Reports

Suppression in vitro: Identification of a Serine-sRNA as a "Nonsense" Suppressor

Abstract. *In a cell-free system, with RNA from a suppressible mutant of bacteriophage R17 as messenger, no functional coat protein of the bacteriophage is synthesized unless serine-accepting soluble RNA from the suppressor strain,* Escherichia coli *S26R1E, is present.*

The molecular mechanism by which the amber suppressor genes in *Escherichia coli* reverse the effects of mutations in other genes recently has been shown to be a misreading of the genetic code. The amber suppressor genes constitute one class of suppressors; there is evidence that other suppressors in *E. coli* (*1*, *2*) and in yeast (*3*) act in a similar manner.

Mutations suppressible by amber suppressor genes can occur in many different cistrons, regardless of the cistron's function. These amber mutations—mutations suppressible by amber suppressor genes—have been isolated in the rII region of bacteriophage T4 (*4*), in the entire T4 genome (*5*), in the *E. coli* structural gene specifying alkaline phosphatase (*6*), in the bacteriophage lambda (*7*), and in the RNA-containing (*8*) bacteriophage f2 (*9*).

Benzer and Champe (*10*) and Garen and Siddiqi (*6*) first suggested that the amber mutation produces a nonsense codon—a nucleotide triplet which does not code for any amino acid and thus interrupts polypeptide synthesis in a nonpermissive cell (one lacking an amber suppressor). They further postulated that this codon would be read as an amino acid code word in a permissive (suppressor-containing) host, thereby permitting protein synthesis to continue. This hypothesis received strong support from the work of Brenner and his colleagues, who showed that amber mutants in the head protein of bacteriophage T4D produce a normal yield of released chain fragments in a nonpermissive *E. coli* host, but make a mixture of about 60 percent complete protein and 40 percent fragments in the permissive host (*11*).

Four genetically distinguishable amber suppressor genes in *E. coli* have been identified (*12*). The action of one of these, the *su-A* suppressor gene contained in strain S26R1E of Garen and Siddiqi, has been investigated extensively. In the T4D head protein (*13*), in alkaline phosphatase (*12*), and in f2 coat protein (*14*), a glutamine or tryptophan residue in the wild-type protein is always replaced by serine in the mutant protein produced in the *su-A*–containing host. Thus, the amber nonsense codon has arisen through mutations from triplets coding for glutamine and tryptophan. An alteration of the protein-synthesizing machinery of the permissive host has changed its ability to recognize the nonsense codon, so that part of the time it is recognized as a code word for serine.

Since amber suppression is based on the misreading of the code in the permissive cell, an alteration of any component that guarantees the accuracy of reading might result in suppression. Four possibilities are: (i) an altered aminoacyl synthetase which transfers an amino acid to a class of sRNA molecules which normally recognizes the nonsense triplet, but does not bind any amino acid; (ii) a new species of sRNA specific for a particular amino acid, yet capable of recognizing the nonsense codon; (iii) mutant ribosomes which distort the natural mRNA-sRNA complex, thereby permitting the recognition of a nonsense codon by a normally occurring sRNA species (*2*); and (iv) the presence of base analogs or an inaccurate RNA polymerase, which would produce an altered messenger (for example, fluoracil inserted in place of uracil) (*4*).

We show here that the component active in suppression in strain S26R1E is a serine-accepting sRNA species not present in the nonpermissive isogenic strain S26. This result was made possible by the development of an assay, in vitro, for suppression by S26R1E of an amber mutation in the coat-protein cistron of the RNA-containing bacteriophage R17. The RNA isolated from this mutant directs the synthesis of functional coat protein in a cell-free protein-synthesizing system derived entirely from the permissive host S26R1E. No functional protein is produced in a system derived from S26. However, a mixed system, consisting of serine-accepting sRNA from S26R1E added to an otherwise nonpermissive system, produces functional coat protein.

Preparation of the assay system. *Escherichia coli* bacterial strains S26 and S26R1E, an isogenic pair obtained from A. Garen, are nonpermissive (Su−) and permissive (Su+), re-

