



Sense and Nonsense in the Genetic Code

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Sense and Nonsense in the Genetic Code

Three exceptional triplets can serve as both chain-terminating signals and amino acid codons.

Alan Garen

The recent elucidation of the genetic code, shown in Table 1, marks a notable milestone in biology (1). This code designates the relations between the 64 possible codons (2) present in messenger RNA and the 20 amino acids present in proteins. The RNA codons are derived by transcription of complementary codons in DNA, which is the primary genetic material of most organisms (the only exceptions known are certain viruses in which messenger RNA is used directly as the genetic material).

Most of our present knowledge about the code has been obtained from studies with *Escherichia coli*, in which synthetic polyribonucleotides (rather than natural messenger RNA) are added to cell extracts containing the components required for protein biosynthesis in vitro (and presumably in vivo) (3). The polynucleotides in such experiments are either triplets, which can bind a specific transfer RNA species to ribosomes, or longer chain polymers (with random or defined base sequences), which can direct the incorporation of amino acids into polypeptides. A critical assumption for this approach to the deciphering of the code is that the coding properties of polynucleotide codons in vitro are the same as those of messenger RNA codons in vivo, allowing the extrapolation

from in vitro to in vivo coding assignments. There is convincing support for this assumption from two lines of evidence, one showing that amino acid substitutions occurring in proteins as a result of mutations can be attributed to base changes which are consistent with the coding assignments for the amino acids (4-6), and another showing that the RNA component of an RNA phage acts in vitro as well as in vivo as a messenger for the coat protein of the phage (7). It should be noted that a coding assignment based on results in vitro does not necessarily prove that the codon is actually used in vivo; there is evidence that an organism can have the capacity to translate a codon but not incorporate it into its own code (see 6).

It is implicit in a triplet code, which provides a potential surplus of triplets for 20 amino acids, that the code contains degenerate codons (that is, different triplets coding for the same amino acid) or nonsense triplets (triplets which do not code for any amino acid), or both. It is immediately apparent from a glance at Table 1 that the code exhibits extensive degeneracy, as much as six-fold for some amino acids. There also are three triplets, UAG, UAA, and UGA, designated as nonsense, and it is this aspect of the code which is the main subject of this review.

Nonsense Mutants

Protein biosynthesis is a sequential process during which a peptide chain grows unidirectionally, by increments of one amino acid, from the amino-terminal toward the carboxy terminal residue (8). Accordingly, if a nonsense triplet is present at any of the positions in messenger RNA which code for the amino acid residues of a peptide chain, a gap will appear in the chain and cause premature termination of chain growth. Nonsense triplets do not normally occur within the coding regions of messenger RNA, but they can be generated from certain codons by mutation. The resulting mutants are called nonsense mutants, as distinguished from missense mutants which result from the transformation of a codon for one amino acid into a codon for another amino acid.

Since nonsense mutants cannot be produced selectively, a procedure is required for their identification in a population that may contain other classes such as missense, frame-shift, and deletion mutants. The problem can be simplified to some extent by restricting attention to mutants that can be reverted to the parental type by exposure to the base-analog mutagens 2-aminopurine or bromouracil (9); these mutants should comprise only the nonsense and missense classes.

To distinguish nonsense mutants from missense mutants, four procedures have been used with bacteriophage and bacteria. Evidence obtained by these procedures has firmly established the existence of nonsense mutants and has confirmed the hypothesis that the mutants produce chain-terminating nonsense triplets. The experimental details are as follows.

1) *Pleiotropic mutant phenotype*. Because of the polarity of messenger RNA, which is translated unidirectionally starting from the 5'-end of the molecule (5), it is possible for one nonsense triplet to block translation of an extended region of the RNA molecule. The extent of the block will depend on

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Table 1. The genetic code. Each amino acid listed in the table is coded for by an RNA triplet (codon) which has a nucleotide sequence as follows. The 5'-terminal nucleotide appears in the column on the left, the middle nucleotide appears in the top row, and the 3'-terminal nucleotide appears in the column on the right. The symbols *N1* (*amber*), *N2* (*ochre*) and *N3* designate the three nonsense triplets, UAG, UAA, and UGA. The symbols in the tables are explained in reference (2).

5'-terminal	Middle nucleotide				3'-terminal
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	<i>N2 (Ochre)</i>	<i>N3</i>	A
	Leu	Ser	<i>N1 (Amber)</i>	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

the distance between the nonsense triplet and the next site in the RNA at which translation can again be initiated. In the extreme case where there is no site for reinitiating translation, the block will extend over the entire region of the RNA molecule between the nonsense triplet and the 3'-terminal end.

The first evidence of this kind for the occurrence of nonsense mutants was obtained by Benzer and Champe working with mutants of bacteriophage T4, involving the two contiguous A and B genes of the rII region (10). In the standard phage strain, the two genes are probably transcribed as separate messenger RNA molecules, which are translated independently into separate A and B proteins. Consequently, any mutation (including the nonsense type) occurring in one gene will have no effect on the physiological function controlled by the other gene. There exists an exceptional rII mutant in which portions of both genes are deleted; the remaining portions of the two genes appear to act as a single gene, presumably transcribed as a single messenger RNA specifying one protein molecule (Fig. 1). This protein retains the function of the B protein, but has lost the function of the A protein.

The special significance of the deletion mutant is that it can be used to test for the effect on the B gene of mutations occurring in the remaining portion of the A gene. If, in the deletion mutant, a single messenger RNA for the A and B genes is translated in the sequence A to B [as suggested by results of Crick *et al.* (11)], a nonsense triplet

appearing in the A region of the RNA could block the subsequent translation of the B region (Fig. 1). Benzer and Champe found that some of the base-analog revertible mutations in the A gene eliminated the function controlled by the B gene. They concluded that these were nonsense mutations acting as shown in Fig. 1. Although their evidence did not provide the proof for this conclusion, it was sufficiently persuasive to arouse widespread interest in the subject of genetic nonsense, and it introduced a valuable methodology for the identification of nonsense mutations.

The results obtained with rII mutants of bacteriophage T4, as summarized in Fig. 1, suggest that a nonsense triplet in one region of a messenger RNA can prevent the translation of another region of the RNA molecule. In the T4 rII case, both regions are transcribed from a segment of DNA which functions as a single gene (the combined A and B genes which are integrated by a deletion). It has been shown with bacteria that a cluster of several genes can operate as a polygenic unit of transcription, forming a messenger RNA molecule containing information for specifying more than one peptide chain (12, 13). If the entire RNA molecule is translated sequentially, a nonsense triplet causing premature chain termination of one of the peptide chains could exert a pleiotropic effect, blocking the formation of one or more of the other peptide chains specified by the molecule (Fig. 2). This effect will be polarized, preventing continuation of growth in one direction only, toward the regions

that are subsequently translated. Thus the existence of polygenic species of messenger RNA, transcribed as a unit from several genes, provides a means of extending the pleiotropic test for nonsense mutations; instead of the test being limited to a single gene, as in the T4 rII experiments, the responses of different genes in a cluster to a mutation in one of the genes can be examined.

It has been found in studies of several gene clusters in bacteria—notably the lactose (14), tryptophan (13), histidine (15), and pyruvate dehydrogenase (16) clusters—that certain mutations, revertible by base-analog mutagens, produce a pleiotropic mutant phenotype involving more than one of the genes in the cluster. There is polarity associated with the pleiotropic effect which is generally in accordance with the map order of the genes; only the genes located along one direction from the mutation usually are affected (17). This is the expected result for nonsense mutations which act as shown in Fig. 2, but it is not a proof of the postulated mechanism. Pleiotropic mutations have also been described for an RNA phage, in which the genetic material acts directly as a polygenic messenger RNA (18).

