

Communicated by Richard B. Setlow, April 10, 1978

ABSTRACT **Two different mutations that greatly diminish the rate of synthesis of the gene *0.3* protein of bacteriophage T7 have been characterized. One is in the initiator triplet for the *0.3* protein, changing it from AUG to ACG. This mutation was found to have little effect on binding of ribosomes to the *0.3* mRNA *in vitro*, although *0.3* protein synthesis was greatly depressed *in vitro* as well as *in vivo*. A suppressor mutation that partially restores the wild-type rate of synthesis was found to lie within the *0.3* RNA but not close to the mutant ACG (more than 64 nucleotides away). The second mutation is a G-to-A transition located 11 bases to the 5′ side of the initiator AUG. This change eliminates a possible five-base pairing with a sequence near the 3′ end of 16S ribosomal RNA, an interaction previous workers have proposed to be important for initiation of protein synthesis. This mutation causes the site of ribosome binding to shift about 15 bases to the 3′ side, centering on an internal AUG, but this new site has only a poor potential interaction with 16S RNA. A suppressor mutation that restores the rate of *0.3* protein synthesis to essentially wild-type levels and also restores wild-type ribosome-binding behavior was found to lie adjacent to the original mutation, creating a new four-base complementarity with 16S RNA. These results provide strong support for the idea that a pairing interaction between mRNA and 16S RNA is involved in specific initiation of protein synthesis in *Escherichia coli* and indicate that this interaction may be important in selecting the site in mRNA at which the ribosomes bind.**

At least two base-pairing interactions of mRNA are thought to be involved in specific initiation of protein synthesis in *Escherichia coli:* the AUG initiation codon pairing with the anticodon loop of fMet-tRNA, and a sequence to the 5′ side of the AUG pairing with a sequence near the 3′ end of the 16S ribosomal RNA (1–3). We have discovered mutations that change the nucleotide sequence in each of these two regions of the mRNA for the gene *0.3* protein of bacteriophage T7. The effects of these mutations on ribosome binding, polysome formation, and rate of protein synthesis are reported here.

MATERIALS AND METHODS

T7 RNAs and Proteins. Procedures for isolation and growth of T7 strains having mutations within gene *0.3* have been described (4), as have techniques for labeling and electrophoretic analysis of T7 RNAs and proteins (5). ^{32}P-Labeled *0.3* RNA (25–50 Ci/nmol) was synthesized *in vitro* and purified by preparative electrophoresis on 4% polyacrylamide slab gels (6).

Preparation of D159 Fragments. An RNA fragment containing the first 100 or so nucleotides from the 5′ end of the *0.3* RNA was prepared by hybridizing intact *0.3* RNA to the DNA of a deletion mutant (D159) and digesting away the unpaired nucleotides with RNase T1 (EC 3.1.4.8) or pancreatic RNase (EC 3.1.4.22). To increase the efficiency of hybridization, the DNA was first digested with the gene *6* exonuclease of T7, which removes nucleotides sequentially from the 5′ end of double-stranded DNA (7). Hybridizations were for 16 hr at 65° in 0.5 ml of 0.3 M NaCl/0.03 M Na_3 citrate/0.1% sodium dodecyl sulfate containing 50 μg of the treated DNA and 40 μg of carrier *E. coli* tRNA, after which the mixture was treated with RNase (4 μg/ml) for 90 min at room temperature. The hybrids were collected on nitrocellulose filters, residual RNase was inactivated by incubating with ICH_2COO^-, and the protected region of the RNA (termed the D159 fragment) was released from the hybrids by DNase digestion and purified by electrophoresis on 7% polyacrylamide gels (8).

Ribosome Binding. Binding of ribosomes to ^{32}P-labeled *0.3* RNA or D159 fragments was analyzed essentially as described by Steitz (9). Reaction mixtures (50 μl) contained 350 μg of low-salt washed ribosomes from *E. coli* BL15 (10), 5 μg of carrier f2 RNA, and 13 μg of fMet-tRNA (Boehringer), charged and formylated as described (11). Sucrose gradient centrifugation was used to detect binding and to isolate fragments that had been protected from RNase digestion (9).