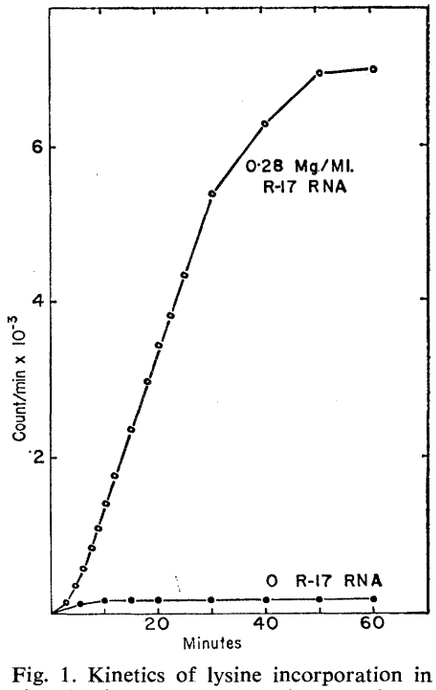

Fig. 1. Kinetics of lysine incorporation in vitro in the presence and absence of R17 RNA. The 1-ml reaction mixture contained 0.25 ml of "preincubated" *E. coli* B S-30, 0.8 mg of stripped sRNA from *E. coli* B, and 0.5 μc of C^{14}-lysine (1.93×10^7 count min^{-1} $\mu mole^{-1}$). Each point represents the radioactive material in 50 μl of reaction mixture, which was precipitable by hot trichloroacetic acid (TCA).

Table 1. Capacity of sRNA's to accept amino acids; results in mμmole of amino acids per milligram of sRNA. The conditions used for these assays were those of Berg *et al.* (*26*).

Amino acid	sRNA		
	Su+	Serine-accepting	Leucine-accepting
Alanine	2.21	0.024	0.025
Arginine	2.14	.026	.022
Isoleucine	0.895	.015	.083
Leucine	3.51	.028	3.26
Serine	1.81	1.64	0.027

spectively. *Escherichia coli* strain C600G, a permissive host used as an indicator strain by Campbell (*7*), was obtained from M. Meselson and converted to F+ so that it would support the growth of R17. *Escherichia coli* B and *E. coli* Hfr_1 are nonpermissive strains. Strain Hfr was obtained with a stock of R17 from A. Graham.

For the preparation of *E. coli* extracts (S-30) (*8*), cells were grown in a glycerol-casamino acid medium (*15*). Cells were harvested during exponential growth, washed twice in tris-magnesium buffer (0.01*M* magnesium acetate and 0.01*M* tris, *p*H 7.5), and frozen until used. The S-30 extracts were prepared according to the method of Nirenberg and Matthaei (*16*) except for two modifications: (i) NH_4Cl was used in place of KCl and (ii) 1.5 ml of tris-magnesium buffer per gram of cells was used instead of 3.0 ml per gram.

The S-30 extracts were "preincubated" (*8*) with an ATP-generating system, chilled, and immediately used in incorporation experiments. For cell-free protein synthesis, the reaction mixtures contained per milliliter: one-fourth volume of preincubated S-30, 0.003 mmole ATP, 0.002 mmole GTP, 0.005 mmole phosphoenolpyruvate, 20 μg pyruvate kinase, 4×10^{-5} mmole of each amino acid, 0.010 mmole magnesium actetate, 0.075 mmole of NH_4Cl, 0.05 mmole tris (*p*H 7.8), 0.01 mmole glutathione, stripped sRNA (*8*), and R17 RNA. The temperature of incubation was 36°C.

Total sRNA was prepared by the procedure of Zubay (*17*). The biological activity of all sRNA species except those protected by the attachment of a chosen amino acid to the sRNA terminal adenylic acid residue was destroyed by oxidation with periodate. Stripped Su+ sRNA was charged with (esterified to) either serine or leucine, saturating amounts of Su− activating enzymes being used. The reaction was stopped after 20 minues at 35°C by precipitation with ethanol. The precipitate was suspended in distilled water and dialyzed for 2 hours at 4°C. The RNA solution was then centrifuged at 15,000 rev/min for 20 minutes in a Serval SS-2 centrifuge to remove denatured protein. The supernatant was made 0.1*M* with sodium acetate, *p*H 4.7. A 12-fold excess of sodium periodate was added to the sRNA solution, and the oxidation was allowed to proceed for 30 minutes at 30°C in the dark. The reaction was stopped by the addition of excess glucose, and the mixture was precipitated with ethanol. The precipitate was dissolved in distilled water, and the solution was dialyzed for 24 hours. To test the capacity of the oxidized sRNA preparations to accept amino acid, the sRNA's were first stripped by incubating with 0.1*M* tris, *p*H 8.8, at 37°C for 3 hours. The oxidation procedure was adopted to assure protection of sRNA charged with serine. P. C. Zamecnik pointed out to us that the normal procedure for periodate oxidation is not successful because of the oxidation of the amino and hydroxyl groups of serine and the subsequent lability of the oxidation product.