A detailed quantitative analysis of pleiotropic mutations in the Z gene of the lactose cluster showed that the extent of the block of the function controlled by another gene in the cluster varied with the relative position of the mutation within the Z gene. The block became progressively weaker as the position of the mutation was shifted closer to that of the adjoining gene (14; for similar results with the tryptophan cluster, 13). A practical consequence of this position effect is that the pleiotropic action of a nonsense mutation located close to an adjoining gene might not be detected. An interpretation of the position effect, according to the mechanism illustrated in Fig. 2, is that premature chain termination occurring in the first gene to be translated in a polygenic messenger RNA has a certain probability of blocking reinitiation of translation of the RNA; this probability is directly proportional to the distance between the point of chain termination and the beginning of the next gene in the sequence.

Thus, on the basis of the polarized pleiotropic effect of some of the base-analog revertible mutations in phage and bacteria, it has been tentatively concluded that these are nonsense mu-

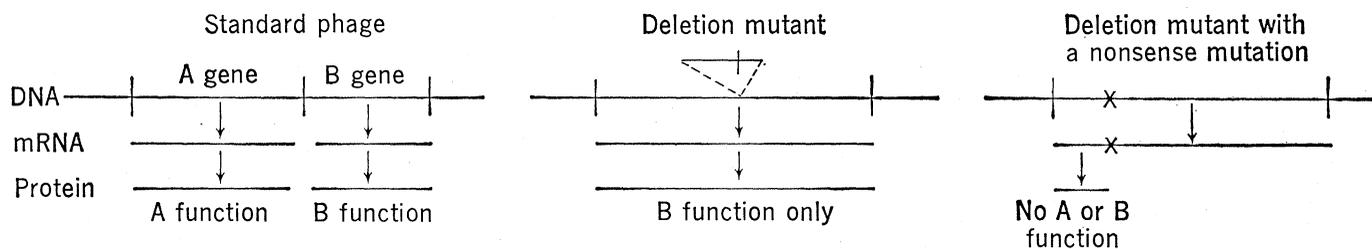


Fig. 1. Pleiotropic effect of a nonsense mutation in a T4 rII deletion mutant. In the standard bacteriophage strain, the A and B genes of the rII region are shown to be transcribed as separate messenger RNA molecules which are translated into separate proteins. In the deletion mutant, a single messenger RNA molecule is formed which is translated into a single protein capable of performing the B function but not the A function. In the deletion mutant containing a nonsense mutation in the A gene, a protein fragment is produced which cannot perform either the A or B functions (10).

tations. Other experiments confirming this conclusion are discussed later in relation to suppression; they show that the pleiotropic mutations respond to certain suppressor genes in *Escherichia coli*, which reverse the chain-terminating action of nonsense triplets.

2) *Extreme negative-mutant phenotype.* Another approach to the problem of distinguishing between a nonsense mutant and a missense mutant is to assay for the capacity of the mutant to perform certain functions controlled by the gene in which the mutation occurs. Two of the functions which are especially useful for this purpose are the formation of immunological cross-reacting material (CRM) and intragenic complementation. These functions are not likely to be retained by a nonsense mutant which can synthesize only a fragment of the protein required for the functions, while a missense mutant which synthesizes the protein intact might retain at least one of the functions. In a study done contemporaneously with the T4 rII experiments of Benzer and Champe, Garen and Siddiqi (19) showed that some of the alkaline phosphatase structural mutants of *Escherichia coli*, which are revertible by base-analog mutagens, have an extreme negative phenotype totally lacking in phosphatase enzymatic activity, CRM, and complementation activity, and that they are therefore possible nonsense mutants. These properties alone are not

sufficient to identify a nonsense mutant because some missense mutants could also have an extreme negative phenotype. The significant aspect of the studies with alkaline phosphatase is that all of the mutants belonging to a certain suppressible class (which is a criterion for nonsense mutants discussed in the following section) have the extreme negative phenotype (19). This generalization applies not only to the suppressible phosphatase mutants but also to the same class of suppressible mutants involving other genes of bacteria (13, 15) and T4 phage (20). Furthermore, most of the extreme negative mutants which can be reverted by base-analog mutagens appear to be nonsense mutants by the same criterion of suppression.

3) *Suppression.* Two independent criteria for identifying the mutations in phage and bacteria which appear to be nonsense were described above. A connecting link between these criteria is the phenomenon of suppression of a mutation by the action of certain specialized bacterial suppressor genes, resulting in the restoration of a normal (or partially normal) phenotype despite the persistence of the original mutation. Both the T4 rII mutations and the alkaline phosphatase mutations, tentatively classified as nonsense (see sections 1 and 2, above), are suppressible in certain bacterial strains (10, 19). When the T4 rII mutations were tested in a suppressor strain active with alkaline phosphatase

tase mutations, a positive response was obtained (21), an indication that mutations involving two genes with unrelated functions can be suppressed by the same strain (known to contain a single active suppressor gene). Evidently, suppression is specific for the mutation and not for the gene in which the mutation occurs. (This generalization is not strictly valid, since the mechanism of suppression, as discussed below, acts during translation and therefore cannot affect genes which are not translated, such as the structural genes for transfer RNA.)

Suppression is now the preferred method for identifying nonsense mutations in phage and bacterial genes. The principal reason for the preference is that the genetic and biochemical properties of the suppressor genes involved are well defined; and, as discussed in the following section, the genes act by reversing the chain-terminating effect of nonsense triplets in messenger RNA. Thus, suppression by these genes is a valid criterion for a nonsense mutation. Another reason is that the techniques of testing for suppression are simple and rapid. In describing the techniques, it is helpful to distinguish two ways of designing the suppression test: one which is applicable only to mutations in genes having an essential, indispensable role in the growth of an organism, and the other which is applicable only to mutations in genes that, under suitable culture conditions, are unessential

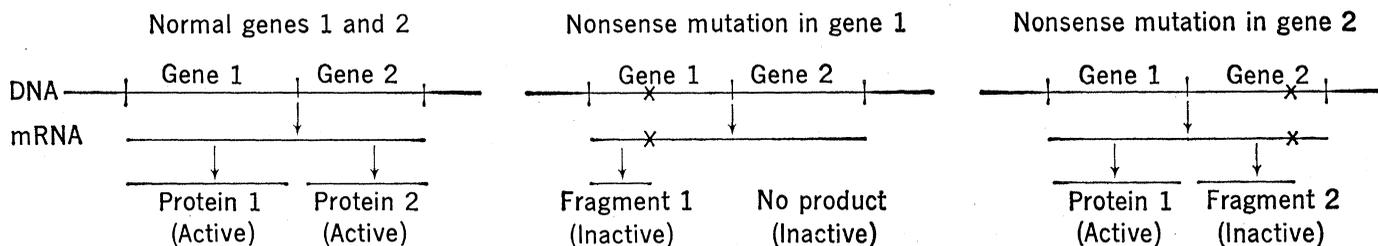


Fig. 2. Polarized pleiotropic effect of a nonsense mutation in a polygenic messenger RNA. A hypothetical example of a cluster of two genes which are transcribed as a single messenger RNA. The direction of translation is assumed to be from 1 to 2.