RNA Sequence Analysis. D159 fragments labeled separately with the four [α-^{32}P]ribonucleoside triphosphates were subjected to pancreatic RNase and RNase T1 fingerprint analysis, and the sequences of the individual oligonucleotides and their neighboring bases were determined by standard techniques (12).

RESULTS

Mutations Affecting Initiation of *0.3* Protein Synthesis. The *0.3* RNA of T7 is an early RNA about 600 nucleotides long (13) that specifies two proteins. The 5′ two-thirds of the *0.3* RNA specifies the *0.3* protein, which is responsible for overcoming host restriction (4), and the 3′ one-third specifies a protein of unknown function (unpublished data), which will be referred to as the *0.4* protein. The *0.3* protein is about 120 amino acids long (unpublished data) and is made in large amounts during T7 infection; the *0.4* protein is smaller and is made in smaller amounts. Each of these two proteins apparently is synthesized from its own ribosome binding and initiation site in the *0.3* RNA (14).

We have found T7 mutants that seem to be deficient in initiation of synthesis of the *0.3* protein. Five such mutants, representing at least two genetically distinct sites near the NH_2-terminal end of the *0.3* protein, were found among a group of 11 *0.3* mutants isolated from a stock that had been mutated by two cycles of growth in the presence of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. All five mutants (represented by CR17 and CR35 in Fig. 1) had a greatly reduced rate of synthesis of *0.3* protein (about 1/6 to 1/12) but normal synthesis of *0.4* protein. Some *0.3* protein was still made, as shown by comparison with deletion or chain-terminating *0.3* mutants (Fig. 1). These mutants also plated somewhat better on restricting hosts than

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. §1734 solely to indicate this fact.

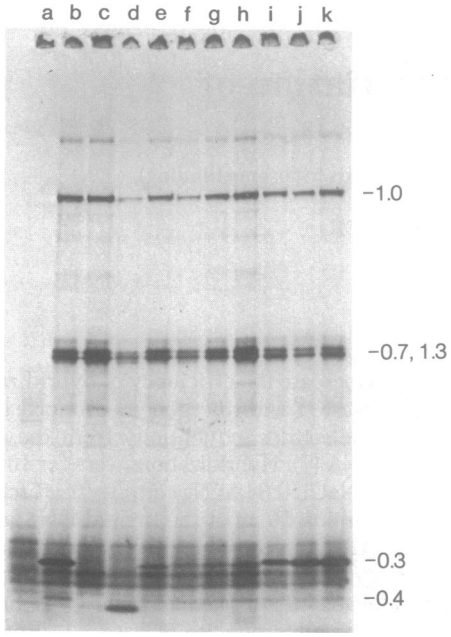

FIG. 1. Synthesis of T7 early proteins by different T7 mutants. Infection, labeling, sample preparation, and gel electrophoresis were essentially as described (5). Cells were *E. coli* B, UV-irradiated before infection; labeling was with 20 μCi of [^{35}S]methionine per ml for 0–8 min after infection; electrophoresis was on a 10–20% polyacrylamide gradient slab gel with 5% stacking gel and a discontinuous Tris·Cl/glycine buffer system containing 0.1% sodium dodecyl sulfate. Positions of different T7 early proteins are indicated at the right; the T7 mutant used for infection is indicated above each track: a, uninfected; b, wild; c, D364; d, CR3b; e, CR17; f, CR35; g, CR35a; h, CR35b; i, CR17-1; j, CR35a-1; k, wild. D364 is a deletion mutant that lacks both the *0.3* and *0.4* proteins but no others. CR3b (4) carries a chain-terminating mutation in the *0.3* protein but does not affect synthesis of the *0.4* protein (all of the T7 early proteins were relatively weakly labeled after infection with CR3b in this experiment). The other mutants are described in the text.

did chain-terminating *0.3* mutants, consistent with the finding that a small amount of *0.3* protein seems to be made.