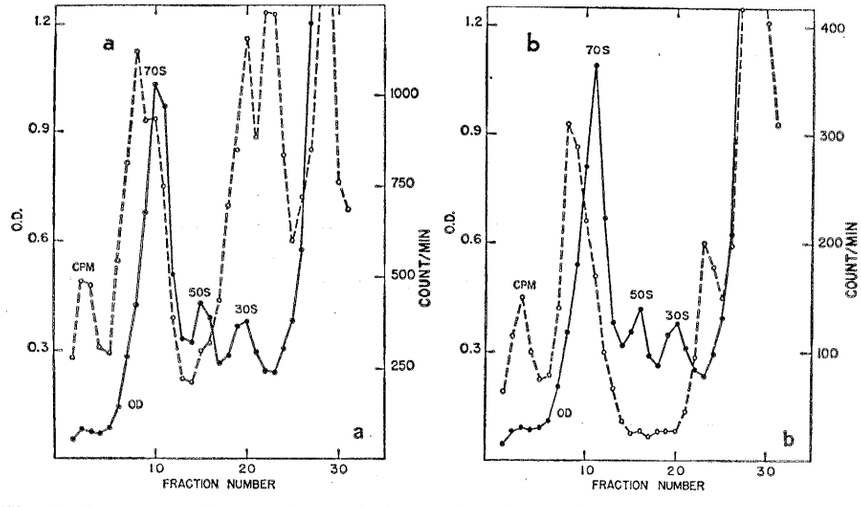

Fig. 2. Sucrose-gradient analysis of the total amino acid–incorporating system after 20-minute incubation with R17 RNA and either C^{14}-lysine and C^{14}-arginine or H^3-histidine. The 200-μl reaction mixture contained 50 μl of preincubated *E. coli* B S-30, 104 μg of R17 RNA, 160 μg of stripped *E. coli* B sRNA. (*a*) The reaction mixture contained 0.2 μc of C^{14}-lysine (7.25×10^7 count min^{-1} $\mu mole^{-1}$) and 0.2 μc of C^{14}-arginine (7.75×10^7 count min^{-1} $\mu mole^{-1}$); (*b*) the reaction mixture contained 5 μc of H^3-histidine (5.25×10^8 count min^{-1} $\mu mole^{-1}$). A 100-μl portion of the reaction mixture was layered on a 5-ml linear sucrose gradient (5 to 20 percent) and centrifuged at 38,000 rev/min for 2.3 hours at −12°C. Each fraction was first assayed for optical density at 260 mμ and then treated with 6 percent TCA at 90°C for 20 minutes to prepare the samples for radioactivity analysis. Of the acid-insoluble (hot TCA) radioactive material originally put on the gradient, 85 percent was recovered. The optical density of the 30*S* peaks in this and succeeding experiments is higher than might be expected because of the absorption of phage RNA.

Bacteriophage R17 was grown and purified according to the procedure of Gesteland and Boedtker (*15*). Titers of 1 to 3×10^{12} pfu/ml (*8*) were routinely obtained. The R17 RNA was extracted with phenol and then precipitated from the extract with ethanol. The ratio of the absorbancy at 260 mμ to that at 280 mμ of the R17 RNA so prepared is between 1.9 and 2.0.

For the isolation of R17 amber mutants, a concentrated phage suspension was incubated in 0.2*M* $NaNO_2$, *p*H 4.6, at 37°C for 8 to 9 hours. After dilution to stop the reaction, the suspension of phage was plated on S26R1E; individual plaques were picked, and each was tested for growth on S26. After nitrous acid treatment of a phage suspension which originally contained 10^{13} viable particles per milliliter, 10^5 particles per milliliter survived. Among the survivors, about 1 to 3 in 500 were mutants.

For the growth of the R17 mutant am11B, a mutant stock containing 2×10^{11} pfu/ml, 0.8 percent revertants, was used to infect 16-liter cultures at a phage–bacteria ratio of about 3 to 1, and an optical density of 0.6 to 0.8. Stocks thus obtained all contain 5 to 7×10^{11} pfu/ml, with 1 to 2 percent revertants. The phage particles were purified in the same manner as wild-

type phage and the percentage of revertants remained constant throughout this procedure.

Amino acids labeled with C^{14} or H^3 were obtained from New England Nuclear Corporation, Boston, Mass. The specific activities of the C^{14} amino acids in $\mu c/\mu mole$ were: alanine, 100; arginine, 239; isoleucine, 221; lysine, 210; serine, 120; H^3-histidine had a specific activity of 1.1 $mc/\mu mole$. Pyruvate kinase, ribonuclease, and deoxyribonuclease were obtained from Worthington Biochemical Corporation.

Development of the assay for amber suppression. The kinetics of C^{14}-lysine incorporation in vitro, with a preincubated S-30 extract in the presence and absence of added R17 RNA, are shown in Fig. 1. Under optimum conditions, there is a 30- to 50-fold stimulation of amino acid incorporation with the addition of R17 RNA.

If we assume that the product synthesized in vitro has an amino acid composition similar to that of R17 coat protein (*18*), then we can calculate that approximately 260 amino acids per ribosome are being incorporated into protein. This corresponds to two molecules of phage coat protein per ribosome in the reaction mixture. From Fig. 1, one can see that preincubation virtually eliminates protein synthesis mediated by endogenous *E. coli* messenger. All experiments reported here were performed with a preincubated S-30 homogenate.