Table 2. Triplets related by a single base substitution to the *amber* and *ochre* triplets.

Nonsense triplet	Related triplet		
UAG (<i>amber</i>)	AAG (Lys)	UCG (Ser)	UAC (Tyr)
	CAG (Gln)	UUG (Leu)	UAU (Tyr)
	GAG (Glu)	UGG (Trp)	UAA (<i>ochre</i>)
	AAA (Lys)	UCA (Ser)	UAC (Tyr)
UAA (<i>ochre</i>)	CAA (Gln)	UUA (Leu)	UAU (Tyr)
	GAA (Glu)	UGA (Trp)	UAG (<i>amber</i>)

for growth. For technical reasons, the first way of testing for suppression is feasible with bacteriophage but not with bacteria, while the second has been used both with phage and bacteria. The experimental details, in brief, are as follows.

For the first suppression test involving essential phage genes, mutants are produced and propagated in a host strain containing an active suppressor gene (*Su*⁺ strain) and tested for growth in a host strain without an active suppressor gene (*Su*⁻ strain). The evidence for suppression is the inability of the mutant to grow in the *Su*⁻ host (20). A reverse procedure is required for the second test involving mutations in unessential genes of bacteria or phage. As

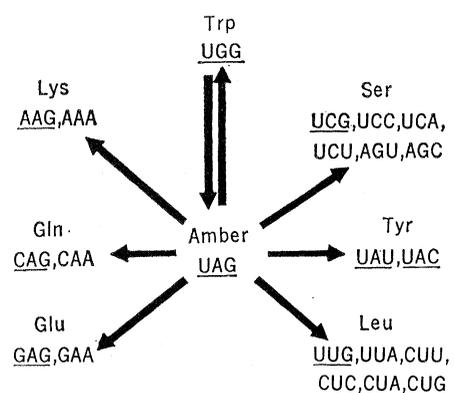


Fig. 3. Identification of an *amber* nonsense triplet as UAG. The diagram summarizes the pattern of amino acid substitutions in the alkaline phosphatases produced by revertants of a phosphatase *amber* mutant (32). Each substitution occurred at the same position, which is occupied by a tryptophan residue in the standard phosphatase molecule. All of the triplets designated from studies in vitro as codons for the substituted amino acids are listed; the underlined codons are those related to UAG by a single base change. It is evident that UAG is the only triplet which has this relationship to at least one of the codons for each of the seven substituted amino acids. See (2) for abbreviations.

applied to bacteria, the defective mutants (for example, phosphatase-negative) are isolated in an *Su*⁻ strain, and afterward the mutant genes are transferred, by means of mating or transduction, to an *Su*⁺ strain. The evidence for suppression is the appearance of the mutant phenotype only in the *Su*⁻ strain, whereas in the *Su*⁺ strain the normal phenotype (for example, phosphatase-positive) is at least partially restored (19).

An important feature of the first method of isolating nonsense mutants is that it is generally applicable to all genes which specify an essential protein. The nonsense mutants obtained in this way with the bacteriophage T4 (20), with a single-stranded DNA phage (22), and with an RNA phage (23), can be used to identify most, and possibly all, of the genes of these organisms.

4) *Fragment formation*. Although the three tests for nonsense mutations described above have given consistent results, they are based on indirect criteria. What is lacking is direct evidence for premature chain termination caused by a nonsense mutation, namely, the isolation of a protein fragment specified by a mutant gene. Fragments are difficult to detect because the assays developed for the intact protein generally do not work with a fragment. The first successful isolation of fragments was achieved with nonsense mutants of bacteriophage T4, involving the gene that specifies the major subunit of the head protein of the phage (24). The special characteristic of this protein, and the key to the success of the experiment, is that it represents the major component synthesized in cells infected with T4, and it can therefore be detected directly without prior purification. The experiment was performed with a group of the head protein mutants classified as nonsense by the criterion of suppression. The results were dramatic and convincing. The cells infected with a nonsense mutant produced a precisely defined fragment of the head protein, the size of the fragment correlating with the relative position of the nonsense mutation in the head protein gene. In subsequent work with nonsense mutations in the coat-protein gene of the RNA bacteriophage f2 (18), in the structural genes for β -galactosidase of *Escherichia coli* (25), and in the structural gene of alkaline phosphatase of *E. coli* (25a), fragments of the proteins specified by these genes have also been found.

The chain-terminating mutations that cause fragment formation in bacterio-

phages T4 and f2 are suppressed in a suppressor strain of bacteria, with the result that an intact protein is synthesized instead of a fragment (18, 24). Other experiments on the biochemistry of suppression (see below) show that the intact protein synthesized in a suppressor strain can have an amino acid substitution at a position in the protein which corresponds to the position of the chain-terminating mutation (26-28). These results indicate that suppression occurs at the stage of translation of a triplet which functions, in the absence of suppression, as a chain-terminating nonsense triplet. Thus, the first goal in the study of nonsense in the genetic code has been achieved with the demonstration of the occurrence of mutations which produce nonsense triplets in messenger RNA.

Nonsense Triplets

With the availability of well-characterized nonsense mutants, the problem shifts to the identification of the nonsense triplet (or triplets) causing premature chain termination. The first mutants selected for the analysis of nonsense triplets were classified together on the basis of their capacity to respond to three of the suppressor genes of *Escherichia coli*, *Su1*, *Su2*, and *Su3* (29, 30). The mutants in this class are called *amber* when they occur in T4 phage (20, 31) and *N1* when they occur in the alkaline phosphatase gene of *E. coli* (29); since the phage terminology has gained wide acceptance, it is adopted for this review.

Techniques adequate for nucleotide-sequence determination with cellular DNA or messenger RNA have not yet been developed. Therefore, indirect methods are required to identify a nonsense triplet. Two different methods have been employed for this purpose, the choice being determined by the potentialities and limitations of the materials involved. One method used with alkaline phosphatase nonsense mutants (32) is based on the triplet assignments in the genetic code (Table 1); in this method, the nonsense triplet is altered by mutations, and the new triplets derived in this way are identified by the amino acid substitutions they cause in the alkaline phosphatase molecule. If the triplets related to the nonsense triplet by a base substitution are known, it is possible to deduce the nonsense triplet. In the other method, chemical mutagens which appear to produce specific

base substitutions in T4 DNA are used (31); the nonsense triplet is deduced by an analysis of the pattern of induction and reversion of nonsense mutations by the mutagenic agents. At present, the genetic code probably provides a more reliable frame of reference than the specificity of chemical mutagenesis. As expressed in a review on mutagenesis (9): "At one time it was hoped that mutation studies would lead to a solution of the coding problem, but, with the development of in vitro protein-synthesizing systems, the situation is reversed." It was encouraging to find that results with the two methods were in agreement in identifying the *amber* nonsense triplet as UAG (31, 32). The evidence obtained for this assignment from experiments with an alkaline phosphatase *amber* mutant is summarized in Fig. 3. The assignment of UAG as the *amber* triplet has been made at five different mutant sites in the alkaline phosphatase gene (6) and at eight different sites in the head-protein gene of phage T4 (33). Thus, UAG appears to be the only *amber* triplet.