As might be expected from the heavy mutagenesis, both CR17 and CR35 carry secondary mutations, which were detected by changes in electrophoretic mobility of certain T7 late proteins. The secondary mutations of CR17 were not removed for this work, but CR35 was found to have a secondary mutation within the *0.3* RNA, as detected by changes in electrophoretic mobility of the *0.3* RNA in certain buffer systems. CR35b, a strain from which all of the detectable secondary mutations of CR35 were removed, was produced by introducing the gene *1* mutation *am*193 (15) into CR35 and then removing it again by crossing with wild-type T7. CR35b remained deficient in ability to synthesize *0.3* protein (Fig. 1), but it plated somewhat better on restricting hosts than did CR35 (although still less well than wild-type T7). Strain CR35a was produced by the same genetic manipulations as CR35b, but it remained indistinguishable from CR35.

Polysome Profiles. The deficiency in synthesis of *0.3* protein after infection of CR17 and CR35b is reflected in the polysome profile. A nonrestricting host, *E. coli* C, was infected at 30° in the presence of $^{32}PO_4$. Six minutes after infection, lysates were prepared and polysome profiles were analyzed according to procedures described by Godson and Sinsheimer (16). Centrifugation was at 4° through 15–30% sucrose gradients for 30 min at 49,000 rpm in a Beckman SW 50.1 rotor. The position of full-length ^{32}P-labeled *0.3* RNA in the polysome profile was determined by gel electrophoresis of each fraction from the sucrose gradient (not shown). The *0.3* RNA from a wild-type infection was found near the bottom of the centrifuge tube, indicating association with many ribosomes, but the *0.3* RNA from a CR17 or CR35b infection was near the top of the tube, close to the position of 70S ribosomes. This difference should reflect a change in the number of ribosomes bound to the *0.3* region of the mRNA, because synthesis of the *0.4* protein is unaffected by these mutations (Fig. 1). The finding that apparently full-length *0.3* RNA of CR17 and CR35b was complexed with relatively few ribosomes is consistent with the idea that these mutations affect the rate of loading of ribosomes onto the message rather than the stability of the *0.3* RNA.

***In Vitro* Protein Synthesis.** T7 early RNAs were synthesized *in vitro* by *E. coli* RNA polymerase from wild-type, CR17, or CR35a DNA and used to program a cell-free protein synthesizing system from *E. coli* BL15, essentially as described (10). When RNAs from CR17 or CR35a were used, the same depression of *0.3* protein synthesis observed *in vivo* was found (not shown). Again, selective degradation of the mutant *0.3* RNAs does not seem to be involved, because electrophoretic analysis of the labeled RNAs indicated that the wild-type and mutant *0.3* RNAs have similar half-lives in the protein-synthesizing system.

Sequence Analysis of 0.3 RNA. In order to understand how the CR17 and CR35 mutations might be affecting initiation of *0.3* protein synthesis, the change in nucleotide sequence produced by each of these mutations was determined. Previous work on the sequence of the RNase III cleavage site at the 5′ end of *0.3* RNA (unpublished data) and on the ribosome binding site at the beginning of the *0.3* protein (14) had determined the sequence of the first 52 nucleotides from the 5′ end of the *0.3* RNA. In addition, the *0.3* protein had been purified and the sequence of amino acids at its NH_2 terminus had been determined (unpublished data), confirming that the *0.3* protein is initiated at the AUG triplet proposed by Steitz and Bryan (14). This AUG triplet lies 35 bases from the 5′ end of the RNA (Fig. 2).

Rather than using full-length *0.3* RNA to determine the nucleotide changes caused by the CR17 and CR35 mutations, we prepared D159 fragments containing approximately the first 100 nucleotides from the 5′ end of the *0.3* RNA. We knew that the CR17 and CR35 mutations should lie within this fragment because both could recombine with D159. Pancreatic RNase fingerprints of the D159 fragments of wild-type, CR17 and CR35a are shown in Fig. 3 *A–C*. Fingerprints of the D159 fragment of CR35b were exactly the same as those of CR35a (not shown), demonstrating that the secondary mutation in the *0.3* RNA of CR35 and CR35a does not lie within the D159 fragment.