After 20 minutes of incubation with R17 RNA as messenger, newly synthesized polypeptide sedimented in six discrete regions, with sedimentation constants of approximately 112*S*, 85*S*, 70*S*, 30*S*, 20*S*, and less than 4*S* (Fig. 2*a*).

The radioactivity which is associated with fractions sedimenting at 70*S*, 85*S*, and 112*S* represents newly made chains attached to ribosomes. The 85*S* and 112*S* peaks arise from the combination of an R17 RNA chain with one and two ribosomes, respectively; the 70*S* peak represents ribosomes from which the RNA messenger has been detached or degraded, but which have not released their polypeptide product. All the more slowly sedimenting material consists of protein products released from ribosomes. Particularly relevant is the newly synthesized material which sediments at 30*S*.

We believe that this material consists of complete coat protein for the following reasons: (i) autoradiograms of peptides resulting from digestion of the 30*S* material by trypsin reveal a peptide pattern (fingerprint) identical to that obtained from coat protein (*19*); (ii) sucrose-gradient analysis of cell-free reaction mixture in which the radioactive label was exclusively in histidine reveals (Fig. 2*b*) that no radioactivity is present in the 30*S* peak. This is in agreement with the fact that the coat protein of R17, like that of f2 and MS2, does not contain histidine (*18*).

The rapid rate of sedimentation of this coat protein synthesized in vitro suggests that it consists either of a coat protein aggregate or of protein subunits bound to the R17 RNA which, in the reaction mixture, normally sediments at about 30*S*. Mild

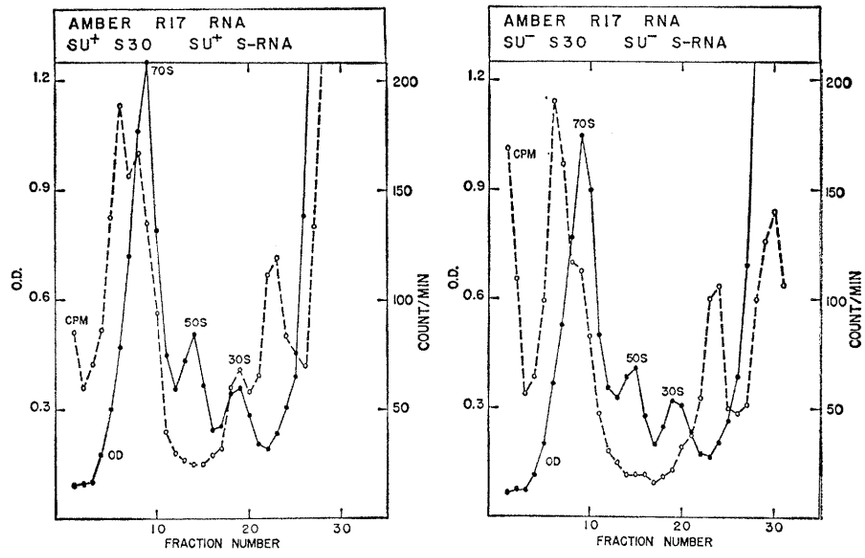

Fig. 3. Sucrose-gradient analyses of amino acid incorporation directed by am11B RNA. Each reaction mixture contained per 200 μl: 100 μg of am11B RNA containing less than 2 percent contamination from wild type, 0.2 μc of C^{14}-isoleucine (8.4×10^7 count min^{-1} $\mu mole^{-1}$), and either 50 μl of preincubated Su^+ S-30 and 160 μg of stripped Su^+ sRNA (left) or 50 μl of preincubated Su^- S-30 and 160 μg of Su^- stripped sRNA (right). A 100 μl sample of the reaction mixture was layered on a 5-ml sucrose gradient after a 20-minute period of incubation at 36°C. The gradients were centrifuged at 38,000 rev/min for 2.4 hours at −12°C. Approximately 85 percent of the acid-insoluble (hot TCA) radioactive material that had been put on the gradients was recovered.