Further experiments on suppression of nonsense mutants, involving additional suppressor genes, revealed a new class of mutants which could be suppressed by some, but not by all, of the suppressor genes for *amber* mutants (31, 34). The new mutants are called *ochre* when they occur in T4, and *N2* when they occur in the alkaline phosphatase gene of *Escherichia coli*; as before, the phage nomenclature is used in this review. *Ochre* mutants can be transformed into *amber* mutants by mutation, indicating that different nonsense triplets are involved and that they are interrelated by a single base substitution (31, 34). Identification of the *ochre* triplet as UAA was accomplished by the same two methods used for the *amber* triplet, namely chemical mutagenesis of T4 (31) and amino acid substitution determinations with alkaline phosphatase (35). The experiments with alkaline phosphatase are discussed in detail because of certain interesting features they reveal about the code.

The nine triplets related by a single-base substitution to the *amber* triplet UAG are tabulated in the first part of Table 2. Eight of the triplets can be accounted for as codons for the amino acid substitutions shown in Fig. 3 (on the assumption that tyrosine substitutions are specified in some cases by UAC and in others by UAU). The remaining codon, UAA, appears not to have coding activity in vitro, and, since

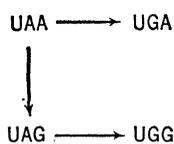


Fig. 4. A procedure for producing the triplets UAA, UAG, UGG, and UGA at the same position in alkaline phosphatase mRNA. The UAG triplet was derived from UAA by isolating a phosphatase *amber* mutant from a phosphatase *ochre* mutant. By further mutation of the *amber* mutant, the UGG triplet can be derived from UAG, and similarly, by mutation of the *ochre* mutant, the UGA triplet can be derived from UAA.

it is related to UAG, it is the prime candidate for the *ochre* triplet. In the second part of Table 2, the nine triplets related to UAA are listed. One is the *amber* triplet. Two others, the tyrosine codons UAC and UAU, are also related to UAG. The remaining six triplets are related only to UAA and not to UAG; however, five of these specify amino acids which are also specified by triplets related to UAG. The exception is UGA which does not appear to have coding activity in vitro (3). Thus, only one difference can be expected when *amber* and *ochre* nonsense mutants are analyzed by the amino acid substitution method, namely the appearance of tryptophan among the substitutions derived from an *amber* mutant but not from an *ochre* mutant. This expectation was realized in experiments with alkaline phosphatase *amber* and *ochre* mutants (6, 35). However, it is always a possibility in this kind of analysis that a particular substitution might not appear because it is incompatible with the activity of the protein molecule. To examine this possibility, the procedure outlined in Fig. 4 was followed. Both *amber* and *ochre* triplets were produced at the same positions in the alkaline phosphatase gene; each triplet was then altered by mutations, and the resulting alkaline phosphatase protein species were analyzed for amino acid substitutions occurring at the same position in the protein. Tryptophan substitutions did occur by alteration of the *amber* triplet but not of the *ochre* triplet; in contrast, the other six amino acids listed in Table 2 occurred as substitutions by alteration of both *amber* and *ochre* triplets. These results show that the alkaline phosphatase molecule can accept a tryptophan substitution at the positions analyzed. The fact that a tryptophan substitution did not occur with *ochre* mutants despite an extensive search is, for the reasons previously cited, consistent

with the assignment of UAA as the *ochre* triplet.

From the position that the triplet UGA occupies in the genetic code (Table 1), it might be expected to code either for tryptophan or cysteine. That UGA is not a tryptophan codon is indicated by the results, discussed in the preceding section, of amino acid substitution analyses with alkaline phosphatase *ochre* mutants (6, 35) and also from experiments with T4 *ochre* mutants (36). Furthermore, there is no evidence that UGA can code in *Escherichia coli* for any other amino acid either in vitro (3) or in vivo (6, 35). The possibility that UGA might be a third nonsense triplet was examined by means of mutations in the T4 rII region which, on the basis of chemical mutagenesis criteria, appear to produce a UGA triplet (37). The principal finding was that one of these mutations in the *rIIA* gene exerted a pleiotropic effect on the *rIIB* gene when tested by the technique of Benzer and Champe (Fig. 1). Since this behavior conforms to one of the well-established criteria for nonsense mutations, UGA has been assigned as a third nonsense triplet in *E. coli*. However, in another organism, the guinea pig (liver cells), coding experiments in vitro suggest that UGA may be an effective cysteine codon (3).

Thus, the three triplets UAG, UAA, and UGA, produced by mutations in certain strains of bacteriophage and bacteria, can act as nonsense triplets. The coding results in vitro (Table 1) indicate that there are no additional nonsense triplets in *Escherichia coli*, since all remaining triplets have a coding assignment. However, nonsense is not an absolute property of a triplet but depends on a number of experimental factors that enter into an in vitro system, such as the organism used to test for translation of a triplet, the suppressor genotype of the selected strain of the organism, the ionic composition of the medium, and the sensitivities of the assay techniques for measuring coding activity. Therefore, the possibility remains that one or more triplets other than UAG, UAA, and UGA might be found to act as a nonsense triplet in some organisms.

Suppressor Genes for Nonsense Mutations

The coding behavior of nonsense triplets is genetically controlled in bacteria by certain suppressor genes which

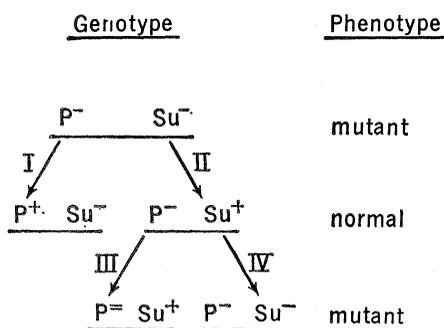


Fig. 5. A procedure (29) for the genetic analysis of suppressor genes for phosphatase nonsense mutations, showing pathways I, II, III, and IV which are discussed in the text.

determine the capacity of the cell to translate the triplets. The properties of bacterial suppressor genes have been studied by the procedure outlined in Fig. 5 for obtaining suppressor mutants (29). For these studies, nonsense mutations in the structural gene for alkaline phosphatase were used as a marker to detect the suppressor mutants (nonsense mutations in other genes can also be used for this purpose, since the specificity of suppression pertains to the mutation and not to the gene in which the mutation occurs). The procedure starts with a suppressor-negative *Su*⁻ strain containing a phosphatase nonsense mutation. The first step is the isolation of revertant strains having a normal phosphatase phenotype. Genetic mapping shows that the revertants can

Table 3. Response of *amber* and *ochre* nonsense mutations to suppression. The amino acid inserted by a suppressor gene was determined by analysis of the protein produced as a result of suppression. Experimental details are reported in the following references: *Su1*⁺ (26), *Su2*⁺ and *Su3*⁺ (27), *Su4*⁺ (28), *Su5*⁺ (32). The tabulated efficiencies of suppression were estimated by the amount of alkaline phosphatase protein (measured as CRM) produced as a result of suppression of a phosphatase-negative mutant (29, 34). These efficiencies have also been estimated by the extent of reversal of chain termination in an *amber* mutant of phage T4 (30, 33).