Approximately half of the oligonucleotides from the wild-type D159 fragment could be ordered from previous results, giving the sequence to nucleotide 52. The remaining oligonucleotides could be placed in a unique order from a combination of nearest neighbor information and knowledge of the sequence of the first 21 amino acids from the NH_2 terminus of the *0.3* protein. The sequence of the first 101 nucleotides of the wild-type *0.3* RNA and the first 21 amino acids of the *0.3* protein are given in Fig. 2.

The nucleotide sequence of each mutant D159 fragment was found to differ from that of wild type by a single base. In CR17 a U-to-C transition had occurred at nucleotide 36, changing the initiator AUG triplet to ACG; in CR35 a G-to-A transition had occurred at nucleotide 24, in the center of a five-base sequence (nucleotides 22–26) that is complementary to a five-base sequence near the 3′ end of the 16S ribosomal RNA (Fig. 2). Thus, these mutations lie in two regions of the mRNA thought to be

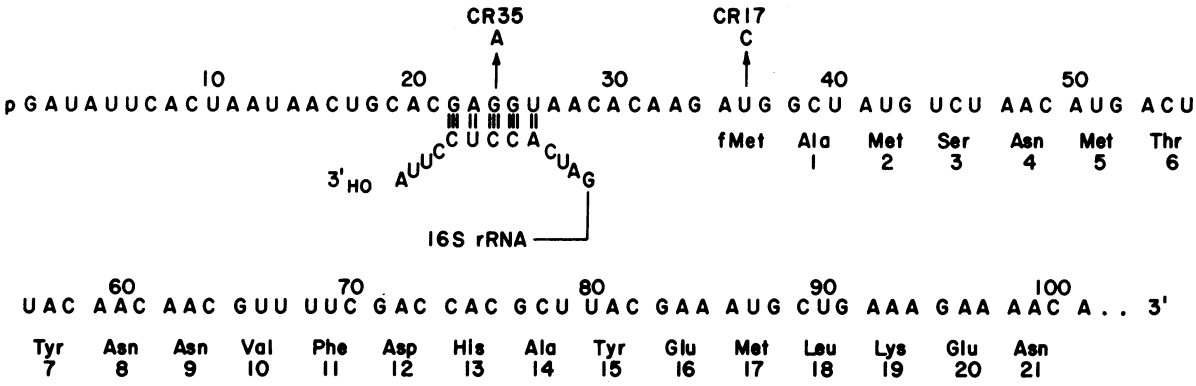

FIG. 2. Nucleotide and amino acid sequences near the beginning of the *0.3* protein. The nucleotide sequence beginning at the 5′ end of the *0.3* RNA and extending to the D159 deletion was determined as described in the text. The amino acid sequence was determined from the purified *0.3* protein, which lacks the initial fMet (unpublished data). The nucleotide sequence at the 3′ end of the 16S ribosomal RNA (2) is also given, to show the potential pairing between the *0.3* RNA and the ribosomal RNA (14). The nucleotide changes caused by the CR17 and CR35 mutations are indicated. The CR35a-1 suppressor mutation changes nucleotide 23 of CR35 from A to G, producing the sequence GGAG for nucleotides 22–25.

involved in the initiation of synthesis of the *0.3* protein (1–3, 14).

Suppressor Mutations. Several spontaneous phenotypic revertants were identified as large plaques in the background of small plaques that form when high concentrations of CR17 or CR35a are plated on a lawn of *E. coli* B, a restricting host. (This type of screening was not possible with CR35b because the background of small plaques was too heavy.) Two such derivatives, CR17-1 and CR35a-1, were analyzed. Both plated well on hosts that have an active restriction system, both produced much more *0.3* protein than the original mutants (Fig. 1), and both gave polysome profiles in which the *0.3* RNA returned to nearly the wild-type position (not shown). In all three tests, CR35a-1 seemed to approach wild-type behavior more closely than did CR17-1.

Sequence analysis showed that CR17-1 and CR35a-1 each retained its original mutation but had acquired a second mutation. The additional mutation in CR35a-1 was an A-to-G transition at nucleotide 23, which creates a new four-base complementarity with bases near the 3′ end of 16S ribosomal RNA, nucleotides 22–25 of CR35a-1 being GGAG (see Fig. 2). CR17-1 had no base change relative to CR17 within the first 101 nucleotides, but fingerprints of the entire *0.3* RNA showed that CR17-1 had acquired a base change in another part of the *0.3* RNA, relative to both wild type and CR17. Further work will be needed to determine the exact location of this base change.