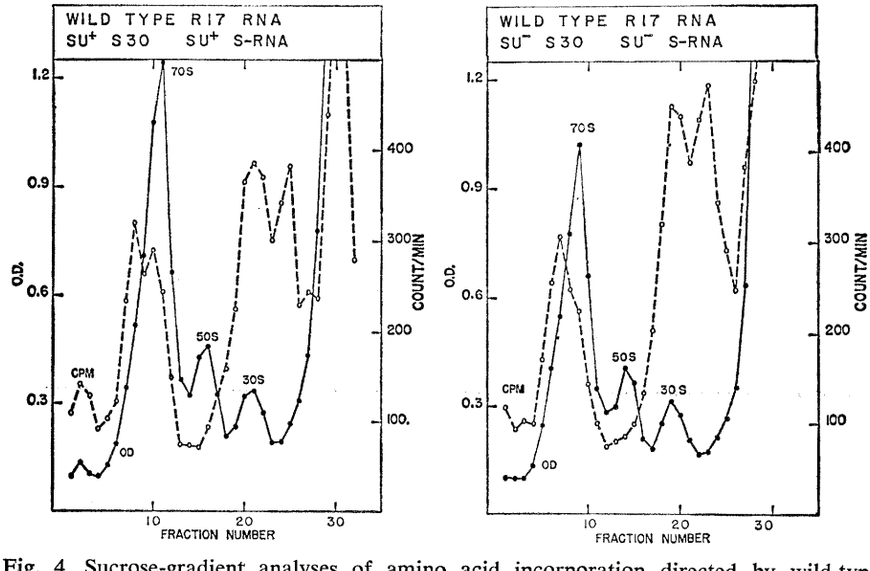

Fig. 4. Sucrose-gradient analyses of amino acid incorporation directed by wild-type RNA in systems derived from Su^+ and Su^- bacteria. These experiments were run in parallel with, and under the same conditions as, those described in Fig. 3. In each 200-μl reaction mixture there were 104 μg of wild-type R17 RNA. On the left is the result of incorporation in the Su^+ system, on the right, that in the Su^- system.

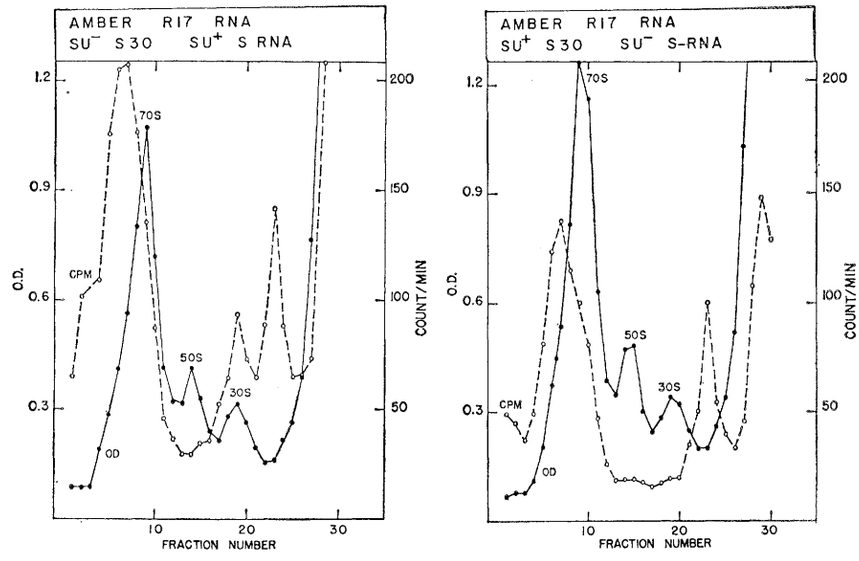

Fig. 5. sRNA mixing experiment. The sucrose-gradient analysis of protein synthesis in vitro with am11B RNA as messenger was run in parallel with, and under the same conditions as, the experiment shown in Fig. 3. The only difference was that to the reaction mixture containing 50 μl of preincubated Su$^+$ S-30, was added 160 μg of stripped Su$^-$ sRNA (right) and to the reaction mixture containing Su$^-$ S-30 was added stripped Su$^+$ sRNA (left).

treatment with ribonuclease (0.2 μg/ml for 3 minutes at 0°C) of an incubation mixture prior to its being layered on a sucrose gradient shifts the newly synthesized 30*S* material to the top of the gradient, thereby demonstrating that it was previously bound to free R17 RNA molecules.

The nature of the 20*S* peak is not yet clear. It definitely is not coat protein, since (i) it contains histidine and (ii) its autoradiogram is different from, and far more complex than, that of coat protein (*19*). Thus it must arise either from a mixture of different proteins or from a protein of high molecular weight relative to that of coat protein. It is most tempting to believe that the 20*S* material might be R17-specific RNA synthetase.

Notani, Engelhardt, Konigsberg, and Zinder (*14*) have unambiguously demonstrated that a number of their amber mutations in f2 are in the coat-protein cistron. All of the coat-protein mutants (su-3 class) grow on strain S26R1E, but not on strain S26 or on strain C600. In contrast, a number of other mutants (su-1 class), identified by complementation tests as belonging to another cistron, grow on strain C600 as well as on S26R1E. A search was undertaken for R17 amber mutants (*20*) which had the same plating and complementation properties as the su-3 group of f2. Among those found, one, am11B, was chosen for studies in vitro. Parallel with the experiments in vitro, we have undertaken chemical experiments to demonstrate directly a difference in the amino acid composition of its coat protein. Fingerprinting of tryptic digests, a separation of the tryptic peptides by electrophoresis in one dimension followed by chromatography in the second dimension, reveals the disappearance of one normal peptide and the appearance of a new one compared to wild-type R17 coat protein. This result gives us reason to believe that am11B is a coat-protein mutant. The results of the studies in vitro that are described next confirm this identification.