Suppressor gene	Nonsense mutation	Amino acid inserted	Efficiency of suppression (%)
<i>Su1</i> ⁺	<i>Amber</i>	Ser	28
	<i>Ochre</i>	0	0
<i>Su2</i> ⁺	<i>Amber</i>	Gln	14
	<i>Ochre</i>	0	0
<i>Su3</i> ⁺	<i>Amber</i>	Tyr	55
	<i>Ochre</i>	0	0
<i>Su4</i> ⁺	<i>Amber</i>	Tyr	16
	<i>Ochre</i>	Tyr	12
<i>Su5</i> ⁺	<i>Amber</i>	(Basic)	5
	<i>Ochre</i>	(Basic)	6

occur in either of two ways. One is by a mutation which transforms the nonsense triplet into another triplet which is a codon in the *Su*⁻ strain (pathway I in Fig. 5). Revertants obtained in this way provided the alkaline phosphatase protein used for deducing the *amber* and *ochre* nonsense triplets by the amino acid substitution method (Fig. 3 and Table 2). The second way in which revertants occur is by a suppressor mutation that produces a suppressor-positive *Su*⁺ strain capable of translating the nonsense triplet (pathway II). The revertants obtained in the second way comprise the strains needed to analyze the genetic and biochemical properties of suppressor genes for nonsense mutations. In addition to revertants that suppress phosphatase nonsense mutations, revertants have been isolated on the basis of their suppressor activity for nonsense mutations in other genes of *Escherichia coli* and T4 (30, 38).

The revertants which suppress *amber* or *ochre* mutations occur at several different map positions. In this review the three positions shown in Fig. 6, involving five suppressor genes, are discussed. Each suppressor gene designates a region of the map controlling a single suppressor function. The two genes *Su2* and *Su5*, which are located at one of the map positions, are closely linked, but for the biochemical reasons discussed below they appear to be functionally distinct. The evidence for separate *Su3* and *Su4* genes is that their map positions, although closely linked, are sufficiently separated to make it unlikely that only a single gene is involved (34).

The five suppressor genes in Fig. 6 differ in certain physiological and biochemical properties. All five of the genes suppress *amber* mutations, but only two, *Su4* and *Su5*, also suppress *ochre* mutations (Table 3). None of the genes suppress the UGA class of mutations. Another difference is in the efficiency of suppression of nonsense mutations, that is, the extent of restoration of the synthesis of the intact protein molecule. The efficiencies range from 5 to about 60 percent among the five genes (Table 3). These values are a measure of the relative rates of translation of a nonsense triplet in different suppressor strains. A third difference is in the amino acid residue incorporated into a protein as a result of suppression. The remarkable finding is that four of the five genes specify the incorporation of different amino acids (Table 3).

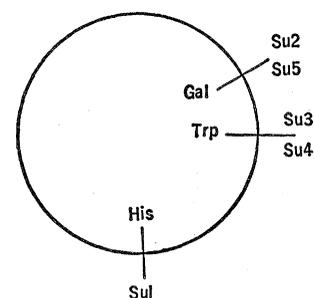


Fig. 6. Genetic map locations of five *Escherichia coli* suppressor genes for nonsense mutations. The *Su1*, *Su2*, and *Su3* suppressor genes (29) correspond to the suppressor genes designated by other investigations as *suI*, *suII* and *suIII* (33).

Thus, a suppressor gene controls not only the capacity of a cell to translate a nonsense triplet but also the coding specificity of the triplet. For example, the same *amber* triplet can either be nonsense if all the suppressor genes are *Su*⁻, or it can specify any one of the four amino acids, serine, glutamine, tyrosine, or a basic residue, depending on which of the suppressor genes is *Su*⁺ (Table 4) (39). There are also other *Escherichia coli* suppressor genes for *amber* and *ochre* nonsense mutations, in addition to the five described in Table 3; it is likely that biochemical studies with these suppressors will reveal new amino acids which can be specified by the nonsense triplets.

The efficiency of suppression of a nonsense mutation can depend on the relative position of the mutation within a gene as well as on the potency of a suppressor gene. Such a position effect has been demonstrated for one of the *amber* mutations in the structural gene for alkaline phosphatase; that mutation responds poorly to suppression in comparison to other *amber* mutations (6). The efficiency of translation of the *amber* triplet (and also other triplets) may be influenced by its nearest-neighbor nucleotide sequence.

A suppressor gene for mutations producing the UGA nonsense triplet has been found in *Escherichia coli* (40). The efficiency of suppression of the UGA class of mutations is high, about 60 percent, but there is no suppressor activity for either *amber* or *ochre* mutations. At present there is no information about either the map position of this suppressor gene or the amino acid which is specified by UGA as a result of its suppressor activity.

The mutational pathway II (Fig. 5), by which suppressor revertants are obtained, apparently generates a unique

change in each suppressor gene, since independently isolated *Su*⁺ revertants for the same gene fail to produce *Su*⁻ recombinants in pairwise genetic crosses. This genetic behavior can now be understood in terms of the biochemistry of the suppressor gene product (see below). The mutational pathway IV in Fig. 5, by which *Su*⁻ mutations are induced in an *Su*⁺ strain, yields a different result; studies of the *Su1*⁺ gene have shown that *Su*⁻ mutations occur at several separate sites within the suppressor gene (29). The *Su*⁻ mutants derived in this way provide material for fine-structure genetic analyses of suppressor genes and, as discussed below, also for biochemical analyses of the suppressor gene product.

Biochemical Basis of Suppression

Having established that suppressor genes control the specificity of translation of certain triplets, the desirability of studying suppression with the techniques of protein biosynthesis in vitro becomes evident. The experiments in vitro were initiated before the nonsense triplets were identified, and for that reason synthetic polynucleotides were not used initially as the message in vitro. Instead, the experiments were designed to use nonsense mutants of an RNA phage, since it had been shown that the RNA of the phage acts as a message in vitro for the coat protein of the phage (7). For this purpose *amber* mutants, suppressible in an *Su1*⁺ host strain, were isolated (41). The availability of the phage *amber* mutants, combined with the knowledge that the amino acid specified by the *amber* triplet in the *Su1*⁺ suppressor strain is serine (Table 3), set the stage for a test in vitro of suppression. Successful results were obtained by two groups, by Cappecchi and Gussin working in J. D. Watson's laboratory (42) and by a collaborative effort between the laboratories of N. D. Zinder and A. Garen (43). Both groups were able to demonstrate that a transfer RNA (tRNA) for serine was the active suppressor component in an *Su1*⁺ strain. The results of the latter group showed that in the absence of the suppressor tRNA a small fragment of the coat protein was formed, and that the addition of tRNA from the *Su1*⁺ suppressor strain resulted in the formation of a complete protein molecule with a serine residue replacing a glutamine residue normally present in the

Table 4. Effects of five suppressor genes on the translation of a messenger RNA containing the *amber* triplet UAG. The original RNA is shown to contain the tryptophan codon UGG, from which the *amber* triplet is derived by mutation. The amino acid specified by the *amber* triplet as a result of suppression by the *Su5*⁺ gene is a basic residue which has not been identified but probably is lysine. See (2) for abbreviations.

Messenger RNA	Polypeptide	Suppressor genotype				
		<i>Su1</i>	<i>Su2</i>	<i>Su3</i>	<i>Su4</i>	<i>Su5</i>
AUGUACUGGGUCGUU	Met.Tyr.Trp.Gly.Val	-	-	-	-	-
AUGUACUAGGUCGUU	Met.Tyr	-	-	-	-	-
AUGUACUAGGUCGUU	Met.Tyr.Ser.Gly.Val	+	-	-	-	-
AUGUACUAGGUCGUU	Met.Tyr.Gln.Gly.Val	-	+	-	-	-
AUGUACUAGGUCGUU	Met.Tyr.Tyr.Gly.Val	-	-	+	-	-
AUGUACUAGGUCGUU	Met.Tyr.Tyr.Gly.Val	-	-	-	+	-
AUGUACUAGGUCGUU	Met.Tyr.(Lys).Gly.Val	-	-	-	-	+

molecule. Other suppressor tests in vitro on the tRNA derived from the remaining four suppressor strains listed in Table 2 suggest that the mechanism of suppression is the same in all of the strains tested, a specific tRNA from each suppressor strain apparently being the active suppressor component (44).