Ribosome Binding. We investigated the ability of the *0.3* RNAs of wild type, CR17, and CR35a to bind to ribosomes *in vitro*. Steitz and Bryan (14) showed that bound ribosomes protect two regions of wild-type *0.3* RNA from RNase digestion: site *a*, the initiation site for the *0.3* protein, and site *b*, the presumed initiation site for the *0.4* protein. We find that ribosomes bind well to all three RNAs and to the D159 fragments derived from them and that such binding protects portions of the RNA from digestion by either pancreatic RNase or RNase T1. The protected fragments from [α-^{32}P]GTP-labeled RNAs were isolated from 70S ribosome complexes, digested to completion with the same RNase, and fingerprinted. The results of representative analyses with pancreatic RNase are shown in Fig. 3 *D–I*. Oligonucleotides from sites *a* and *b* are easily distinguished because the D159 fragments contain only site *a*.

The oligonucleotides from site *b* were the same for all three RNAs, identical to those described for wild-type *0.3* RNA by Steitz and Bryan (14). The approximate limits of site *a* in the sequence of Fig. 2 can be determined from knowledge of the oligonucleotides in the protected fragment, the specificities of the nucleases, and the positions of the labeled phosphates. Site *a* of wild type or CR17 was found to contain at least nucleotides 17–50 and not to extend past nucleotide 52, in excellent agreement with the results of Steitz and Bryan (14). However, site *a* of CR35a had shifted perhaps 12–15 bases from the wild-type position, containing nucleotides 34–62 and not extending beyond nucleotides 27–65. It can be seen from the pancreatic RNase fingerprints of Fig. 3 that, relative to wild type or CR17, the protected fragment from CR35a contained little U(G), AC(G), or GAAGU(A) (from nucleotides 17–26), about the same amount of AAGAU(G) (32–36), and also a new oligonucleotide, AAC(G) (62–64). This 12- to 15-base shift suggests that ribosomes may bind to CR35a RNA in a position to initiate at the in-phase AUG at nucleotides 50–52, 15 bases from the AUG used for initiation in wild type.

Exactly the same protection patterns were observed for the site *a* region when purified D159 fragments were used instead of full-length *0.3* RNA (Fig. 3 *G–I*). Therefore, the observed binding and protection behavior does not appear to be influenced by nucleotides outside the first 100, and the shift in protection of CR35 RNA must be due to the mutation at nucleotide 24. The CR35a-1 suppressor mutation shifts the ribosome protection of site *a* back to the wild-type position (not shown), a strong indication that pairing with the 16S ribosomal RNA is an important determinant of ribosome binding (1–3).

As a more quantitative test of whether the CR17 or CR35 mutations might decrease efficiency of ribosome binding to site *a*, we compared the ability of ribosomes to bind labeled RNA from an equal mixture of D159 fragments from wild-type and mutant *0.3* RNAs. The nuclease step was omitted to eliminate the possibility that it could cause a decrease in recovery of bound RNA. The relative amounts of RNAs bound were determined both by using specifically labeled RNAs and by quantifying oligonucleotides characteristic of each RNA. The results indicated that, under the conditions used, the CR17 mutation had no effect on binding efficiency at site *a* and the CR35 mutation had only a small effect, decreasing binding by perhaps 15–40%. Changes in variables that might influence binding efficiency, such as concentration of ribosomes, fMet-tRNA, or competitor f2 RNA, have not been investigated systematically, but preliminary indications are that binding of ribosomes to CR35 RNA is decreased, relative to CR17 or wild-type RNA, as the concentration of fMet-tRNA is decreased.