When am11B RNA is used as messenger in the cell-free system, there are no gross differences either in the kinetics or in the final amount of protein synthesis between the Su+ (S26R1E) and Su− (S26) systems. Under conditions in which synthesis mediated by wild-type RNA is identical in the two systems, there is a 15 to 20 percent greater incorporation of amino acids in the Su+ than in the Su− system when RNA from mutant phage is used.

The reaction mixtures are supplemented with stripped sRNA (about 1 mg/ml) isolated from Su+ and Su− bacteria. This addition stimulates protein synthesis threefold, regardless of whether the mRNA used is from wild-type or am11B bacteriophage. The influence of added sRNA on the degree of amino acid incorporation is an important factor in the success of experiments described below.

Sucrose gradient analyses of Su+ and Su− reaction mixtures, incubated with am11B RNA, are shown in Fig. 3. Each reaction mixture contained

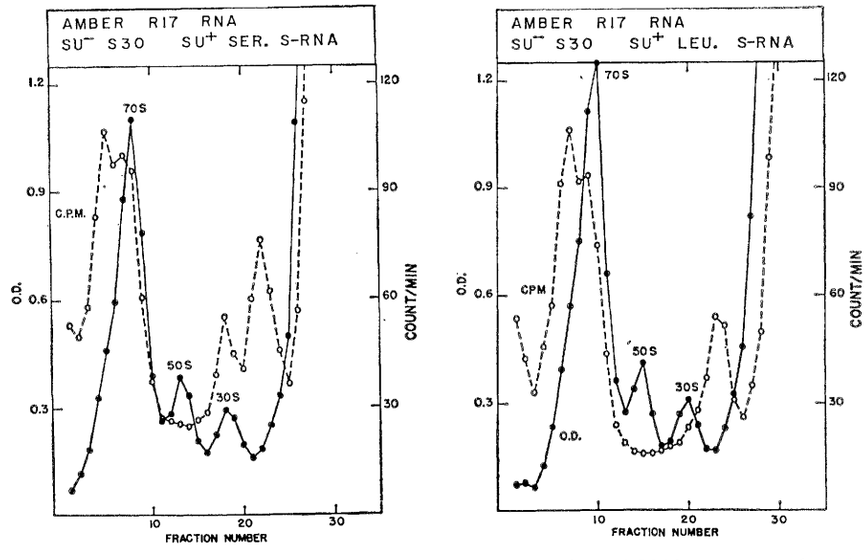

Fig. 6. Addition of Su$^+$ serine- or Su$^+$ leucine-accepting sRNA's to an otherwise nonpermissive protein-synthesizing system directed by am11B RNA. In each 200-μl reaction mixture there were 95 μg of am11B RNA, 50 μl of preincubated Su− S-30, 120 μg of stripped Su$^-$ sRNA, 0.2 μc of C^{14}-isoleucine (8×10^7 count min^{-1} μmole^{-1}), and approximately 5 μg of Su$^+$ sRNA having only serine-accepting activity (left) or leucine-accepting activity (right). A 100-μl sample of the reaction mixture was put on a 5-ml sucrose gradient after a 20-minute period of incubation at 36°C. Approximately 85 percent of the acid-insoluble (hot TCA) radioactive material, which had been put on the gradients, was recovered.

added sRNA which was homologous to the S-30 used. A difference between the two gradients is apparent. The 30*S* peak, previously identified as R17 coat protein synthesized in vitro and bound to R17 RNA, is absent in the gradient of the Su− reaction mixture (right side of Fig. 3). This observation is consistent with the hypothesis that am11B is a mutation in the R17 cistron specifying coat protein. As a control, the experiment was performed in parallel tubes with wild-type R17 RNA as messenger (Fig. 4). Since both bacterial strains permit the growth of wild-type phage, both the 30*S* and 20*S* proteins should be synthesized in vitro in either system when wild-type RNA is used as messenger. This is, in fact, observed.

We have repeated all of these experiments, with identical results, using different preparations of S-30's, sRNA's, R17 wild-type and am11B RNA's, and also using amino acids other than isoleucine as label. These results confirm prior indications that suppression occurs during messenger RNA translation; and most important, the presence or absence of the 30*S* peak constitutes as assay for suppression in vitro.

The amount of 30*S* protein synthesized in the Su+ system is reduced relative to that of the 20*S* protein when am11B RNA is used as messenger. This reduction can be used as a measure of the efficiency with which the Su+ system can suppress the amber mutation in vitro. The level observed is consistent with the observation that suppression of amber mutations in the T4 head protein by the *su-A* suppressor gene is approximately 60 percent efficient in vivo (*11*).