The results of the suppressor tests in vitro prove that certain species of tRNA in the *Su*⁺ strains analyzed are the cellular components directly responsible for suppression. What remains unresolved by these analyses is the role of the suppressor gene in the formation of the tRNA. One possibility is that a suppressor gene acts directly as a structural gene for the tRNA. An alternative possibility is that a suppressor gene acts indirectly by specifying some product, perhaps an enzyme, which transforms an inactive tRNA into one with suppressor activity.

The function of a suppressor gene has been revealed by recent biochemical studies involving the *Su3* gene. This gene occurs in the region of the *Escherichia coli* genome which is transducible by the phage $\phi 80$. Transduction by $\phi 80$ is of the "restricted" type in which certain bacterial genes from one region either replace some of the phage genes of the transducing particle, or are added to a complete phage genome. The $\phi 80$ particles with transducing activity for the *Su3*⁺ gene provide a source of phage DNA, containing the *Su3*⁺ gene, which can be used for hybridization tests between DNA and tRNA. The results of such hybridization tests reported by two laboratories (45) showed that *Su3*⁺ DNA could hybridize specifically with tyrosyl tRNA. Since tyrosine is the amino acid specified by the *amber* triplet as a result of suppression by the *Su3*⁺ gene, this is the expected result if the *Su3*⁺ gene is a structural gene for a tyrosyl tRNA.

Purified fractions of two major

species of tyrosyl tRNA, called species I and II, were equally effective in the hybridization tests. Thus, as a method for identifying the product of a suppressor gene, hybridization cannot distinguish between the different species of tRNA molecules with the same acceptor activity, presumably because of the close similarity of their base sequences. Furthermore, the tyrosyl tRNA obtained from both an *Su3*⁺ and *Su3*⁻ strain proved equally effective in hybridizing with the DNA of the transducing phage. This is not a surprising result in view of the fact that the two strains differ by a single suppressor mutation, and therefore the two tRNA molecules presumably differ by only a single base substitution.

Further biochemical studies of the tRNA specified by a suppressor gene require purified material. An attempt to purify the seryl tRNA, specified by the *Su1* gene, was only partially successful and led to the discouraging conclusion that this tRNA was present as a minor species containing less than 10 percent of the amount of seryl tRNA in a major species (46). A similar conclusion was reached in the case of the tyrosyl tRNA specified by the *Su3* gene (33). The purification of such minor components presents a formidable experimental obstacle. This obstacle was overcome by an ingenious use of the $\phi 80$ transducing phage carrying the *Su3* gene (33). It was found that, in a cell infected with both the transducing phage and a nontransducing $\phi 80$ phage, conditions could be established in which the tyrosyl tRNA specified by the *Su3* gene becomes the predominant species synthesized by the infected cell, thereby greatly simplifying its purification. With this procedure, enriched samples of tyrosyl tRNA specified by the *Su3*⁻ and *Su3*⁺ genes (the two genes differing by a single mutation) were prepared, and these were tested for their

Table 5. A model for the biochemical effect of certain suppressor mutations. The general assumption is that each suppressor gene is a structural gene for a tRNA species, and that an *Su⁻* mutation alters the anticodon of the tRNA. In the case of the tRNA specified by the *Su3⁻* gene, it has been shown that the anticodon is GUA, rather than the alternative possibility AUA (47). For further details see the text and reference (56).

Sup- pres- sor gene	Anti- codon in tRNA	Matching codon in mRNA	Amino acid specified
<i>Su1⁻</i>	CGA	UCG	Ser
<i>Su1⁺</i>	CUA	UAG	
<i>Su2⁻</i>	CUG	CAG	Gln
<i>Su2⁺</i>	CUA	UAG	
<i>Su3⁻</i>	GUA	UAU, UAC	Tyr
<i>Su3⁺</i>	CUA	UAG	
<i>Su4⁻</i>	AUA or GUA	UAU and/ or UAC	Tyr
<i>Su4⁺</i>	UUA	UAA, UAG	
<i>Su5⁻</i>	UUU	AAA, AAG	Lys
<i>Su5⁺</i>	UUA	UAA, UAG	

capacity to bind to ribosomes in the presence of various nucleotide triplets (33). The triplets UAU and UAC, which are standard codons for tyrosine, stimulated binding of the tRNA specified by the *Su3⁻* gene, while the *amber* triplet UAG stimulated binding of the tRNA specified by the *Su3⁺* gene, and the *ochre* triplet UAA did not stimulate binding of either of these tRNA species. These results suggest that the two tyrosyl tRNA species have different anticodons, the species specified by the *Su3⁻* gene containing the anticodon GUA and the suppressor species specified by the *Su3⁺* gene containing the anticodon CUA. (The convention for designating an anticodon is the same as is used for a codon, placing the 5'-terminal nucleotide at the left and the 3'-terminal nucleotide at the right, although the codon-anticodon pairing occurs in an antiparallel orientation.)

This difference in the anticodons of the tyrosyl tRNA specified by the *Su3⁻* and *Su3⁺* genes was demonstrated by comparative base-sequence analyses of the purified tRNA. The tRNA specified by the *Su3⁻* gene contains the triplet GUA in the anticodon loop, which is replaced in the tRNA from the *Su3⁺* gene by the triplet CUA (47).

The impressive biochemical results obtained with the tyrosyl tRNA of the *Su3* gene support the general mechanism of suppressor gene function shown in Table 5. The basic premise is that, for all of the suppressor genes that act on *amber* or *ochre* mutations, the phenotypic difference between the *Su⁻* parental strain and the *Su⁺* revertant strain results from a change in the sequence of the suppressor tRNA. It is

further assumed that the anticodon of the tRNA specified by the *Su⁻* gene is complementary to one of the standard codons for the amino acid which the tRNA carries. This would account for the finding that the four amino acids known to be specified by *amber* and *ochre* triplets as a result of suppression (Table 3) are normally coded for by triplets which are related to the two nonsense triplets by a single base substitution. Accordingly, there probably exist other, still unidentified, suppressor genes for both *amber* and *ochre* mutations which specify leucyl and glutamyl tRNA species, and a suppressor gene for *amber* mutations which specifies a tryptophanyl tRNA.

The mechanism of suppression shown in Table 5, in conjunction with the "wobble" hypothesis of codon-anticodon specificity proposed by Crick (48), provides an adequate explanation for the fact that the *Su1⁺*, *Su2⁺*, and *Su3⁺* genes suppress only *amber* mutations, while the *Su4⁺* and *Su5⁺* genes suppress both *amber* and *ochre* mutations (Table 3). According to Crick's hypothesis, a CUA anticodon should be capable of interacting only with UAG because of the postulated stringency in the GC pairing when C is at the 5' end of the anticodon, in contrast to a UUA anticodon which should be capable of interacting both with UAG and UAA because of the reduced stringency (that is, "wobble") permissible when uracil occupies the 5'-end position in the anticodon.

Suppressor mutations which act as shown in Table 5 are potentially dangerous to a cell, since these may produce alterations in anticodons required for the translation of some of the standard codons. Unless there is available to the cell an additional tRNA species containing an anticodon which is identical (or functionally equivalent) to the anticodon altered in the *Su⁺* strain, a suppressor mutation would be a lethal event. In the case of the tyrosyl tRNA specified by the *Su3⁻* gene, the GUA anticodon is also present in two other species of tyrosyl tRNA which are unaffected by the suppressor mutation and remain available to the *Su3⁺* strain for translating the standard tyrosine codons UAC and UAU (47).