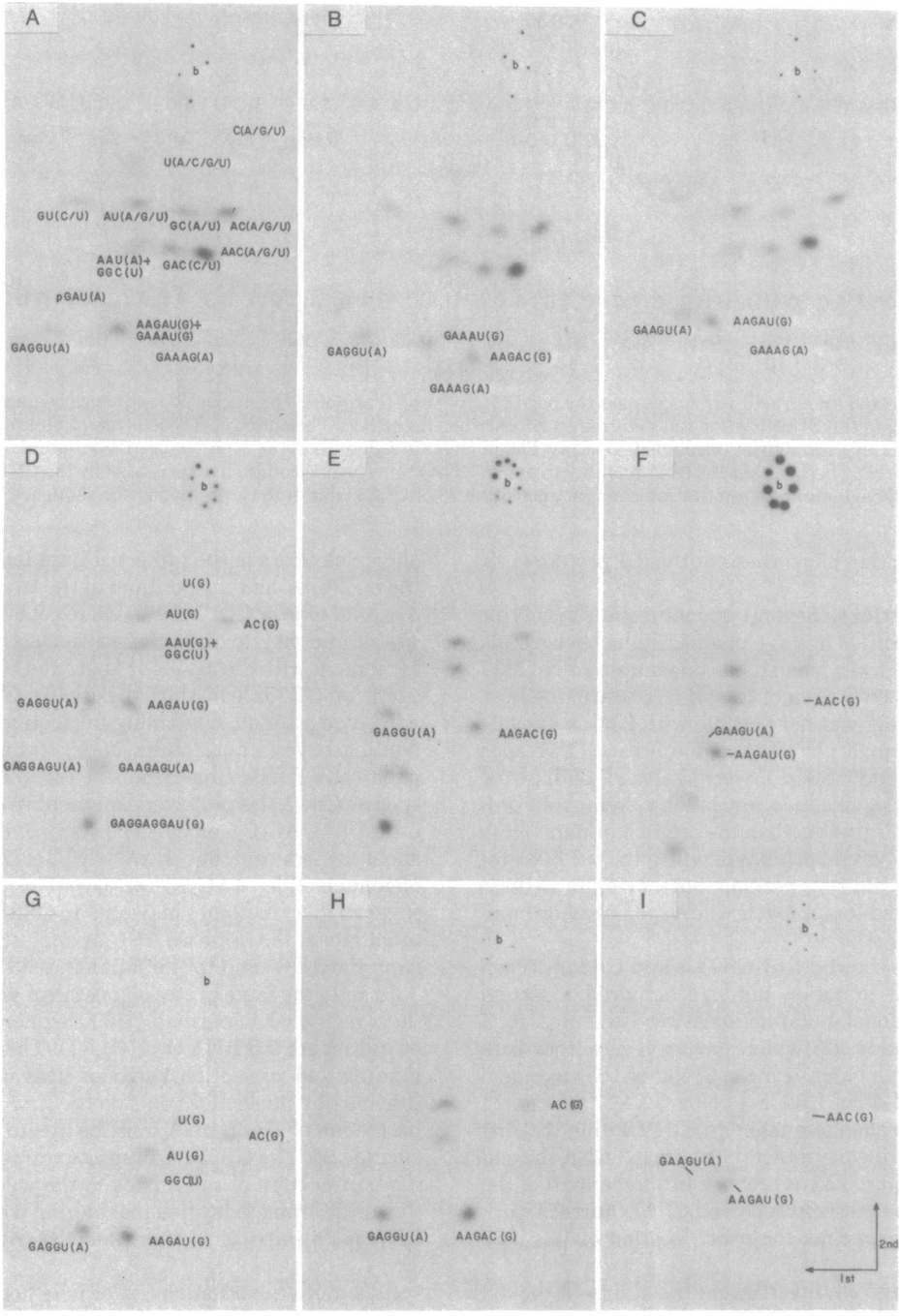

FIG. 3. Pancreatic RNase fingerprints of D159 fragments and of ribosome-protected regions of the *0.3* RNAs of wild type (*A*, *D*, and *G*), CR17 (*B*, *E*, and *H*), and CR35a (*C*, *F*, and *I*). Fingerprints were obtained by electrophoresis on cellulose acetate at pH 3.5 followed by homochromatography on DEAE thin layers (Brinkmann, Cel 300 DEAE) with Homomix c (12). The position of the xylene cyanole FF dye is indicated by "b" in each pattern. (*A–C*) Fingerprints of the entire D159 fragments (uniformly labeled with ^{32}P). (*D–F*) Fingerprints of ribosome-protected regions of full-length *0.3* RNA, containing oligonucleotides from both sites *a* and *b* (14) (labeled with [α-^{32}P] GTP). (*G–I*) Fingerprints of ribosome-protected regions of the D159 fragments of *0.3* RNA, containing oligonucleotides from only site *a* (labeled with [α-^{32}P]GTP). The sequences and nearest neighbors of all the oligonucleotides found for the wild-type RNAs are given. Sequences of selected oligonucleotides are also given for the CR17 and CR35a RNAs; all other oligonucleotides have the same sequence as their wild-type counterparts. The entire sequence of the D159 fragment of wild-type *0.3* RNA is given in Fig. 2.

DISCUSSION

Our results provide strong support for the idea that specific initiation of protein synthesis in *E. coli* involves a sequence in the mRNA that is capable of forming base pairs with the 16S ribosomal RNA (1–3). A five-base complementarity between the *0.3* RNA of T7 and the 16S RNA had been identified previously [(14); Fig. 2], and we have identified two mutations within this sequence of the mRNA: the CR35 mutation which changes the center base, interrupting the complementarity; and the CR35a-1 suppressor mutation which changes a neighboring base and creates a new four-base complementarity at essentially the same site. The CR35 mutation not only causes a great decrease in rate of synthesis of *0.3* protein but also shifts the position of the ribosome-binding site observed *in vitro;* the CR35a-1 suppressor mutation restores both the wild-type rate of synthesis and the wild-type position of ribosome binding. This suggests that pairing between the mRNA and 16S RNA may