With this assay for suppression in vitro, we are in the position, by means of mixing experiments, to discover which component or components of the protein-synthesizing system are responsible for the suppression of the amber mutation. Experiments in which Su+ sRNA was added to the incubation mixture containing Su− S-30 (Fig. 5, left), and vice versa (Fig. 5, right), were run in parallel with those shown in Figs. 3 and 4. Since addition of Su+ sRNA to the S-30 prepared from Su− cells permits synthesis of functional coat protein (protein capable of binding to the added R17 RNA), and since, conversely, the Su+ S-30 cannot produce 30*S* protein in the presence of Su− sRNA, suppression must be caused by a difference in the respective sRNA pools in Su+ and Su− bacteria.

Weigert and Garen (*12*) and Notani *et al.* (*14*) have shown that suppression by S26R1E results in the insertion of serine into mutant protein, usually in place of a glutamine residue in the wild type. This fact, coupled with our finding that suppression in this strain is due to a difference between its sRNA pools and those of the isogenic strain S26, leads us to predict that the specific sRNA acting as the suppressor is a new or altered serine-accepting sRNA capable of inserting serine at the nonsense site. To test this hypothesis, we prepared Su+ sRNA which contained only biologically active serine-accepting sRNA's. This we achieved by charging stripped Su+ sRNA with serine, using an unfractionated, nucleic acid–free mixture of amino acid activating enzymes (*21*) prepared from S26 (Su−) bacteria. As a control, a second portion of Su+ sRNA was charged with leucine.

The two sRNA preparations were then treated with sodium periodate to destroy any uncharged sRNA by oxidation of the 2′- and 3′-hydroxyl groups on the ribose of the terminal adenylic acid residue of sRNA (*22*). To test the effectiveness of this method in protecting only a specific sRNA, the oxidized sRNA was stripped, and the amino acid–accepting activity for each of five different amino acids was examined and compared to that of the original Su+ sRNA (Table 1). Protection of the serine- and leucine-accepting sRNA's is better than 90 percent, whereas other sRNA species were destroyed to the extent that their residual accepting activity was only 1 percent. The anomaly with isoleucine could be due either to contamination by C^{14}-leucine of the C^{14}-isoleucine used to test the isoleucine-accepting activity, or to contamination by C^{12}-isoleucine of the C^{12}-leucine employed for protection. The addition of sRNA's which have the 2′- and 3′-hydroxy groups of ribose oxidized to aldehydes does not inhibit protein synthesis in vitro.

The effect of adding either Su+ serine sRNA or Su+ leucine sRNA to the protein-synthesizing system mediated by am11B RNA is shown in Fig. 6. The reaction mixtures contained Su− S-30, 0.9 mg per milliliter of Su− sRNA, and 0.5 mg per milliliter of either Su+ serine-accepting or Su+ leucine-accepting sRNA. The results clearly implicate Su+ serine-sRNA as the requirement for suppression of amber mutations by S26R1E. The experiment was repeated with wild-type RNA instead of am11B RNA as messenger, and no appreciable difference between the serine and leucine gradients was observed.

Molecular models for suppression. The C^{14}-labeled polypeptide sedimenting in the 30*S* region of the sucrose gradient has been identified by fingerprinting as phage coat protein. Its high sedimentation value is due to the binding of subunits of phage coat protein, synthesized in vitro, to the phage RNA, which was added as messenger. This formation of a 30*S* complex between input phage RNA and protein synthesized in vitro allows us to examine selectively the synthesis of intact coat-protein molecules and thus to develop an assay for suppression in a cell-free system.

The assay consists of testing the capacity of the amino acid incorporating system to read the nonsense codon in the coat-protein cistron of the R17 amber mutant am11B as a serine codon, and thereby to permit the synthesis of intact phage coat protein. We have shown that the only requirement for this translation of the amber codon is the addition of an Su+ serine-accepting sRNA to the otherwise totally Su− system.

Based on studies of the pattern of mutation to, and reversion from, nonsense, Brenner *et al.* and Garen (*23*) have proposed that the amber triplet is UAG (*8*). This triplet could, in fact, be unreadable, or it could be a specific signal to terminate a completed polypeptide chain. In the first case, there would be no sRNA species able to recognize the amber codon, and chain termination might be merely a means of freeing ribosomes for the synthesis of new protein. In the second case, one might imagine an sRNA species capable of recognizing the UAG triplet, but unable to bind any amino acid.

With these possibilities in mind, two types of models can be proposed for the reading of the amber codon by a specific serine-accepting sRNA. These can be designated as (i) precise-reading models and (ii) ambiguous-reading models.