There is no reason to believe that alteration of tRNA is the only cellular mechanism available for suppressing a nonsense mutation. The complex sequence of events involved in the translation of a triplet provides other possibilities. For example, alteration of

ribosomes can affect the specificity and fidelity of translation (49). There has not yet been any conclusive demonstration that suppression of nonsense mutations does occur by alteration of components other than tRNA, although suggestive observations along these lines have been reported (50).

Fine-Structure Analysis of Suppressor tRNA

The genetic and biochemical methods that have been developed for studying the fine-structure details of suppression are also applicable to an analysis of the relation between the structure and function of the tRNA that is specified by a suppressor gene. Consider the mutational pathway IV in Fig. 5, by which *Su⁻* mutations are induced in an *Su⁺* gene. The phenotypic effect of an *Su⁻* mutation is the reduction or complete elimination of suppressor activity (29). When a suppressor gene is a structural gene for tRNA, this phenotypic effect must result from base substitutions occurring in the suppressor tRNA which affect its capacity to participate in the translation of a nonsense triplet. There are several ways that mutations might inhibit the suppressor activity of a tRNA. One is by alteration of the anticodon. The fact that *Su⁻* mutations occur at more than three different genetic sites within a suppressor gene (29) indicates that the anticodon cannot be the only region of the suppressor tRNA affected by the mutations. Evidently, other essential functions of the tRNA molecule, such as its amino acid acceptor and transfer activities, are subject to inactivation by mutation. Thus, *Su⁻* mutations can provide a family of differently altered forms of the same species of tRNA, involving base substitutions at various sites in the molecule, which is ideal material for biochemical studies of the structural requirements for the various functions of the molecule.

Minor Species of tRNA

A striking conclusion from fractionation experiments with suppressor tRNA is that these tRNA species are, at least in the two cases analyzed (33, 46), minor components containing less than 10 percent of the tRNA in a major species having the same amino acid acceptor activity. The small amounts of suppressor tRNA may explain why the effi-

ency of suppression is less than 100 percent (Table 3). The rate of translation of a nonsense triplet is probably limited by the amount of suppressor tRNA available.

Identification of a minor species of tRNA is difficult to achieve without a specific assay such as suppression. For example, the seryl tRNA specified by the *Su1* gene and the tyrosyl tRNA specified by the *Su3* gene fractionate along with major species having the same amino acid acceptor activity (46, 47). These components might not have been detected without the availability of the suppressor assay. Probably other minor species of tRNA also exist.

The presence of both major and minor species of tRNA indicates that there is a mechanism in *Escherichia coli* for regulating the rates of transcription of the structural genes for tRNA. It remains to be shown whether the regulation of tRNA synthesis involves the same kind of control mechanism operating in protein synthesis (51). Analysis of tRNA regulation would be considerably advanced if regulatory mutants were available. Such mutants are difficult to identify because a change in the amount of tRNA synthesized is not readily detectable. The assay for suppression could provide a method of screening for mutants with altered rates of suppressor tRNA synthesis, since the efficiency of suppression, which is a measurable quantity, appears to be correlated with the amount of a suppressor tRNA.

Chain Termination of Protein Biosynthesis

During the process of protein biosynthesis, the growing peptide chain is covalently linked to a tRNA which is attached (probably noncovalently) to the messenger-ribosome complex. The finished protein molecule is ultimately released unlinked to tRNA. When premature chain termination is induced by a nonsense triplet, either *amber* or *ochre*, the protein fragment apparently undergoes the complete reaction sequence involved in normal chain termination. The evidence now available, all from experiments in vitro, indicates that the fragment is not associated with any tRNA (18, 52).

Because virtually nothing is known about the mechanism of normal chain termination, which is one of the critical steps in protein biosynthesis, attention

has been focused on premature chain termination in nonsense mutants as a potential model system for understanding the normal process. The central question is whether any of the nonsense triplets are signals for normal chain termination. There are two reasons why it appears necessary to assume that certain sequences are reserved for this purpose. First, in a polygenic messenger RNA which specifies more than one protein, a terminating signal is needed to prevent the linkage of one protein to another. The chain-initiating triplet AUG, which specifies formylmethionine (53), is, in principle, sufficient for this purpose since formylmethionine cannot form a peptide bond with the preceding amino acid specified by the message. However, when AUG occurs in an internal position in a message rather than at the 5'-terminal position, it acts as a codon for methionine and not for formylmethionine (3). Therefore, additional information must be associated with AUG to identify it as a chain-initiating formylmethionine codon for the internal genes of a polygenic messenger RNA.

A second reason for assuming that a chain-terminating signal exists is that a mechanism is needed for releasing newly synthesized polypeptide chains from the messenger-ribosome complex. There is no information at present about how a release mechanism might operate; possibly additional components, such as special tRNA or protein species, are involved.

The sequence in messenger RNA which normally signals chain termination in *Escherichia coli* has not been identified. However, there are experiments bearing indirectly on the possible role of nonsense triplets in chain termination. If nonsense triplets do perform this essential function, suppression of the function could be harmful to the cell. For two of the nonsense triplets, UAG and UGA, suppression can be as efficient as 60 percent and still not affect cell growth (29, 33, 40), a finding which argues against a normal chain-terminating role for these triplets. By the same reasoning, UAA is a candidate for this role, since even low levels of suppression of the chain-terminating action of this triplet are inhibitory to cell growth (33, 34).

Another pertinent experiment involves a new class of mutants of the RNA phage. The mutants grow in an *Su⁻* strain but are inhibited in *Su⁺* strains in which chain termination by

UAG is strongly suppressed (54). One explanation for this mutant phenotype is that the mutation has transformed a UAA triplet, normally used for chain termination, into UAG. Accordingly, since UAG can cause chain termination as well as UAA in an *Su⁻* strain, phage growth is normal in this strain; in the *Su⁺* strain there is an inhibition of phage growth because a critical chain-terminating site is exposed to the action of a strong suppressor.

Thus, there is suggestive evidence from experiments on suppression that UAG and UGA do not normally serve as chain-terminating triplets in *Escherichia coli*. Furthermore, it is a reasonable guess that these triplets are entirely absent from all messenger RNA. The suppression experiments also suggest that UAA is needed as a nonsense triplet, and therefore that it might be a normal signal for chain termination. The present evidence on these points, however, is inconclusive.

Dispensable and Indispensable Suppressor Genes

It has been shown, as discussed in a preceding section, that the *Su3* suppressor gene is a structural gene for a minor species of tyrosyl tRNA. This tRNA species, and also the minor species of seryl tRNA specified by the *Su1* gene, appear to be dispensable components, since suppressor mutations which alter the anticodon or affect the activity of the molecules in other (unidentified) ways are not lethal to the cell. In addition to these dispensable tRNA species, there probably are also indispensable tRNA species which cannot be altered without causing cell death, notably the species required for translation of the standard codons of a cell. However, in a diploid cell containing two copies of the structural gene for an indispensable tRNA, a mutation in one of the genes need not be lethal as long as the cell retains a normal copy of the gene. *Escherichia coli* cells are usually haploid and therefore cannot tolerate such mutations, but some of the episomal strains of *E. coli*, which have multiple copies of certain regions of the bacterial chromosome, might contain structural genes for indispensable tRNA species in duplicate. In that case, it should be possible to obtain viable mutants of the episomal strains with altered forms of indispensable tRNA species. The problem remains of identifying the mutants.