be important both in selecting the site in mRNA at which ribosomes bind and for determining the efficiency of initiation of protein chains. Further work will be required to determine how these two effects are related and whether pairing between mRNA and 16S RNA may be involved in more than one step in the initiation process.

The new ribosome-binding site in the *0.3* RNA of CR35 would center initiation on another in-phase AUG, nucleotides 50–52. The longest perfect complementarity between the sequence on the 5′ side of this AUG and the appropriate sequence in 16S RNA is only two bases, involving the GG at nucleotides 37–38. However, nucleotide 53 is A, and Taniguchi and Weissmann (17) found that the efficiency of ribosome binding increased when the nucleotide following an AUG initiation codon was changed to an A, presumably because AUGA can form an additional base pair with the anticodon loop of fMet-tRNA. Perhaps a strong interaction between fMet-tRNA and the AUGA at nucleotides 50–53, together with the relatively poor interaction between mRNA and 16S RNA, is sufficient to bind the *0.3* RNA to the ribosome at this site. It should be possible to establish from the NH_2-terminal amino acid sequence whether the small amount of *0.3* protein made after infection by CR35 is initiated at this internal AUG.

The CR17 mutation changes the AUG initiation codon for the *0.3* protein to ACG and causes a drastic decrease in rate of synthesis of this protein, both *in vivo* and in a cell-free protein-synthesizing system. However, *0.3* RNA containing this mutation seems to bind ribosomes *in vitro* at the same site and with the same efficiency as does wild-type *0.3* RNA. This suggests that loss of the AUG does not affect the ribosome-binding step and that the defect in initiation is at a later stage. It is possible that the interaction between mRNA and 16S RNA may cause ribosomes to bind at this site even in the absence of an AUG at the initiation site. On the other hand, the *0.3* initiation site has a second in-phase AUG at nucleotides 41–43, just two codons to the 3′ side of the AUG normally used (Fig. 2); perhaps this second AUG is close enough to participate in binding to the ribosome but is unfavorably located for efficient initiation of protein synthesis. It should be possible from the NH_2-terminal amino acid sequence to distinguish whether the small amount of *0.3* protein made after infection by CR17 begins at the ACG or at the second AUG.