One precise-reading model could be based on a mutation in a serine-accepting sRNA anticodon, enabling it to recognize the amber codon. Since there is a UCG serine codon (*24*), the change of a single base in the corre-

sponding serine-accepting sRNA would permit it to pair exactly with the amber codon UAG. However, that species of sRNA capable of recognizing the UCG serine codon would now be eliminated unless there existed more than one cistron for the UCG-specific sRNA. Furthermore, this model does not explain the release of a large proportion of polypeptide fragments in the Su^+ cell, unless we include the additional hypothesis that an amber-specific chain-terminating sRNA exists.

A second precise-reading model is based on the assumption that an sRNA species exists that normally recognizes the amber codon as chain-terminating. A modification of this sRNA could enable it to accept serine. The production of a mixture of complete and incomplete protein would be a consequence of this model if the charging of the altered amber sRNA with serine were inefficient—that is, if not all such sRNA molecules became charged.

In the case of the ambiguous-reading model, no assumption about the nature of chain termination is necessary. Suppression could be caused by a modification of a serine-accepting sRNA, which destroys the specificity of its attachment to a codon without altering its anticodon. For example, modification of the overall structure of the serine-accepting sRNA species which recognizes the serine codon UCG might permit this sRNA species to recognize either the nonsense (UAG) or the serine (UCG) codon. This model requires no additional hypotheses to explain the production of a mixture of fragments and completed chains in the Su^+ cell.

The amber suppressor mutation, in the second and third models, could be a mutation either in the sRNA cistron or in a gene coding for an enzyme which could modify the serine-accepting or amber-specific sRNA species assumed to act as the suppressor. Likely candidates for such a task are enzymes which produce the unusual bases in sRNA (for example, methylated bases, pseudouracil, or dihydrouracil). Our results do not allow us to distinguish between enzymatic and genetic modification of the specific sRNA involved. It is possible, therefore, that the new serine-accepting sRNA is only the indirect product of the *su-A* suppressor gene.

Finally, the conditions selected for the mixing experiment (Fig. 6) enable us to determine whether the suppressor mutation is dominant in vitro. Since, in these experiments, the incubation mixtures contained a twofold excess of Su^- serine-accepting sRNA over Su^+ serine-accepting sRNA, we conclude that the *su-A* suppressor is dominant. This is consistent with the findings of Signer, Beckwith, and Brenner (*25*) that a cell heterozygous for amber suppressor gene is permissive.

MARIO R. CAPECCHI
GARY N. GUSSIN
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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8. Abbreviations and definitions: RNA, ribonucleic acid; mRNA, messenger RNA; sRNA, soluble RNA; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; pfu, plaque forming units; S-30, a bacterial homogenate from which whole cells, cell-wall fragments, and cell-membrane fragments have been removed by centrifugation at 30,000*g* for 30 minutes; "preincubation," refers to incubation of the S-30 in the absence of labeled amino acids to eliminate amino acid incorporation directed by endogenous *E. coli* messenger RNA; "charged sRNA," is sRNA to which an amino acid is attached by a phosphate-ester linkage between the terminal adenylic acid of sRNA and the carboxyl group of the amino acid; "stripped sRNA," is sRNA from which the amino acid has been removed by hydrolysis of the phosphate-ester linkage; UAG and UCG are symbols representing the sequence of nucleotides in specific codons, the letters represent the nucleotides uridine, adenosine, guanosine, and cytidine 5′-monophosphate; TCA, trichloroacetic acid.
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Pasteurella pestis: Role of Pesticin I and Iron in Experimental Plague

Abstract. *Loss of the genetic determinant for pesticin I in* Pasteurella pestis *results in concomitant loss of the plague coagulase and fibrinolytic factor. The median lethal dose for mice of an isolate lacking only these activities is increased by factors of about 10^1, 10^4, and 10^7 cells when administered by the intravenous, intraperitoneal, and subcutaneous routes, respectively. Virulence of the aforesaid strain can be enhanced in mice treated with 40 μg of ferrous iron. This response resembles that of* Pasteurella pseudotuberculosis, *a closely related species that normally lacks pesticin I.*

Burrows and co-workers (*1*) have described four properties that are essential for full virulence in *Pasteurella pestis*, the causative agent of bubonic plague. These properties are the genetic potentials that permit synthesis of purines (Pu^+), virulence antigens (VW^+), and capsular antigen ($F1^+$) and permit formation of pigmented colonies on synthetic medium (P^+). The median lethal doses in mice and guinea pigs of mutants lacking each of these determinants are shown in Table 1. Production of F1 antigen is not essential for virulence in the mouse, and strains that are Pu^+, VW^+, and P^- can be restored to full virulence in this animal by injection of ferrous ion (*2*).

Wild-type strains of *P. pestis* produce at least two bacteriocin-like substances. The first, termed pesticin I (PI), prevents growth of certain strains of *Pasteurella pseudotuberculosis* (*3*, *4*)