Consider the mechanism of suppres-

sion outlined in Table 5. There are seven amino acids with codons which are related to the *amber* triplet UAG by a single base substitution (Table 2). The anticodons of the major tRNA species normally involved in translating these seven codons can be altered by mutation to yield suppressor tRNA which can translate the *amber* triplet. If the suppressor mutant is isolated in an appropriate episomal strain, it should be possible to obtain a heterozygote suppressor strain (that is, Su^+/Su^-) in which the suppressor gene is a structural gene for a major species of tRNA. Suppressor strains of this kind can provide genetic and biochemical information about the structural genes for some of the major, indispensable tRNA species. Experiments along these lines are now in progress.

Hindsight and Foresight

The study of nonsense triplets and their suppression has involved two related but experimentally distinct problems, one concerned with the nature of the genetic code and the mechanism of its translation, and the other with the chemistry of transfer RNA. With regard to the first problem, the results of genetic and biochemical experiments on nonsense triplets have contributed to an understanding of the genetic code in several ways. The identification of the three nonsense triplets in *Escherichia coli*, UAG, UAA, and UGA, filled in certain gaps which were left unresolved by coding experiments in vitro and thus helped to complete the code. The demonstration of a polarized chain-terminating effect of nonsense triplets in protein biosynthesis confirmed the sequential mechanism of translation of messenger RNA, and revealed an interdependence in the translation of different genes in a polygenic messenger RNA molecule. The elucidation of the mechanism of suppression of nonsense triplets showed that suppressor mutations, by altering the anticodons of certain minor species of tRNA, could introduce a limited degree of variability into the code. [In this review the important work on suppression of certain missense mutations in bacteria has not been discussed. The role of tRNA in the suppression of the missense mutations is discussed in reference (55).]

There are three outstanding questions related to nonsense triplets and suppression which remain unanswered. (i) Are

any of the nonsense triplets involved in the normal process of chain termination of protein biosynthesis, and, if not, what is the signal for chain termination? (ii) What is the physiological role of the minor species of tRNA specified by a suppressor gene? These species appear to be redundant in the Su^- strain which also contains major tRNA species with the same anticodons and amino acid acceptor activities. (iii) Can suppression of nonsense triplets occur by genetic alteration of components other than tRNA, such as ribosomes and activating enzymes? Needless to say, these intriguing questions are currently under intensive study.

With regard to the second problem—the chemistry of tRNA—there has emerged from the study of suppression an elegant and powerful methodology for correlating the structure and function of this important macromolecular component of cells. By introducing mutations into a suppressor gene (Fig. 5), altered forms of a single species of tRNA can be obtained which differ structurally by a single base substitution in the polynucleotide chain, and functionally in the reaction specificities of the molecule. With the sophisticated techniques of tRNA chemistry now available to identify these base substitutions, it should be possible to elucidate in fine-structure detail the sites in a tRNA molecule which determine its capacity to accept a particular amino acid and to incorporate it into a growing protein chain.

References and Notes

1. For a comprehensive picture of the extensive work on this subject, see "The genetic code," *Cold Spring Harbor Symp. Quant. Biol.* **31**, entire volume (1966).
2. A glossary of some of the specialized terms used in this review follows. *Transcription*, the biosynthesis of an RNA containing a base sequence specified by a DNA template; *translation*, the biosynthesis of a protein containing an amino acid sequence specified by a messenger RNA template; *messenger RNA*, the cellular RNA species which act as templates for protein biosynthesis (as distinguished from ribosomal and transfer RNA); *complementary base pairs*, the specific pairing relationships between bases in double-stranded nucleic acids, which pair adenine with thymine or uracil, and guanine with cytosine; *codon*, the fundamental coding unit in DNA or RNA (a nucleotide triplet) which specifies the incorporation of an amino acid into protein; *deletion mutant*, a mutant resulting from the deletion of a segment of the genetic material; *frame-shift mutant*, a mutant resulting from the addition or deletion of one, or a few, nucleotides in the genetic material, which shifts the "reading frame" of the messenger RNA (it should be noted that a deletion mutant could also be classified as a frame-shift mutant if the deleted region is not extensive and does not contain three, or a multiple of three, nucleotides); *polygenic messenger RNA*, a species of messenger RNA which contains, in a single molecule, the information transcribed from more than one gene—a gene in this context is meant to designate a sequence of genetic information which is translated as a single polypeptide; *intragenic complementation*, the capacity of two functionally defective forms of the same gene (containing different mutations) to function cooperatively when present together in a heterozygous cell—complementation can occur when the gene product is a polymeric protein composed of identical subunits, allowing for the formation in a heterozygous cell of hybrid molecules containing subunits derived from different mutant forms of the gene; *mating*, the transfer of genetic material from a donor to a recipient bacterium by direct cell-cell contact; *transduction*, the indirect transfer of genetic material between bacteria by means of a phage vector. Abbreviations used are U, uracil; C, cytosine; A, adenine; G, guanine; tRNA, transfer RNA; mRNA, messenger RNA; the amino acids are referred to in the table and in sequences as follows: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; and Val, valine.
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the world's population to radioactive iodine in the fallout from nuclear testing or from accidents with nuclear reactors.

Risk of Thyroid Neoplasms after Irradiation in Childhood

Studies of populations exposed to radiation in childhood show a dose response over a wide dose range.

Louis H. Hempelmann

Until relatively recently, the thyroid gland has been classified as a radioreistant organ (1). This classification was based on the fact that large doses of radiation are needed to depress secretory function and to produce histological evidence of tissue damage. Recent studies of late radiation effects indicate that, under certain conditions, neoplastic transformation of the gland can be initiated by radiation doses lower than those formerly considered to be damaging to tissues (2). With respect to this type of radiation damage, the thyroid should be regarded as one of the more radiosensitive organs in the body. This radiosensitivity was not recognized earlier because such radiation injury leading to

the subsequent development of grossly apparent neoplasms is not apparent unless the damaged thyroid cells proliferate for many cell generations. Because parenchymal cells divide only infrequently in the normal adult gland, conditions are unfavorable for the expression of cellular damage induced by radiation. However, in children whose glands are proliferating rapidly (2) or in adult animals with glands stimulated by goitrogens (3) the cells injured by radiation and with abnormal growth potential are able to develop into gross neoplastic lesions. The radiosensitivity of the growing thyroid glands of children is of interest because of the potential exposure of large segments of

Risk of Malignant Transformation after Radiation Exposure

Attempts to obtain an accurate estimate of man's chance of developing thyroid neoplasms after exposure to radiation have been hampered by three uncertainties. (i) Information about the dose response is unsatisfactory because data on incidence of thyroid neoplasms in man after exposure to different doses are limited. (ii) The radiation doses to the thyroid gland used in calculations of risk may be questioned in most studies dealing with children given x-ray treatments to the chest or neck. In these instances, the dose to the thyroid was derived from the x-ray dose in air without taking into account whether the gland was inside or outside the primary beam. In that the radiation dose in tissues falls rapidly at the periphery of the x-ray beam, the location of the gland with respect to the beam is usually more important in determining the thyroid dose than the dose in air is. (iii) The criteria used in diagnosing malignancy were not always uniform. However, because of the need to assess

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