If the AUG at nucleotides 41–43 is involved in binding ribosomes to the *0.3* RNA of CR17, this would provide a possible explanation for the difference between our results and those of Taniguchi and Weissmann (17), who found that a change in the initiation codon for the Qβ coat protein from AUG to AUA completely abolished ribosome binding (although an additional mutation in the next nucleotide restored some binding). The Qβ initiation site (17) has no nearby AUG, either in or out of phase, to participate in binding. Alternatively, the difference in ability of the two RNAs to bind ribosomes after loss of the AUG could reflect a difference in potential interaction between mRNA and 16S RNA: the T7 site has a possible interaction of five consecutive pairs whereas the Qβ site would be able to form only three base pairs plus a terminal U·G. This interpretation would suggest that the interaction of the AUG with fMet-tRNA is more important for binding at sites that have a relatively unfavorable interaction with 16S RNA. The shift in the ribosome-binding site as a result of the CR35 mutation would also be consistent with this idea.

The CR17-1 suppressor mutation, which permits an increased rate of synthesis of *0.3* protein from RNA containing the ACG mutation of CR17, lies within the *0.3* RNA but is not within the first 101 bases from the 5′ end. It is not clear how a mutation so far away influences initiation, but perhaps the conformation of a critical portion of the *0.3* RNA is affected. Determining the sequence around this suppressor mutation, and the location of the initiation codon used in CR17-1, might provide some insight into the mechanism of this suppression.

Gene *0.3* of T7 has several advantages for further work on initiation of protein synthesis. Mutations that decrease the rate of *0.3* protein synthesis are fairly easy to identify, as are secondary mutations that restore higher rates of synthesis. The *0.3* RNA and protein are well characterized, easy to prepare, and readily identified by gel electrophoresis. The *in vivo* effects of mutations affecting initiation of synthesis of the *0.3* protein are reflected in cell-free protein-synthesizing systems, making it possible to dissect the biochemical effects of the mutations. The *0.3* RNA is also translated in cell-free protein-synthesizing systems from eukaryotes (18), so effects of these mutations on initiation of protein synthesis in eukaryotic systems can also be tested.

We thank H. D. Robertson and J. A. Steitz for helpful discussions, H. D. Robertson for the gift of f2 RNA, and N. Delihas for help in preparing fMet-tRNA. We thank W. C. Crockett, B. Lade, G. McGovern, and O. Ritter for able technical assistance. This research was carried out at Brookhaven National Laboratory under the auspices of the Department of Energy. E.B.P. received support from Grant 1 T32-CA0-9121-03, awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

1. Steitz, J. A. (1978) in *Biological Regulation and Development*, ed. Goldberger, T. (Plenum, New York), Vol. 1, in press.
2. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **72**, 1342–1346.
3. Steitz, J. A. & Jakes, K. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4734–4738.
4. Studier, F. W. (1975) *J. Mol. Biol.* **94**, 283–295.
5. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 237–248.
6. Dunn, J. J. (1976) *J. Biol. Chem.* **51**, 3807–3814.
7. Kerr, C. & Sadowski, P. D. (1972) *J. Biol. Chem.* **247**, 311–318.
8. Barnes, W. M., Reznikoff, W. S., Blattner, F. R., Dickson, R. C. & Abelson, J. (1975) *J. Biol. Chem.* **250**, 8184–8192.
9. Steitz, J. A. (1969) *Nature* **224**, 957–964.
10. Dunn, J. J. & Studier, F. W. (1975) *J. Mol. Biol.* **99**, 487–499.
11. Dubnoff, J. S. & Maitra, U. (1971) in *Methods in Enzymology*, eds. Moldave, K. & Grossman, L. (Academic, New York), Vol. 20, pp. 248–261.
12. Barrell, B. G. (1971) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. L. & Davies, D. R. (Academic, New York), Vol. 2, pp. 751–795.
13. Simon, M. N. & Studier, F. W. (1973) *J. Mol. Biol.* **79**, 249–265.
14. Steitz, J. A. & Bryan, R. A. (1977) *J. Mol. Biol.* **114**, 527–543.
15. Studier, F. W. (1969) *Virology* **39**, 562–574.
16. Godson, G. N. & Sinsheimer, R. L. (1967) *Biochim. Biophys. Acta* **149**, 489–495.
17. Taniguchi, T. & Weissmann, C. (1978) *J. Mol. Biol.* **118**, 533–565.
18. Anderson, C. W., Atkins, J. F. & Dunn, J. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2752–2756.